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VI NATIONAL CONGRESS OF PHARMACOLOGY

INVITED SPEAKERS

TEACHING PHARMACOLOGY IN THE MEDICAL FACULTY OF MEDICAL UNIVERSITY IN SOFIA

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The Department of Pharmacology and Toxicology at the Medical Faculty in Sofia was found and started education on pharmacology in 1945. It was first department teaching pharmacology in Bulgaria. Our department supported the development of teaching process in field of pharmacology in medical Universities in Varna, Plovdiv, Pleven, Stara Zagora as well as the teaching in colleges for nurses and medical workers. The department has a history of great progress for scientific education on pharmacology. We offers a broad range of educational opportunities from undergraduate studies to graduate research leading toward the M.D. and Ph.D. Pharmacology education involves undergraduate, graduate degree programs, online pharmacology as well as professional education for medical doctors, dentists, nurses and medical workers.

The pharmacology education program of Medical Faculty in Sofia differs from pharmacy programs. Our program combines pharmacology and drug toxicology into a single unit, focusing on pharmacokinetic, pharmacodynamic, toxicology, drug interactions, prescriptions of drugs, etc., for medical students and doctors. The program contains general, systems, integrative pharmacology and drug toxicology. Students of pharmacology are trained for medical doctors as well as for researchers. Medical Faculty in Sofia offers PhD training in Pharmacology & Toxicology. The specialization on Pharmacology is available. Currently, 300 medical students and 200 students from Dental School are taking their classes and exams on pharmacology in our department.

The department competes to attract able students by offering new courses and options. Problem-based learning (PBL) is gaining interest in many medical schools. Although various approaches have been labelled PBL, it remains unclear which approach is most appropriate for pharmacology courses. There are numerous opportunities in the field of pharmacology available to undergraduate students in our department. The department offers additional 7 PBL courses (modules) for students each academic year as followings: 1. Pharmacotherapy of chronic disorders; 2. Pharmacology of Pain; 3. Drug Abuse; 4. Pharmacotherapy of disorders in dentistry; 5. Alternative medicine for therapy of social-related diseases; 6. Homeopathy; 7. AIDS: pharmacotherapy. The courses focused on therapeutic and molecular aspects of various drugs, used for treatment of large spectrum of disorders (neuronal, brain, lung, cardio-vascular, gastro-intestinal, muscular, cancers, immune-related diseases, arthritis, AIDS, etc.). Our lectures demonstrated the correlation of the action and effects of drugs and other chemical agents with the physiological, biochemical, microbiological, immunological or behavioural factors influencing disease. Each of these courses is closely interwoven with the experimental techniques of biochemistry, cellular and molecular biology, microbiology, genetics, pathology, physiology, etc. The role of the tutor in PBL courses also affects the choice of potential tutors. Lectures-based pharmacology classes are typically taught by established lecturers. PBL courses have tutors-professors from our department. The results demonstrated the high level of interest from students to PBL. For example: 220 students took PBL classes on pharmacology in 2008/2009 year. The teaching hours were up to 200. The lectures on pharmacology and pharmacotherapy were given by eight professors and 6 assistant professors. This experience has led to suggestions that advantages of PBL might manifest particularly in areas such as clinical competence and pharmacotherapy.

Our results suggest that there is high rank correlation between assessment of student performance by computer and traditional appraisal by written examination. We incorporated various teaching packages on pharmacology for undergraduate students. The computer-assisted learning is a part of our teaching process. Our department started with multimedia 25 years ago. In this department, the use of computer-assisted learning software incorporating video, sound and animated graphics to

replace animal's experiments started before 30 years. We developed video-films on neuropharmacology, cardiopharmacology, pharmacolgy of pain, lung pharmacology, general pharmacology, etc. and our films demonstrate experimental benefits in teaching on pharmacology and drug toxicology. We also developed the text exams on pharmacology for medical students in Bulgaria, published books with test questions in field of pharmacology. Our department has a high level of respects due to the publishing books on pharmacology. The analysis demonstrated that our department staff published 21 teaching books and up to 200 teaching chapters for medical students, students in dentistry, pharmacy, nurses, etc. in the last 20 years. Moreover, we first proposed the new pharmacology book in Bulgaria with illustrations/figures and textbooks for practice. The department presented chapters on online education on pharmacology and it was first one together with the Department of Biochemistry during the International Meeting in 2000. Then the online pharmacology was developed and accepted in 2008 as a supportive book for teaching. Additionally, medical students have the option to practice via online on teaching/exams materials on pharmacology developed by the department's staff.

We started a new scientific method for education of medical students named "Written pharmacological chapters and competition for presentation" (WPC) in 2004. Each student has to write 4 pages on scientific question in field of pharmacology. Then, seven groups of professors and assistant professors in pharmacology scored the written chapters and selected the best of them for further competition and presentations. The department organizes the scientific-educational workshop each year in May. Students who won the competition presented their posters or oral presentations. It is a big scientific meeting with a lot of students, teachers, and medical doctors. It is a great achievement in education on pharmacology. Posters presented news in various fields in pharmacology, analysis of students, information about scientific studies, etc. Oral presentations of students by multimedia trained them to discuss publicly the scientific data in pharmacology. The results from last competition in 2009 were: 1) 250 students from 3rd medical course wrote the chapters; 2) the average score was 5.50; 3) the best chapters with score 6.00+ wrote 55 medical students; 4) Oral presentations were made by 8 students, posters presented 38 students; 5) 200 students and guests participated in the scientific meeting and discussions. The results demonstrate that factual knowledge in field of pharmacology. Although the presentation of pharmacological facts in a clinical and scientific context is likely to enhance medical student motivation to study pharmacology, based on learning theories a further enhancement of motivation can be expected by this new method in our department. We observed self-determination and responsibilities of the students. We also observed the responsibility of medical students for defining and achieving learning objectives in pharmacology. Taken together, it appears that our new achievement in teaching of pharmacology supported the quality of education.

We started teaching on pharmacology for English speaking students in Medical University in Sofia in 2003. We published programs, tests, lectures, etc in English. The new module on "Core information on pharmacotherapy of chronic disorders" was developed for them in 2007. The department also made the "Writing pharmacological chapter and competition for presentation". The results from last competition of English speaking students were: 1) All 24 students wrote chapters; 2) The average score was excellent (5.75); 3) All students made oral presentation during workshop in May 2009.

The unique form for undergraduate and graduate education on pharmacology, toxicology and pharmacotherapy is the First Science-Educational School (SES) for capable medical students. The department of Pharmacology and Toxicology organized the school in 2007. Approximately 70-80 students took lectures, seminars, workshops, posters discussions, etc. each year. The students with high motivation and qualifications are in the school. The aims are to introduce the clinical, scientific and fundamental news in pharmacotherapy to students, to develop medical doctor-scientist from 3rd year in our university. Students have a chance to learn a lot of from various great Bulgarian and European medical doctors and scientists. The close relationship of the students with Acad. E. Golovinsky, Acad. C. Cvetanov, Acad. P. Vasileva from the Bulgarian Academy of Science plays a

role in their scientific/educational motivation. The school celebrated 2 years in May 2009. Students from the school became qualified for lectures in field of pharmacology, started research and they are in the experimental process for developing of new pharmacological product. The students learned and met great Bulgarian scientists in fields of endocrinology, neurology, pharmacology, cancer research, immunology, psychiatry, ophthalmology, molecular biology, microbiology. The students learned and celebrated the progress in medicine given by our teachers and great scientists as Acad. R. Tzanev, Acad. T. Tashev, Acad. Ch. Nachev, Acad. I. Puhlev, Acad. I. Penchev, Acad. D. Pashov, Acad. V. Petkov, Acad. C. Angelov, Prof. D. Paskov, Prof. C. Stoichev, Prof. N. Shipkovenski, Prof. H. Gelinov, Prof. A. Spasov, etc. The SES students met the leaders in medicine from Bulgaria, USA, Europe and Asia. The observation demonstrated a high level of interest in SES.

The pharmacological studies of our undergraduate and graduate students range from those that determine the effects of chemical agents on cellular mechanisms, to those that deal with toxicology of pesticides, to those that focus on molecular modelling of drugs. The students are also involved in studies on prevention of major diseases by drug therapy. The scientific methods of our education on pharmacology play important roles in both medical and dentist programs. Moreover, standardized exams are specifically designed to test the type of knowledge that is well conveyed in lecture-based learning and problem-based learning formats. Because medicine is an ever more rapidly changing science, a key qualification for tomorrow's physicians is the ability and motivation for life-long learning. In this regard, we consider it important that the shift of responsibility for defining and achieving learning objectives in pharmacology is to prepare students better for independent and long learning. So, we used various formats in both lecture- and problem-based learning programs or in online education and scientific studies for medical students. Although some areas of pharmacology can be adapted easily to this learning style, it might be more difficult for other areas. For example, it will be difficult to obtain a comprehensive view of anti-cancer drugs using both lecture- and problem-based learning programs unless a large variety of cases including several rare cancers. Moreover, some teachers remain sceptical about whether problem-based learning is adequate to convey the numerous facts medical students need to memorize about drugs. For these reasons, we used at least 6 formats (lectures, seminars, problem-based modules, computer/video teaching, WSC and courses at SES). In addition, the department is well known in teaching students for research. Our students presented their research at National and International Meetings. The results demonstrated that 40 students studied and 30 students from our department published their research on pharmacology in recent 5 years. The results also documented that 20 of our students presenting scientific papers received the International and National awards. Moreover, our research students won 8 grants/specializations in Bulgaria, Europe and USA. The research plays an important role in education on pharmacology of medical students in Medical University in Sofia.

ONCOPHARMACOLOGY

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The most essential characteristic of oncology and correlated diseases in human beings is their unique complexity. The major components of such complexity include:

- cancer at cellular, gene and molecular level,
- the characteristics of over 120-130 cancer diseases,

and

- characteristics of at least 490 certified by experts carcinogens: chemical agents, radiation, biological factors.

The most essential characteristic of oncopharmacology is its strong bond with the above mentioned complexity of oncology. The development of oncopharmacology has constantly been in relation with the progress in the major components of oncology. On the other side the progress in oncopharmacology realized a remarkable effect on more successful treatment of oncological diseases in humans. Thirty-forty years back many cancer diseases were treated only with surgery and radiation. In now a days drugs are the prevailing agents for treatment in many neoplasms – cancer in children, cancer in the haematopoietic system, prostate cancer, etc. Twenty-thirty years back the leading mechanism of anticancer drug action was direct killing of the cancer cell with affection of some normal cells, especially from haematopoietic organs. Now a tendency for production of drugs with selective action on cell components, including cell-membrane receptors, intra-cell structures, suppressor genes, and tumour vascularization is firmly established.
Thus, oncopharmacology is an excellent example of a scientific field, motivated from and realized by real problems of human health.

PHARMACOTHERAPY OF SURGERY PAIN

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Surgical pain has heterogeneous character and it includes the elements of somatic, visceral, inflammatory and event neuropathic pain in some diseases. Good recognition of different kinds of pain, their characteristics and neuropath of pain impulses, is important for analyzing of the clinic picture and diagnostic of abdominal diseases, for choosing of methods of pain treatment, anaesthesia and for choosing the therapeutic behaviour. We represent experience of treated in surgery clinic patients with planned and emergency surgical procedures.
The use of analgesics is discussed in different circumstances: a/ acute surgical abdomen, b/ chronic pain, c/ postoperative pain, d/ oncological pain. It depends on the kind and the localization of the pathological processes. Good recognition of characters and aspects of pain is important condition for determination of a good time and resources of pain treatment in diagnostic, operative end postoperative period.
Pain lasting presence in the surgical practice. The concept of "surgical pain" focuses definitely and precisely on pain as a symptom - of unspecified malady, of disease with a clear diagnosis, of acute surgical abdomen, but also trauma or past surgical intervention. Pain is an important source of information on surgical practice. There are two necessary circumstances to manifest - cause and sensitive area of adoption. Among etiological reasons provoked pain in the abdominal cavity arranged: ischemia, spasm of the sphincter or circular muscles; high pressure in the hollow body, but also trauma and inflammation. Specific lesions, which provoke disease, make diversity in the types of pain, which has important implications for the clinician: localization (punctum maximum), durability, versatility in power irradiation and others.
There are different types of pain, depending on their neurological and patophysiological characteristics:

a) Superficially. Associated with damage to the superficially tissues - skin and mucosa. In the skin has a high concentration of neural endings as opposed to mucosa, where pain receptors are less, but there are specific pressure, chemistry- and taste receptors. This raises characteristics important for the surgeon:
- Injury to the skin and mucous membranes is indicated as a wound. The pain varies depending on the type of wound. It is weak and short in cut- and puncture-wounds, and sustained in lacerate wounds, wrench a scalp, burning;
- Pain is local. It may be controlled with superficial or topical anaesthesia. This method is often used for analgesia with anaesthetics of topical or conduction anaesthesia (lidocaine, novocaine, chloroform). He has a temporary effect of analgesia in skin and tissue for a period sufficient to make the operation.

b) Somatic pain. It is associated with damage to tendons, bones, joints, blood vessels, nerves, which have limited number of somatic receptors. It is observed at wrench, sprain, fracture, and is presented as a weak localized, dull, but long. Parietal abdominal pain belongs to this type of pain,. It is associated with inflammation of the parietal peritoneum, which could be well localized. This type of pain caused and processes developing in the abdominal wall or affecting radix mesentery. In surgical practice this type of pain may be felt in cutting, but also in crushing or burning tissue. It is an asymmetric, stable, slowly changing in intensity, long pain. Influence of body movement and pressure, but easier relief when stay still.

c) Visceral pain. It is associated with provocation of visceral nociceptors located in organs and body cavities. It is provoked of tension, ischemia and smooth muscles contraction. Pain character is diffuse, poorly localized, indefinite location, often symmetrical. It is provoked by infarction, colic, cramp, crisis. It may change the situation, depending on body position, which makes the patient restless and seeking a comfortable position.

d) Neuropathic pain. Neuropathic pain has been defined by the IASP as ‘pain initiated or caused by a primary lesion or dysfunction in the nervous system’. However, it may also occur in the absence of injury, if nociceptors themselves and sensory pathways are not functioning properly.

By nature, surgical pain is a composite concept, often uniting different type of pains in one. Hence, pain is typical for surgical wounds, including the operational. However, it is present as a major symptom among the five signs of inflammation. Somatic pain is typical for a wide range of diseases such as acute appendicitis, peritonitis and others. For example, inflammation in the biliary system (cholangitis, cholangiolitis) is not associated with pain until Glisson's capsule is not involved. It represents the parietal peritoneum, in which the liver becomes sensitive to palpation and sucsusio hepatis is positive. Stage of acute pus inflammatory process causes sepsis and a reduction of pain. A typical example of visceral pain is renal crisis, and ischemic myocardial infarction. Neuropathic pain was observed in severe chronic pancreatitis with induration and fibrosis of pancreatic tissue and deposition of stones.

Different pain characteristics, associated with peculiarities in neuroanatomy. Somatic abdominal pain is associated with nociceptors in peritoneum of front abdominal wall or diaphragm, in radix mesenteri surface or in the abdominal wall.

There are two primary ascending nociceptive pathways. Suddenly and strong pain impulses are delivered by medium-diameter lightly myelinated A-delta fibres, while feeling of slow pain is delivery by small-diameter, slow conducting non-myelinated C-fibres. The cell bodies of nociceptive afferents that innervate the trunk, limbs and viscera are found in the dorsal root ganglia, while those innervating the head, oral cavity and neck are in the trigeminal ganglia and project to the brain stem trigeminal nucleus. These define 2 primary ascending nociceptive pathways. The central terminals of C- and A-delta fibres convey information to nociceptive-specific areas within lamina I and II of the superficial dorsal horn. The spinothalamic pathway ascends from primary afferent terminals in lamina I and II, via connections in lamina V of the dorsal horn, to the thalamus and then to the somatosensory cortex. Part of the ways, which deliver impulses of spinothalamic pathway, make diffuse contacts with somatosensory cortex and temporal lob. This determines diffuse character of pain and temperature feeling.

According to the form of causes and manifestations surgical pain can be acute or chronic - persistent with long-term periodic events. It differs in its characterization of the so-called "cancer pain" because of the type, characteristics and methods of analgesia. The importance of surgical pain is not limited only by its description as a diagnostic symptom. Clarify the pattern of pain is directly related to the placement of the indications for surgery. Thus, for example, diagnostics of the acute surgical abdomen include over 50 diseases. In some of them, pain is a sign of rapid alert for timely, often life-saving operation. In other cases pain symptoms do not require emergency surgery or fully treated by drugs. The presence of surgical pain is consequential last sign of urgency.

Attention should be given to inflammation as pathological phenomenon. On the one hand, the inflammatory process leads to pain symptoms with certain characteristics. On the other hand, progressive inflammation causes the lower intensity of pain. This should be considered. For example, acute pain associated with acute inflammatory of gall-bladder have two reasons: an inflammatory irritation of the parietal peritoneum and distension of the gall-bladder. In the development of gangrenous inflammatory gall-bladder and perforation , pain disappears, though briefly interrupted, because distension ofparietal peritoneum was stopped, but this is not a soothing symptom.

The means of pharmacological treatment of pain are various:

1. Intraoperative and postoperative pain is influenced by different types of anaesthesia. It may be local, loco-regional or direct effects of the drug on pain receptors. Second notes spinal or epidural anaesthesia, which interrupts the reflex arc of the second level of neurons. Third is the total intravenous or endotraheal anaesthesia direct impact on the SNS and the exclusion of both the perception of pain and responses to it.

2. For the treatment of postoperative pain, and surface, somatic, visceral or neuropathic pain, one might use:

- Analgesics (Acetysal, Analgin, Paracetamol, etc.);
- NSAIDs (Ibuprofen, Diclofenac, Naproxen, Ketorolac, Dynastat, etc.);

- Opioids (Codeine, Diacetylmorphine, Dihydrocodeine, Fentanyl, Metadone, Morphine, Lydol, Tramadol, etc.).

Analgesia in surgical procedures as a practice has its specificity. In suspected acute surgical abdomen, use of analgesics is contraindicated. It may totally mask the clinical picture and lead to errors in diagnosis, and the urgency of the necessary emergency action. Presence of chronic neuropathic pain is associated with two phenomena - the addiction and gradual familiarization to the effect of painkillers with the appropriate agent to increase the dose or changing it. Once clarified, the diagnosis of visceral or somatic nature of pain should be alert for the use of painkillers in conjunction with spasmolytics and/or antibiotics, according to the nature of the underlying disease.

Drug design, development and delivery of bioactive compounds

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The process of drug discovery involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. In the past most drugs have been discovered either by identifying the active ingredient from traditional remedies or accidental discovery by careful observer. Drug discovery is complex process involving various scientific backgrounds. Each step in the drug discovery is thoroughly controlled and documented, a requirement to receive the authorization to continue the development process (1). The process is extremely costly, around $800 million dollars for one candidate drug to reach the market (2). In addition, the process is risky and the success is very low, only one out of thousands compounds is examined and will be approved for sale. Among those few that do reach the market, only one third will be successful enough to have a positive return on investment (3).
Methods for discovering new drugs have exceptionally developed over the past decades. Instead of randomly screening candidates, a rational drug design process is applied. It is the inventive process of finding new medications based on the knowledge of the biological target (3). This approach involves advanced technologies such as computer-aided drug design (CADD), combinatorial chemistry (CC), High Throughput Screening (HTS) and genetic engineering (4). An example in rational drug design is imatinib, a tyrosine kinase inhibitor designed specifically for the *bcr-abl* fusion protein that is characteristic for Philadelphia chromosome-positive leukaemias (chronic myelogenous leukaemia and occasionally acute lymphocytic leukemia) (5). Imatinib is substantially different from previous anticancer drugs, as most chemotherapeutic agents simply target rapidly dividing cells (both cancer and normal).
Drug design frequently but not necessarily relies on computer modeling techniques. This type of modelling is often referred to as **computer-aided drug design**. There are two major types of drug design. The first is referred to as **ligand-based drug design** and the second **structure-based drug design**.

Ligand based drug design (LBDD) relies on knowledge of the structural characteristics of molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore, which defines the indispensable structural characteristics a molecule must possess in order to bind to the target. In other words, a model of the biological target may be built based on the knowledge of what binds to it and this model in turn may be used to design new molecular entities that interact with the target. Modelling techniques for prediction of binding affinity are reasonably successful. However there are many other properties such as bioavailability, metabolic half-life, lack of side effects, etc. that first must be optimized before a ligand can become a safe and efficacious drug. These other characteristics are often difficult to optimize using rational drug design techniques (1).

Structure based drug design (SBDD) is becoming an increasingly successful techniques used to discover and develop new therapeutic compounds. Typically, the process involves a repetitive cycle of steps initiated by the determination of the structure of a target receptor. Structure-based drug design relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. Using the structure of the biological target, one or more ligands are designed, synthesized and assayed as potential agonists or antagonists of a receptor. The structures of successful ligands are then determined and used to suggest improvements in the ligand. This cycle is repeated until inhibitors with the desired affinity, specificity and pharmacokinetic properties are obtained. The power of SBDD has been demonstrated most clearly in the AIDS arena, where structural knowledge of HIV-1 protease fueled the successful design and development of five protease inhibitors (1).

Computer-aided drug design (CADD) uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules. The fundamental goal is to predict whether a given molecule will bind to a target and if so how strongly. Molecular mechanics and/or molecular dynamics are often used to predict the conformation of the small molecule and to model conformational changes in the biological target that may occur when this molecule binds to it (4). Ideally, the computational method should be able to predict affinity before a compound is synthesized and hence in theory only one compound needs to be synthesized. The reality however is that present computational methods provide at best only qualitative accurate estimates of affinity. Therefore, in practice, it still takes several iterations of design, synthesis, and testing before an optimal molecule is discovered. On the other hand, computational methods have accelerated the process of discovery by reducing the number of iterations required and in addition have often provided novel small molecule structures (6).

Identification of biologically active compounds

Two main approaches exist for the finding of new bioactive chemical entities from natural sources: both random collection and screening of materials, or exploitation of ethno-pharmacological knowledge in the selection. The former approach is based on the fact that only a small part of Earth's biodiversity has ever been tested for pharmaceutical activity, and organisms living in a species-rich environment need to evolve defence and competition mechanisms to survive. One example of a successful use of this strategy is paclitaxel. It was identified from Pacific yew tree *Taxus brevifolia*. Paclitaxel is now approved for the treatment of lung, breast and ovary cancers, as well as for Kaposi's sarcoma (7). Another important example is artemisin, an antimalarial agent from sweet worm tree Artemisia annua, used as part of a combination therapy for multiresistant Plasmodium falciparum (8).

Nature as source of drugs

Current research in discovering drugs from medicinal plants origin involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's disease, malaria, pain, etc. Several natural product drugs of plant origin have been introduced to the pharmaceutical market, including arteether, galantamine, nitisinone, and tiotropium. Plants have been utilized as medicines for thousands of years. Drug discovery from medicinal plants has traditionally been lengthier and more complicated than other drug discovery methods. Drug discovery from medicinal plants led to the isolation of early drugs such as codeine, digoxin, morphine, and quinine of which are still in use. Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays, and the scale-up of active compounds (9).

Drug development and delivery

Many aspects of drug development are focused on satisfying the regulatory requirements of drug licensing authorities. Pre-clinical development is a stage of research that begins before clinical trials (phase I-III). Once a drug has proved satisfactory after Phase III trials, the whole documentation for the drug is submitted for review and eventual approval to market. Post Marketing Surveillance Trial (Phase IV) is designed to detect any rare and/or delayed adverse effects in a much larger patient population and over a longer time period compared with Phase I-III clinical trials. Harmful effects discovered in Phase IV trials may result in a drug being no longer sold, or restricted to certain uses, like cerivastatin, troglitazone, rofecoxib, cisapride (10).

Since the sequencing of the human genome, thousands of new molecular targets have been identified as important in various diseases. However, despite advances in technology and understanding of biological systems, drug discovery is still a very long process (8-12 years) with a low rate of success.

References

1. Drug design. Wikipedia, The Free Encyclopedia. Accessed by 04 May, 2009. Available at: http://en.wikipedia.org/w/index.php?title=Drug_design&oldid=293094767
2. Dickson M, Gagnon JP. The cost of new drug discovery and development. Disc Med 2004;4(22):172-179.
3. Backman M, Segrestin B. Drug design strategies: The new challenge of discovery departments in pharmaceutical companies. Presented at the International Product Development Management Conference, June 12-14, 2005.
4. Hermann T, Westhof E. Rational drug design and high-throughput techniques for RNA targets. Comb Chem High Throughput Screen. 2000 Jun;3(3):219-34. Review.
5. Müller BA. Imatinib and its successors--how modern chemistry has changed drug development. Curr Pharm Des. 2009;15(2):120-33. Review.
6. Keil M, Marhofer RJ, Rohwer A, Selzer PM, Brickmann J, Korb O, Exner TE. Molecular visualization in the rational drug design process. Front Biosci. 2009 Jan 1;14:2559-83.
7. Geney R, Sun L, pera P, Bernacki RL, Xia S, Horwitz SB, Simmerling CL, Ojima I. Use of the tubulin bound paclitaxel conformation for structure-based rational drug design. Chem & Biol 2005;12(3):339-348.
8. Lee S. Artemisinin, promising lead natural product for various drug developments. Mini Rev Med Chem. 2007 Apr;7(4):411-22. Review.
9. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci 2005; 78(5):431-41.
10. Schmid EF, Smith DA, Ryder SW. Communicating the risks and benefits of medicines. Drug Dis Today 2007;12(9-10):355-364.

Neurogenesis: A New Therapeutic Approach To Neurodegenerative Disorders

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Neurodegenerative disorders are characterized by neuronal loss in specific regions of the central nervous system. The dogma stating the incapability of nerve cells to divide and reintegrate, which existed until recently, had reduced pharmacotherapeutic efforts to research and creation of ways to compensate neurotransmitter and functional deficits to a certain degree. This is one of the reasons why currently available therapeutic options have only symptomatic action.

Neurogenesis is an important element of cerebral plasticity, the dynamic potential of the brain to reorganize itself during ontogeny, learning, or following damage. During the last decades, proofs have been gathered about the continuous production of neurons throughout life in specific regions, such as the dentate gyrus and the subventricular zone of the lateral ventricles. At the same time, stimulation of neurogenesis is associated with restored function in animal models of neurodegenerative diseases, suggesting that neurogenesis is functionally important. Precursor neural cells have been isolated from the brain of mammals, including humans. Increased neurogenesis in response to cerebral lesions has been found in the striatum, neocortex, corticospinal motor neurons, and in the CA layer of hippocampus. These findings are supposed to shed more light on understanding the aetiology of some neurological disorders. It is believed that dysregulation of neurogenesis in mature age could contribute to the pathogenesis of neurodegenerative diseases. The number of new evidences supporting this idea is growing, and so are the indications that factors promoting neurogenesis could be able to modify the initiation and progression of specific brain lesions. Neurogenesis is thus considered a promising component of new therapeutic strategies in neurodegenerative disorders where functional neurological deficit is related to cellular loss. That is why neurodegenerative disorders could be the ideal candidates for treatment by prevention of apoptosis, by compensation or replacement of specific cell populations. It is then easy to understand the increased interest towards agents and methods which could stimulate endogenous neurogenesis, and towards the assessment of the therapeutic potential of stem/progenitor cells in vitro and in vivo. Therefore, as new conceptions are introduced and established in modern neuroscience, especially the one concerning the functional integration of new neurons in the CNS, directions for search of new therapeutic strategies are changing. Questions related to the influence of known therapeutic options on neurogenesis are being clarified. At the same time, proofs of the therapeutic action of potential new regulating factors are being sought. The introduction of stem cells from embryonic tissue, bone marrow and other sources, after successful proliferation, differentiation and integration into corresponding functional neuronal systems, could lead to a decrease or cessation of pathological deficits.

Regardless of the important progress, the need for increase in knowledge about neurogenesis and neurodegenerative disorders is recognized. Slowly developing, chronically progressive neurodegenerative diseases such as Parkinson's, Alzheimer's, Huntington’s and amyotrophic lateral sclerosis, characterized by a net loss of neurons from specific regions of the central nervous system, are subject to major research. They present evidences of impaired neurogenesis: low presence of stem/progenitor cells, decreased proliferation and differentiation into mature neurons. At the same time, increased neurogenesis is found in the course of progression of Alzheimer’s disease. Neurogenesis in the dentate gyrus is thought to be neurodegenerative stage-dependent and correlated with the severity of neuronal loss. Although increased neurogenesis in these pathological conditions could contribute to the restoration and regeneration of the damaged brain, an inadequate and/or excessive supply of new neurons, or suppressed neurogenesis, could contribute to their physiopathology. To develop successful regenerative treatments for the injured brain, we need to understand more precisely and comprehensively the mechanisms regulating adult neurogenesis under both physiological and pathological conditions.

One of the major challenges in the field of neurodegenerative disease treatment is the assessment of neurogenesis as a target and approach in elaborating new therapeutic strategies. The importance of neurogenesis is proven in several experimental directions. Above all, the study of mechanisms regulating adult neurogenesis under both physiological and pathological conditions is under way. Drugs and factors stimulating neurogenesis could be regarded as essential for patients, which provokes their exploration from such point of view. It appears unlikely that drugs being developed to treat neurodegenerative diseases would be beneficial if they impair neurogenesis. It is admitted that enhancing endogenous neurogenesis at early stages of neurodegeneration may be a valuable strategy to delay neurodegenerative progress.

An important research direction is the influence of known therapeutic agents on endogenous neurogenesis. New mechanisms of regulation and new therapeutic targets are being discovered. Cholinesterase inhibitors for instance could be used to stimulate neurogenesis, as it is known that cell proliferation-linked cholinergic receptors are expressed on neural progenitor cells. Though Dex is neurodegenerative in the developing brain, it increases VEGF which may play a neurotrophic and neuroprotective role. In experimental conditions neurogenesis could be enhanced as well by the anti-inflammatory action of TNF-alpha antagonists (such as etanerecept), or by enriching the environment with granulocyte colony stimulating factor (GCSF).

The varying influence of proinflammatory cytokines, hemokines, neurotransmitters and reactive oxygen species on neural progenitor cells are in the basis of neurogenesis reestablishment observed in NSAID treatment. It is believed that antidepressants enhance neurogenesis in adults by stimulating cell proliferation and increasing the activity of the AMP-CREB cascade and BDNF. Preclinical studies have demonstrated that peripheral GA administration can enhance central BDNF activity and augment neurogenesis, so GA is supposed to act as a potential therapeutic agent for PD and AD.

Proofs for the therapeutic action of new potential agents are being sought. Genetic, pharmacological and environmental factors regulating neurogenesis are also subject to research, aiming at the development of novel treatment to control disease progression. A number of drugs created in order to block degeneration or to provide symptomatic action, stimulate neurogenesis or at least do not impede its mechanisms. Novel therapeutic agents, aimed at blocking degeneration, could have limited efficacy if they block regeneration at the same time. Cytokine treatments may provide a new therapeutic approach for many brain disorders, including neurodegenerative diseases.

Newly invented small cells, modulating the differentiation of neural stem cells, could evolve into orally applicable drugs for neurodegenerative disorders.

Endogenous neurogenesis is regarded as a way to insure the isolation of stem cells, their development and genetic manipulation in vitro for the purpose of cell therapy. Neural stem cells represent a powerful source for the discovery and design of novel approaches to gene therapy of neurodegenerative disorders. Newer, stable cell lines are created, which are able to differentiate in specific directions. These are considered candidates for preclinical studies of cellular replacement and drug testing. A new concept is being established, that in cell-based therapies, stem cells operate not through a unidirectional mechanism (e.g., generating neurons) but rather as cellular mediators of a multitude of biological activities, including stimulation of endogenous neurogenesis through production of neurotrophic factors that could provide a favourable outcome for diverse nervous disorders.

The possibilities for transplantation of stem cells from other sources are also being developed. Embryonic stem cells are an important source as they provide unlimited reserve of specific cell types, subject to further manipulation through genetic engineering. They are expected to play a key role for the creation of novel cell-based therapies. Great efforts will be needed though to materialize the full potential of stem cells.

The specifics and localization of target cell populations in different neurodegenerative diseases provoke the discovery of novel methods for delivery and distribution of therapeutic agents. Intranasal delivery is an example of a practical, non-invasive method of bypassing the blood-brain barrier to deliver therapeutic agents including both small molecules and macromolecules to the brain, such as NAP neuroprotective peptide, insulin, etc., eliminating the need for systemic administration and its potential side effects.

The conception for the role of neurogenesis in contemporary therapeutic strategies in neurodegenerative disorders is still subject to research and approval, aiming at quicker clinical benefits.

References

1. Boucherie C, Hermans E. Adult stem cell therapies for neurological disorders: benefits beyond neuronal replacement? J Neurosci Res 2009; 87(7):1509-1521.
2. Chen Q, Nakajima A, Choi SH, Xiong X, Sisodia SS, Tang YP. Adult neurogenesis is functionally associated with AD-like neurodegeneration. Neurobiol Dis 2008; 29(2):316-326.
3. Crook JM, Kobayashi NR. Human stem cells for modeling neurological disorders: accelerating the drug discovery pipeline. J Cell Biochem 2008; 105(6):1361-1366.
4. Duffau H. Brain plasticity: from pathophysiological mechanisms to therapeutic applications. J Clin Neurosci 2006; 13(9):885-897.
5. Feng Y, Rhodes PG, Liu H, Bhatt AJ. Dexamethasone induces neurodegeneration but also up-regulates vascular endothelial growth factor A in neonatal rat brains. Neuroscience 2009; 158(2):823-832.
6. Kaneko N, Sawamoto K. Adult neurogenesis and its alteration under pathological conditions. Neurosci Res 2009; 63(3):155-164.
7. Kimura T, Hong Nguyen PT, Ho SA, Tran AH, Ono T, Nishijo H. T-817MA, a neurotrophic agent, ameliorates the deficits in adult neurogenesis and spatial memory in rats infused i.c.v. with amyloid-beta peptide. Br J Pharmacol 2009.
8. Rishton GM. Small molecules that promote neurogenesis in vitro. Recent Pat CNS Drug Discov 2008; 3(3):200-208.
9. Srivastava AS, Malhotra R, Sharp J, Berggren T. Potentials of ES cell therapy in neurodegenerative diseases. Curr Pharm Des 2008; 14(36):3873-3879.
10. Vandenbosch R, Borgs L, Beukelaers P et al. Adult neurogenesis and the diseased brain. Curr Med Chem 2009; 16(6):652-666.
11. Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC. Inflammation mediates varying effects in neurogenesis: relevance to the pathogenesis of brain injury and neurodegenerative disorders. J Neurochem 2009; 108(6):1343-1359.

Prolactinoma: current pharmacological treatment and future perspectives

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Prolactinoma is the most common pituitary tumour. Based on tumour size prolactinomas can be divided into two categories: microprolactinomas (with diameter < 10 mm) and macroprolactinomas (with diameter > 10 mm) (24). The main clinical symptoms due to hyperprolactinemia, predominantly, are menstrual disturbancies (oligo-amenorrhoea), galactorrhea and infertility in women and decreased libido and erectile dysfunction in men. Symptoms owing to mass effects (headache, visual field defects and hypopituitarism) are relatively rare and typical for men who are diagnosed with macroadenomas more often than women (10). In contrast to all other hormone-producing hypophyseal adenomas, conservative therapy provides better results than surgical management of prolactinomas. The main goals of treatment are to remove symptoms of the disease, normalize the prolactin levels and reduce tumour size. Because dopamine is the physiological inhibitor of prolactin secretion, long-term treatment with dopamine agonists (DAs) (bromocriptine, qiunagolide, cabergoline) is considered to be the first-choice therapy which can return prolactin levels to normal and shrink the tumour volume in approximately 80 % of patients (4). On the other hand, there is no consensus on how long to maintain the medical therapy**. Bromocriptine** (2-bromo-alpha-ergocryptine mesylate) is an ergot derivative that binds to and stimulates dopamine (D_2) receptors on normal and adenomatous lactotroph cells. The mechanism by which bromocriptine causes tumour size reduction includes an inhibition of transcription of prolactin mRNA and prolactin synthesis which results in a decrease in the number of prolactin secretory granules, involution of the rough endoplasmic reticulum and Golgi apparatus with a decrease in cytoplasmic volume. However, treatment with this medication is frequently associated with side effects such as nausea, vomiting and dizziness even if the recommendations for low initial dose and its gradually increasing are observed. **Quinagolide** is a non-ergot derived dopamine agonist with a chemical structure similar to apomorphine, better tolerated and as effective as bromocriptine. The 22-hour half-life and 24-hour duration of action of quinagolide permit once-daily dosing (1). **Cabergoline** has been shown to be more effective and better tolerated than all other dopamine agonists are. Compared to bromocriptine and quinagolide, prolonged cabergoline therapy is characterized by improved patient compliance as a result of its once or twice weekly administration and the low incidence of above-mentioned side effects (27)**.** Recently, numerous case reports have been published describing the occurrence of valvular lesions in patients treated with high doses of cabergoline (≥ 4 mg total daily dose) for Parkinson's disease (14,23,29). Whether lower doses commonly used in the treatment of prolactinomas (0.25-3 mg/week) are also associated with significant valvulopathy is not clear. Data published thus far in the literature are controversial (2,5,6,12,19). Additional prospective studies with precise echocardiographic assessment and with longer duration of follow-up are required to resolve this question. **Transsphenoidal surgery** is a second-line therapy. Surgical success rates depend on two principal factors: the experience of the neurosurgeon and the tumour size. A radical effect can be expected in microprolactinomas but in macroprolactinomas surgical intervention has only limited success (17,21). In invasive giant prolactinomas (a subset of macroprolactinomas with invasive growth, diameter > 40 mm and extremely high prolactin levels) surgery is usually non-curative compared to conservative therapy with long acting dopamine agonists which results in decrease of prolactin levels, improvement of clinical presentation and visual field defects (7,18,25). Classical surgical indications are resistance or intolerance to therapy with dopamine agonists, pituitary apoplexy or cerebrospinal fluid rhinorrhea, progressive visual loss and symptomatic tumour enlargement during pregnancy that does not respond to dopamine agonist treatment (11). The pharmacological resistance to dopamine agonists therapy can be defined as an absence of serum PRL normalization and/or tumour shrinkage after at least 3 months of treatment with bromocriptine at the dose of 15 mg daily (20,22). True resistance is considered to be due to absence of D_2 receptors on the membrane surface of tumour cells, or abnormalities at a post-receptor level (3,8). **Radioptherapy** is usually not effective and frequently causes serious adverse events such as

hypopituitarism. In most cases, radiotherapy is used as a palliative treatment after failed medical treatment or as a prophylactic postoperative procedure for prevention of residual tumour growth. Recently, it was demonstrated that somatostatin analogues for the SSTR5 subtype suppress PRL release from prolactinoma cell cultures by 30–40% in 66 % of investigated prolactinomas, suggesting that SSTR5 analogues with improved selective binding affinity for these receptor subtypes may be effective in the treatment of prolactinomas (13,15,16,26). Combined therapy with cabergoline and octreotide may be a good therapeutic approach for select cases (10).
Gene therapy represents a potential future perspective for the treatment of pituitary adenomas. In vivo studies have shown a reduction in tumour growth and plasma PRL levels in oestrogen-induced prolactinomas treated with tetracycline-regulated adenovirus carrying the gene of thyrosine hydroxylase (the rate limiting enzyme in dopamine synthesis) (28). Other preclinical *in vivo* models are based upon the method of gene-directed enzyme prodrug therapy, in which the gene encoding thymidine kinase (TK) is delivered to tumour cells, followed by the systemic administration of a nucleoside analog such as gancyclovir, which is converted locally to a cytotoxin by TK (9).

References

1. **Barlier A., Jaquet P.** Quinagolide – a valuable treatment option for hyperprolactinaemia. *Eur J Endocrinol, 2*006; 154 (2), 187-195
2. **Bogazzi F, Buralli S, Manetti L, Raffaelli V, Cigni T, Lombardi M, Boresi F, Taddei S, Salvetti A, Martino E**. Treatment with low doses of cabergoline is not associated with increased prevalence of cardiac valve regurgitation in patients with hyperprolactinaemia. *Int J Clin Pract.* 2008;62(12):1864-9.
3. **Brue T, Pellegrini I, Priou A, et al.** Prolactinomas and resistance to dopamine-agonists. *Horm Res* 1992; 38:84–89
4. **Chanson P, Borson-Chazot F, Chabre O, Estour B**. Drug treatment ofhyperprolactinemia. *Ann Endocrinol (Paris).* 2007; 68(2-3):113-7..
5. **Colao A, Galderisi M, Di Sarno A, Pardo M, Gaccione M, D'Andrea M, Guerra E, Pivonello R, Lerro G, Lombardi G.** Increased prevalence of tricuspid regurgitation in patients with prolactinomas chronically treated with cabergoline. *J Clin Endocrinol Metab.* 2008;93(10):3777-84.
6. **Devin JK, Lakhani VT, Byrd BF 3rd, Blevins LS Jr.** Prevalence of valvular heart disease in a cohort of patients taking cabergoline for management of hyperprolactinemia.*Endocr Pract.* 2008;14(6):672-7
7. **Delgrange E, Maiter D, Donckier J** 1996 Effects of the dopamine agonist cabergoline in patients with prolactinoma intolerant or resistant to bromocriptine. *Eur J Endocrinol* 134:454–456
8. **Delgrange E, Daems T, Verhelst J, Abs R, Maiter D.** Characterization of resistance to the prolactin-lowering effects of cabergoline in macroprolactinomas: a study in 122 patients. *Eur J Endocrinol.* 2009;160 (5):747-52.
9. **Fillat C, Carrio M, Cascante A, Sangro B** 2003 Suicide gene therapy mediated by the herpes simplex virus thymidine kinase gene/ganciclovir system: fifteen years of application. *Curr Gene Ther* 3:13–26
10. **Fusco A, Lugli F, Sacco E, Tilaro L, Bianchi A, Angelini F, Tofani A, Barini A, Lauriola L, Maira G, Pontecorvi A, de Marinis L.** Efficacy of the combined cabergoline and octreotide treatment in a case of a dopamine-agonist resistant macroprolactinoma. *Pituitary.* 2009.
11. **Gillam MP, Molitch M, Lombardi G and Colao** AM. Advances in the Treatment of Prolactinomas. *Endocrine Reviews* 2006; 27 (5): 485-534
12. **Herring N, Szmigielski C, Becher H, Karavitaki N, Wass JA.**Valvular heart disease and the use of cabergoline for the treatment of prolactinoma.*Clin Endocrinol (Oxf).* 2009;70(1):104-8
13. **Hofland LJ, van der Hoek J, Feelders R, van der Lely AJ, de Herder W, Lamberts SW.** Pre-clinical and clinical experiences with novel somatostatin ligands: advantages, disadvantages and new prospects. *J Endocrinol Invest.* 2005;28 (11):36-42.
14. **Horvath J, Fross RD, Kleiner-Fisman G, Lerch R, Stalder H, Liaudat S, Raskoff WJ, Flachsbart KD, Rakowski H, Pache JC, Burkhard PR, Lang AE.** Severe multivalvular heart disease: a new complication of the ergot derivative dopamine agonists. Mov Disord 2004; 19:656–662

15. **Jaquet P, Ouafik L, Saveanu A, Gunz G, Fina F, Dufour H, Culler MD, Moreau JP, Enjalbert A**. Quantitative and functional expression of somatostatin receptor subtypes in human prolactinomas. *J Clin Endocrinol Metab*. 1999;84(9):3268-76
16. **Jaquet P**, **Gunz G**, **Saveanu A**, **Dufour H**, **Taylor J**, **Dong J**, **Kim S**, **Moreau JP**, **Enjalbert A**, **Culler MD**. Efficacy of chimeric molecules directed towards multiple somatostatin and dopamine receptors on inhibition of GH and prolactin secretion from GH-secreting pituitary adenomas classified as partially responsive to somatostatin analog therapy. *Eur J Endocrinol.* 2005; 53(1):135-41.
17. **Jan M, Dufour H, Brue T, Jaquet P.** Prolactinoma surgery. *Ann Endocrinol (Paris).* 2007;68 (2-3):118-9.
18. **Julian R., Davis E, Sheppard ME, Heath DA**. Giant Invasive Prolactinoma*:* A Case Report and Review of Nine Further Cases*. Q J Med* 1990; 74: 227-238
19. **Kars M, Pereira AM, Bax JJ, Romijn JA**. Cabergoline and cardiac valve disease in prolactinoma patients: additional studies during long-term treatment are required. *Eur J Endocrinol*. 2008;159(4):363-7.
20. **Kovacs K, Stefaneanu L, Hovarth E**. Prolactin-producing pituitary tumour: resistance to dopamine agonist therapy. *J Neurosurg; 1995:* 82:886–890
21. **Kreutzer J, Buslei R, Wallaschofski H, Hofmann B, Nimsky C, Fahlbusch R, Buchfelder M.** Operative treatment of prolactinomas: indications and results in a current consecutive series of 212 patients. *Eur J Endocrinol.* 2008;158(1):11-8
22. **Molitch ME.** Pharmacologic resistance in prolactinoma patients*. Pituitary.* 2005;8(1):43-52
23. **Pinero A, Marcos-Alberca P, Fortes J** 2005 Cabergoline-related severe restrictive mitral regurgitation. N Engl J Med 353:1976–1977
24. **Pinzone JJ, Katznelson L, Danila DC, Pauler DK, Miller CS, Klibanski A.** Primary medical therapy of micro- and macroprolactinomas in men. *J Clin Endocrinol Metab*. 2000; 85:3053–3057.
25. **Shimon I., C. Benbassat and M. Hadani.** Effectiveness of long-term cabergoline treatment for giant prolactinoma: study of 12 men. *Eur J Endocrinol,* 2007; 156 (2): 225-231
26. **Shimon I, Yan X, Taylor JE, Weiss MH, Culler MD, Melmed S.** Somatostatin receptor (SSTR) subtype-selective analogues differentially suppress in vitro growth hormone and prolactin in human pituitary adenomas. Novel potential therapy for functional pituitary tumours. *J Clin Invest.* 1997; 1;100(9):2386-92
27. **Verhelst J**, **Abs R**, **Maiter D**, **van den Bruel A**, **Vandeweghe M**, **Velkeniers B**, **Mockel J**, **Lamberigts G**, **Petrossians P**, **Coremans P**, **Mahler C**, **Stevenaert A**, **Verlooy J**, **Raftopoulos C**, **Beckers A**. Cabergoline in the treatment of hyperprolactinemia: a study in 455 patients*. J Clin Endocrinol Metab* 1999; 84(7):2518-22
28. **Williams JC, Stone D, Smith-Arica JR, Morris ID, Lowenstein PR, Castro MG** 2001 Regulated, adenovirus-mediated delivery of tyrosine hydroxylase suppresses growth of oestrogen-induced pituitary prolactinomas. *Mol Ther* 4:593–602
29. **Zanettini R**, **Antonini A**, **Gatto G**, **Gentile R**, **Tesei S**, **Pezzoli G**.Valvular heart disease and the use of dopamine agonists for Parkinson's disease. *N Engl J Med.* 2007l; 4: 356(1):39-46

HOMEOPATHIC NON-SPECIFIC HYPOSENSITIZATION THERAPY OF ALLERGIC RHINITIS IN CHILDREN

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Allergic rhinitis is one of the most wide-spread chronic diseases, the incidence of which may reach a 30%-rate in some populations. The treatment of intermittent (IAR) and persistent rhinitis (PAR) includes: (1) environmental issues of eliminating or avoiding the allergen; (2) pharmacotherapy (local or systemic); (3) immunotherapy. Using recent medical data, the homeopathic hyposensitisation of allergic rhinitis with isomedications is more effective than placebo. In the last several years, the sublingual immunotherapy is one of the first-choice methods in treatment of respiratory allergies in children.

Aim of the study: To investigate and assess effectiveness and safety of the hyposensitisation non-specific homeopathic therapy in treatment of IAR and PAR in comparison with sublingual immunotherapy (SLIT).

Methods: Effectiveness of both therapeutic approaches was compared using several criteria: 1.Subjective assessment of the symptoms T5SS (total five-symptoms score) – the sum of sneezing, rhinorhea, nasal and ocular pruritus, nasal congestion; quality of life - PRQLQ (pediatric rhino-conjuctivitis quality-of-life questionary). 2. Objective criteria: skin-prick tests or specific IgE antibodies (sIgE), nasal eosinophilia, necessity of antihistamine or nasal corticosteroid treatment, exacerbations of concomitant diseases, side effects of the therapy.

Material: 66 children (aged between 6 and 14 years), divided into two groups, depending on the therapy type: 1. Homeopathic group: 18 patients with IAR treated with isopathic homeopathic therapy with Pollens; 15 patients with sensitization against cockroaches and home dust mite treated with Blatta orientalis. Additional medication: Poumon histamine, Apis mellifica and terrain medication. 2. SLIT therapy group: 19 patients with IAR and 14 on a sublingual immunotherapy determined by the leading trigger antigen. Treatment duration in IAR was 3 months and in PAR – 12 months.

Results and discusion: Both methods show a significant improvement in clinical symptoms of allergic rhinitis (T5SS). In the beginning of the homeopathic therapy, in patients with PAR a temporary worsening in clinical symptoms was observed, which later was overwhelmed and lead to significant improvement in their quality of life (PRQLQ), in comparison with the SLIT group. Objective tests for sensitisation did not show a significant difference between both groups. In three patients, local side effects of the SLIT therapy were observed.

Conclusion: Homeopathic non-specific hyposensitization therapy in IAR and PAR in childhood is a safe and effective alternative method of sublingual immunotherapy.

TENDENCIES IN THE DEVELOPMENT OF THE CONTEMPORARY ONCOPHARMACOLOGY – CHALLENGIES AND PERSPECTIVES

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An overall review of the world markets today reveals several negative aspects. The financial crisis, which started in the US at the end of 2007, is gradually growing, affecting the whole world, and every economic and industrial branch, including the pharmacology business. The prognoses for the next few years are up to 20 % of market shrinking, which with an industry estimated at approximately $ 25 billion is a considerable loss. Industrial stagnation is expected in 2009 for a start- from 0 to 5% annual growth in the development of CRO branch. In comparison, this business has been extremely profitable for the last three years – from 2006 to 2009 the income has grown twice. The restricted investment in R&D will cause negative outcomes for a great number of small companies, as many of them will not be able to keep their market positions. The tendencies indicate that the biotechnological companies aim at consolidating the business, which will include merging of the last into the more powerful pharmaceutical companies. The recent contract of cooperation, signed between GSK and the biotechnological group Oxford Biotherapeutics, aiming at providing the financial resources for the discovered 30 target proteins, located on the surface of the cancer cells and elaborating the respective therapeutic antibodies, is an example of this.
According to Randall Stravinsky, Vice-President of Global Investment Research at the American bank Goldman Sachs, a possible solution of the problems could be found. Since the CRO industry is growing faster compared to the biochemical investment, co-operation and funding from outside is considered to be the most profitable strategy. The establishment of bigger CRO companies will lead to long-term market stabilization in spite of the expected short-term instability. This prognosis reflects the general behaviour of the large pharmacological corporations, which diminish the investment in R&D, while the market requires the opposite to be done. The purpose is again the long-term growth, even at the cost of certain losses in the near future. Although the observed current stagnation and the expectation that 2009 will be a difficult year, the pharmacological business will make progress again by the end of 2010, despite the restructuring and the short slowdown in its growth.
The accumulation of new knowledge about the tumour biology and chemistry has led to the discovering of many potential therapeutic targets in Oncology and to the synthesis of substances with potential therapeutic significance during the last decade. The number of new medicines, which were licensed from 1990 to 2004, remains constant due to multi-factorial reason. The investments for studies of the new molecules because of more and more preclinical and clinical trials grow considerably. The early identification of the inefficiency of the new molecules in phase I & II of the clinical trials may lead to considerable decrease in the price of the new medicines. The improved diagnostics in the early phases of the clinical trials with application of positron-emission tomography and computer tomography is an example for the significance of the image diagnostics in the development of new medicines.

Some of the most contemporary tendencies in Oncopharmacology are as follows:
1. Perspectives of application of the monoclonal antibodies in oncology beyond the target effects. Several monoclonal antibodies are used in the oncology practice currently, and many others are in the process of clinical trials. These agents have unique specificity against key molecular targets in the tumour cells or in the tumour microenvironment. The clinical effectiveness of the monoclonal antibodies is due to target-specific mechanisms leading to either neutralization or suppression of the effect of the growth factors or receptor. Several targets, including CD20, the human epidermal growth factor receptor 2, the vascular-endothelial growth factor and the epidermal growth factor receptor are validated in certain tumour diseases on the basis of the effectiveness of

the monoclonal antibodies. Furthermore, the monoclonal antibodies are able to activate immune mediated effector functions, including the antibody dependent cellular cytotoxicity and the complement-dependent cytotoxicity. These functions are the effect of the interaction of the Fc part of the antibody, and they can vary in different antibodies, isotypes and Fc modifications, for example in changes of the glycosylation.

2. **Application of TLR7 and TLR8 ligands for amplification the effect of the cancer immunotherapy.** The role of the Toll-like receptors (TLRs) for stimulation of the innate and adaptive immunity is currently well known. In this connection, TLR ligands are more and more intensively studied for implementation of the immunotherapy or vaccine for adjuvant treatment in oncology. The preclinical data are promising so far. The consequent development of autoimmune disease is one of the main risks.

3. **The newly established role of the Insulin-Like Growth Factor dependent signal pathways as therapeutic targets in oncology.** Numerous experimental models and epidemical studies manifest the role of the Insulin-Like Growth Factor dependent signal pathway for the development of series of tumours. Medicines, representing monoclonal antibodies against insulin-like growth factor-I receptor, as well as small molecular inhibitors of the receptor tyrosin-kinase activity, which modulate this signal pathway, are elaborated. Clinical trials testing these new therapeutic possibilities are in progress.

TOPICAL TREATMENT WITH CALCINEURIN INHIBITORS IN DERMATOLOGY PRACTICE

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Topical calcineurin inhibitors (TCIs) are a new generation of topical immunomodulating agents. They were initially developed for the treatment of atopic dermatitis (AD), a chronic or chronically relapsing skin condition most prevalent in infants and children. These immunomodulatory agents (e.g. tacrolimus, or pimecrolimus) are an alternative to topical steroids. Tacrolimus is the first one of these immunosuppressants. It is an immunomodulator macrolide, which was isolated from *Streptomyces tsukubaensis,* a fungus-like bacterium identified in Japan in mid-1980s and confirmed to have immunomodulatory properties in 1987. The name "tacrolimus" is derived from a combination of the words "Tsukuba" (the name of the mountain from which the soil sample was extracted), "macrolide" (the chemical class), and "immunosuppressant". In the early 1990s, Fujisawa developed an ointment formulation of tacrolimus, the first topical calcineurin inhibitor, specifically developed for the treatment of AD (first reported in the Lancet, 1994). Since then, tacrolimus ointment has been studied in the most extensive and comprehensive clinical development programme in dermatology, with clinical trials conducted in Europe, Japan and North America. As of September 2004, more than 30 clinical studies, both short- and long-term, have investigated the efficacy and safety of tacrolimus ointment in 16 000 AD patients including 3000 children. To date, more than 35 million prescriptions have been written for tacrolimus ointment in Europe alone. Tacrolimus acts as an immunosuppressant by inhibiting the proliferation and activation of $CD4^+$ T helper cells by binding to the cellular receptor known as FK506-binding protein (FKBP). The tacrolimus-FKBP complex further binds to calcineurin, preventing the dephosphorylation of the nuclear factor of activated T cells and blocking the cascade of cytokine gene transcription. This mechanism was likely evolved by *Streptomyces tsukubaensis* , which does not have calcineurin, to inhibit the calcineurin function in their fungal eukaryotic competitors. Other immunomodulatory effects of tacrolimus include the inhibition of mast cell adhesion, the inhibition of the release of mediators from mast cells and basophils, and the down-regulation of the expression of interleukin-8 receptor and FcεRI on Langerhans' cells.

Pimecrolimus is a new, non-steroid, cell-selective, cytokine inhibitor, which belongs to the class of ascomycin macrolactams. It was specifically developed for the treatment of inflammatory skin diseases, such as atopic eczema. It is finally selected among more than 400 derivatives as a macrocyclic natural product derived from *Streptomyces hygroscopicus.* Pimecrolimus is a cell-selective inhibitor of inflammatory cytokines. It primarily targets T cells, which have a key role in the pathology of atopic eczema. In the T cell, pimecrolimus binds to the cytosolic receptor, macrophilin-12, and inhibits calcineurin, a phosphatase required for the translocation of the nuclear factor of activated T cells (NF-AT) to the nucleus. This, in turn, prevents the formation and release of inflammatory cytokines (e.g. IL-2, IL-3, IL-4, IL-8, IL-10, INFγ, TNFα) and the proliferation of T cells in response to T-cell receptor stimulation. Pimecrolimus also prevents the release of inflammatory mediators from activated mast cells (e.g. histamine, tryptase, TNFα). In contrast to pimecrolimus and tacrolimus, corticosteroids have a non-selective mode of action leading to side-effects. They inhibit collagen synthesis by fibroblasts, resulting in skin atrophy. Furthermore, corticosteroids affect Langerhans' cells, which play a key role in the skin immune system. Topical treatment of mouse skin with the corticosteroids clobetasol, betamethasone and hydrocortisone results in elimination of Langerhans' cells from the treated skin, whereas pimecrolimus does not. Therefore, pimecrolimus and tacrolimus is unlikely to interfere with local immunosurveillance.Tacrolimus and pimecrolimus are structurally similar with molecular weight of 822.05 DA and 810.48 DA respectively. TCIs are macrolide lactones. They are more lipophilic than topical corticosteroids. Topical corticosteroids increase their side effects according to their efficacy – the more potent more side effects they will have. That is why they are used for short-term

treatment and have less effective long-term management. Long-term treatment with TCIs shows sustained superiority to corticosteroids. TCIs provide self-limiting, skin-selective treatment, selective mechanism of action, the extent of treatment area is not limited, and there is no clinical or sub-clinical skin thinning.

The topical calcineurin inhibitors were initially developed for the treatment of AD. The goal of treatment is the long-term control of AD by minimizing the frequency and severity of flares. Topical corticosteroids of various potencies have been the mainstay of pharmacologic treatment of AD flares. In the past few years, the introduction of TCIs has provided physicians with an effective, well-tolerated alternative to topical corticosteroids.

TCIs have a favourable safety profile. The most common adverse events are mild-to-moderate application-site reactions, including skin burning, stinging, pruritus, and erythema. Generally, the overall adverse-event profiles of both TCIs and their vehicle are similar. Compared with topical corticosteroids, TCIs have not been found to induce skin atrophy or HPA axis suppression. A single study showed a slight increase in total viral skin infections with pimecrolimus vs the control group; however, this did not reach statistical significance. TCIs can be applied to all skin surfaces, including the neck and face (areas frequently affected in children with AD) and intertriginous areas, compared with mid-potency to high-potency topical corticosteroids, which may have a higher level of absorption in these sensitive skin areas.

The TCI label changes (revised indication and the addition of a boxed warning and patient medication guide) arose from the FDA's concern about a theoretical cancer risk. This concern is based on adverse-effect data from high-dose and prolonged use of oral calcineurin inhibitors for post-transplant immunosuppression, high-dose toxicology studies in animal models, and rare cases of lymphoma and skin malignancy reported in post-marketing surveillance. The FDA-approved boxed warning states that a causal relationship has not been established between the rare cases of malignancy reported and the use of TCIs. However, the label does reiterate that TCIs should not be used for continuous long-term treatment in any age group and that they are not indicated for use in children aged < 2 years. To date, no evidence suggests a causal link between TCI use and malignancy on the basis of extensive safety, pharmacokinetic and toxicology data, and clinical experience with millions of patients. Pharmacokinetic data showed that when TCIs are applied to the affected skin, systemic absorption is low compared with orally administered tacrolimus. Likewise, low systemic absorption of topical pimecrolimus has also been observed in animal and human studies. In 67% of children with moderate-to-severe AD (up to 92% of body surface area affected), pimecrolimus blood levels were undetectable at < 0.5 ng/mL, and in 97%, levels were < 2.0 ng/mL. Among patients who had detectable systemic levels of pimecrolimus after topical administration, the MRHD ($AUC_{0\text{-}24\,h}$) was 37.6 ng•h/mL and 22.8 ng•h/mL for paediatric and adult patients, respectively.

Evidence of the absence of systemic immunosuppression by TCIs was based on several criteria. Studies with tacrolimus ointment showed no effect on cell-mediated immunity or antibody-mediated response to vaccination and no increased rate of cutaneous infection. Similarly, no evidence of pimecrolimus affecting B-cell-mediated vaccine response or T-cell-mediated delayed-type hypersensitivity and no increase in the rate of systemic infections have been reported in the medical literature. Systemic immunosuppression requires sustained levels of an immunosuppressive agent in the blood, which have not been observed in patients receiving TCIs. Considering that the level of systemic exposure of TCIs is very low and systemic immune function is preserved, it is not surprising that a causal relationship has not been established between use of TCIs and the development of any type of malignancy, including skin malignancy and lymphoma.

The most common adverse events (AEs) associated with TCIs in both adults and children were a burning sensation, pruritus and erythema, which were limited to the site of application. All these events were mild to moderate in severity, transient (median duration 15–20 minutes) and did not interfere with therapy. They generally occurred during the first few days of treatment and rapidly resolved as the skin healed. There was no increased risk of any given AE due to age, baseline disease severity, percentage body surface-area affected at baseline or gender. An important consideration for medications used to treat chronic skin diseases is long-term safety and tolerability, particularly.

in children. In the case of TCIs, the excellent tolerability profile demonstrated in short-term studies has been confirmed in long-term clinical trials in both adults and children. No AEs have been reported in long-term studies that had not been identified in the short-term trials.
Since its introduction, anecdotal reports and case series have found TCIs also to be effective and well tolerated in patients with a variety of other skin disorders, including other types of eczema, papulosquamous disorders, disorders of cornification, rosacea, other inflammatory skin conditions, vesiculobullous diseases, vitiligo, connective-tissue diseases, graft-versus-host disease, and follicular disorders. Topical glucocorticoids represent the mainstay of therapy for a variety of skin disorders. However, their clinical use is limited by local and potential systemic side effects, including skin atrophy, telangiectasia, striae, diabetes mellitus, and osteoporosis. Thus, there is a large demand for alternatives, and considerable emphasis has recently been placed on the macrolide lactones. TCIs is approved for use in AD cases, but it has also been used off-label for other skin disorders, mostly as an alternative for topical glucocorticoids.

Use for papulosquamous diseases:

Psoriasis - In a randomized placebo-controlled trial that involved 70 patients with chronic plaque psoriasis, it was found that there was no statistically significant difference in terms of the local psoriasis severity index score between 0.3% topical tacrolimus ointment and placebo ointment applied once daily for 6 weeks. In the same study, 0.005% calcipotriol ointment applied twice daily resulted in statistically significant improvement, as compared with tacrolimus ointment applied once daily. However, the use of tacrolimus ointment only once daily may have contributed to its lack of efficacy when compared to calcipotriol ointment used twice daily. Furthermore, it was applied without occlusion, so the lack of effect may also have been due to the low absorption of the drug through the thick psoriatic scales. This is supported by a subsequent randomized controlled study, involving 16 adults, in which 0.3% tacrolimus ointment with and without diisopropyl adipate as a penetration enhancer was compared (by microplaque assay) with 0.1% betamethasone valerate ointment, 0.005% calcipotriol ointment, and the ointment bases for tacrolimus and betamethasone. Study ointments were applied on descaled lesions under occlusive conditions every 2 to 3 days for 2 weeks. Tacrolimus produced statistically significant reductions in erythema and infiltration, superficial blood flow, and epidermal thickness, when compared with the vehicles. The use of a penetration enhancer did not give much additional benefit. At day 7, both tacrolimus treatments were more effective than calcipotriol treatment, but results were similar by day 14. Betamethasone was more effective than the other active treatments. Because of these results, it was felt that topical tacrolimus is likely effective in the treatment of psoriasis at sites allowing greater absorption, such as the face and intertriginous areas. Topical tacrolimus has also been reported to be useful in treating pustular psoriasis.

Seborrhoeic Dermatitis - The results of a 6-week open-label uncontrolled trial involving 16 adults suggested that 0.1% topical tacrolimus is efficacious in the short-term treatment of seborrhoeic dermatitis. Both lesion severity scores (scaling and erythema) showed statistically significant improvement at the end of the trial; improvement was seen as early as week 2.

Use for Disorders of Cornification:

Ichthyosis Linearis Circumflexa - The first report of the efficacy of topical tacrolimus for the treatment of ichthyosis linearis circumflexa involved a 20-year-old male who did not have the typical atopic manifestations and deformities of the hair shaft seen in Netherton's syndrome and whose lesions improved after topical application of 0.1% tacrolimus twice per day for 2 weeks. At the 1-year follow-up, the disease continued to be managed with no adverse effects.

Lamellar Ichthyosis - A 28-month-old child whose lamellar ichthyosis was treated with 0.1% topical tacrolimus twice daily showed dramatic improvement during the subsequent 7 weeks.

Rosacea - In an open-label trial, 24 patients with erythrotelangiectatic or papulopustular rosacea were treated with 0.1% tacrolimus ointment twice daily as monotherapy for 12 weeks. Erythema was significantly improved in both rosacea subtypes, but there was no decrease in the number of papulo-pustular lesions. Topical tacrolimus has also been used to treat steroid-induced rosacea. In an open-label trial, 24 patients with erythrotelangiectatic or papulopustular rosacea were treated with 0.1% tacrolimus ointment twice daily as monotherapy for 12 weeks. Erythema was

significantly improved in both rosacea subtypes, but there was no decrease in the number of papulopustular lesions. Topical tacrolimus has also been used to treat steroid-induced rosacea.

Use for Other Inflammatory Diseases:

***Pyoderma Gangrenosum* -** The first documentation of success with topical tacrolimus in the treatment of pyoderma gangrenosum reported the use of a 0.5% solution as an add-on medication to systemic glucocorticoids in a patient. Topical tacrolimus has also been used to treat peristomal pyoderma gangrenosum in patients with abdominal stomas, most commonly secondary to inflammatory bowel disease.

***Lichen Planus* -** The possible efficacy of TCIs in the treatment of erosive mucosal lichen planus was first documented in some observational series. Topical tacrolimus is similarly effective for genital lichen planus.

Lichen Sclerosus - Vulvar and anogenital cases of lichen sclerosus have been documented as responding to topical tacrolimus.

Lichen Striatus - There have been two case reports of adults with lichen striatus involving the face, trunk, hip, and thigh being treated with 0.1% tacrolimus ointment once or twice daily. The treatment resulted in dramatic improvement within 2 weeks and a complete resolution after 4 to 6 weeks.

Use for Vesiculobullous Diseases

***Pemphigus Vulgaris* -** There have been anecdotal reports concerning the treatment of pemphigus with topical tacrolimus as an adjunct. In reported cases, refractory lesions on the lip or cheek resolved following the application of 0.1% tacrolimus ointment twice daily for 3 to 4 weeks and the continuation of systemic immunosuppressive therapy whereas ocular lesions improved with the addition of 0.03% topical tacrolimus (applied twice daily) to the systemic regimen.

Use for Pigmentary Disorders

***Vitiligo* -** Several reports concerning patients with all Fitzpatrick skin types have indicated good results; the best responses were achieved on the face and scalp, and there was minimal improvement in other areas. The enhanced effect on the face and scalp may have been due to the stimulatory effects of sun exposure on melanogenesis although patients were instructed to use sunscreen in most cases. It may also reflect the greater penetration of TCIs in these areas or the greater density of follicles, which provides a greater melanocyte reservoir. Since phototherapy remains the most efficacious option for vitiligo, studies have also looked at the combination of phototherapy and topical immunomodulators.

Use for Connective-Tissue Diseases

***Lupus Erythematosus* -** Application of 0.1% topical tacrolimus ointment once or twice daily has been found to be useful in treating refractory skin lesions of various forms of lupus erythematosus, including subacute cutaneous lupus erythematosus and systemic lupus erythematosus. Improvement was observed after 3 to 8 weeks. Of note, the lesions of chronic discoid lupus erythematosus have been shown to be less responsive or to be resistant to topical tacrolimus applied twice daily or even under occlusion. There are some pilot studies for the use of TCIs in dermatomyositis and localized scleroderma with good results.

According to current data, TCIs are safe and efficacious alternative that minimizes the lifetime need for topical glucocorticoid application. However, further studies with long-term follow-up are required to clarify their efficacy, especially for different racial groups and in comparison with other existing therapies, and safety data should continue to be monitored and reported. Given that therapy combining drugs that have different mechanisms of action may enhance the effectiveness of each drug and minimize potential adverse effects, it is worthwhile to conduct large-scale trials to evaluate the use of tacrolimus ointment combined with topical glucocorticoids and the treatment of disease rebound and flares.

TEAR FILM STABILITY TEST A NEW APPROACH FOR EVALUATING TEAR FILM SUBSTITUTES

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Introduction:

Dry eye is one of the most common and underdiagnosed problems in ophthalmic practice.[1, 2] There are many qualitative and quantitative methods for assessment of human tear film, most popular of which are Shirmer's test, meniscus high, tear break up time (TBUT), lid parallel conjunctival folds (LIPCOF), as well variety of laboratory testing.[3] Most established tests are with poor repeatability and number of modifications, which makes the existing publications more difficult for interpretations.[4]

Regardless of the dry eye severity, most commonly the problem is treated by tear film substitutes.[5, 6] There are number of products available, however, there is no established protocol for clinical evaluation of their effect on tear film stability and distribution. Most commonly the therapy is titrated on the basis of subjective reaction of the patient, and comfort is the most evaluated outcome measure. [7, 8]Although it is clear that different tears are having different effect on ocular surface of different subjects there is no established method for evaluation of tear film following application of lubricants.

Tear film break up test, and its number of modifications, is used as a standard in the clinical practice. However, it provides only the time point of the presence of the first dry spot.[9] The position, extent and size of the dry spot is ignored. In order to address this issue a different test called tear film stability test (TFST) was developed and tested.

The purpose of this study is to compare TFST results with the TBUT results for normal subjects, contact lens wearers and "dry eye" subjects and to evaluate the effect of the artificial tears on both tests.

Materials and methods:

Study included 100 subjects, 40 with "normal" tear film, 40 contact lens wearers and 20 diagnosed with "dry eye". After explanation of the study and informed consent, all patients filled McMonnies questionnaire, and were examined clinically using standard protocol. Special tests included TBUT (tear break up time) and TFST (tear film stability test).

McMonnies questionnaire is a 12 item and allows subdivision of the patients into 3 different groups: normal (score under 10), "dry eye suspect" (score 11 to 19) and "dry eye" score over 20 . Furthermore, the "normal" group was subdivided into 3 subgroups: group 1 (score 0-3), group 2 (score 4-7) and group 3 (score 8-10).

TBUP was applied to all patients following the standard protocol established by the Tear Film Society.

TFST is a new approach to tear film distribution and quality, based on tear film distribution at the time point coincidental with the length of mean inter-blink interval. To evaluate TFST one must first measure the mean interblink interval as asking patient to participate in visually demanding task (such as filling an electronic questionnaire in this study) and time the interval for 10 blinks. Then divide the interval in 9 and use the value as a mean interblink interval. Subsequently in patients eye a small amount of fluorescein is instilled using a semi-dry pre-prepared fluoriscein stripe. The patient is asked to stare after few blinks and a picture is taken at the time point of the interblink interval starting last blink. The picture is then qualitatively and quantitatively analysed.

Tear film stability evaluation is based on zone and extent. There are 4 zones A, B, C, D (fig 1) and extent is based on the following 6 grades:

Grade 1 – normal homogenous tear film	Grade 4 - dry area up to 1/2 per zone
Grade 2 – few dry spots with small size	Grade 5 - dry area up to 3/4 per zone
Grade 3 – dry area up to ¼ per zone	Grade 6 – total dryness

Subsequently the 40 healthy individuals (no contact lenses, no dry eye) were included in blind experiment applying two types of artificial tears and evaluation of their effect by independent examiner. The eyes were randomly assigned to medications 1 and 2 and tear film was evaluated 60 minutes after application. Medication 1 was medication with preservative, medication 2 was preparation in monodoses.
All statistics was done using SPSS computerized analysis.

Results:
For healthy subjects a positive correlation was found between results from the questionnaire and objective tear film evaluation (TBUT) – table 1. This correlations were confirmed by statistical analysis ($p=0.0002$, Student T test). In contact lens wearers the mean value from McMonnies questionnaire was 6.3 ± 3.6. Positive correlation between the subjective results and objective findings was established again (table 2). In dry eye group such a correlation was not found (table 3). Tear film stability test was positive correlated with the results for all three groups including the questionnaire score and TBUT. The correlation between the test and currently the most popular investigation TBUT, for all three groups, is presented as figures 2, 3 and 4. The results demonstrating effect of the two different tear film substitutes are presented in table 4. Statistical analysis revealed no difference in general effect of the two preparations, however, overall improvement of the TBUT and TFST was significant.

Discussion:
Dry Eye is still one of the most commonly underdiagnosed conditions in day to day clinical practice. This challenging syndrome may be associated with severe eye problems, especially in case of contact lens wear.[10, 11] That is why diagnosis and treatment are so important.
There are many methods to evaluate and diagnose dryness, however, most of them are difficult on application to the busy clinic, some are very expensive, and majority are very difficult to standardize. It is very complicated to interpret simple methods like Shirmer's test for example.[12, 13] The tear break up time (TBUT) and its modification and correlation with other parameters appear to be the most recommended and widely used clinical test. The proposed tear film stability test is simple, more informative and with better repeatability. The best way to apply this test is to take a snapshot at the time point of mean intrerblink interval. Subsequently this snapshot may be analysed and re-analysed by one or many examiners.
On the basis of aforementioned clinical test, a diagnosis of dry eye would be made, but most importantly would be specified as evaporative or tear insufficiency dry eye.[14] The former is more common especially in relation to contact lenses. As far the diagnosis is made, the practitioner mast select a proper therapeutic regimen. The most commonly used medications are tear film substitutes. They may be drops, gels and even spray. Recently, some peroral medications are also used. The second line measures are to prevent drainage by temporary or permanent punctual occlusion.[6] In evaporative dry eye one of the most important treatment modality is lid hygiene. Special approach is required when practitioner deals with contact lens related dry eye. The contemporary materials and designs will be discussed in order to highlight the options for reducing and treating dryness. Regardless of the selected therapy, practitioner must follow on its effectiveness. The results of the current study showed good predictive value of both tear film stability test and tear break up time. In the published literature, only tear break time has been used.
To treat dry eye practitioner must be familiar with all diagnostic methods, apply them properly, identify the underlying reason(s) for dry eye and treat the condition with the best possible therapy. Treatment should be titrated individually, as dry eye is dependent on many environmental, biological and other factors. Today, treating dry eye is more like an art….
Conclusions: Evaluation of the tear film is a key investigation for all patients. The proposed test for tear film stability is easy and more informative then all other tests.

References:
1. McMonnies, C.W. Contact Lens & Anterior Eye, 2007. 30(1):37-51.
2. Montes-Mico, R. J. Cataract & Refractive Surgery, 2007. 33(9): 1631-5.
3. The Ocular Surface, 2007. 5(2):75-92.
4. Montes-Mico, R., J.L. Alio, W.N. Charman. Invest. Ophthalmology & Visual Science, 2005. 46(5): 1615-9.
5. Malet, F., D. Karsenti, P. Pouliquen. Eye & Contact Lens: Science & Clinical Practice, 2003. 29(1): 38-43.
6. Levine, M.R. Intern. Ophthalmology Clinics, 1978. 18(3): 101-19.
7. Narayanan, S., et al. Eye & Contact Lens: Science & Clinical Practice, 2005. 31(3): 96-104.
8. Nichols, J.J., et al. Cornea, 2002. 21(5): 469-75.
9. Glasson, M.J., et al. Invest. Ophthalmology & Visual Science, 2003. 44(12): 5116-24.
10. Doughty, M.J., et al. Ophthalmic & Physiological Optics, 2005. 25(2): 119-27.
11. du Toit, R., et al. Optometry & Vision Science, 2001. 78(6): 455-62.
12. Dogru, M., et al., Amer. J. Ophthalmology, 2004. 138(3): 381-8.
13. Morgan, P.B., A.B. Tullo, N. Efron. Eye, 1995. 9(Pt 5):615-8.
14. Su, S.-B., et al. BMC Public Health, 2006. 6:303.

Table 1 Results for the "normal" subjects group (N=40, n=80) including McMonnies questionnaire score, TBUT, TFST.

Group		***Normal***			***"Dry eye" suspect***
		Group1	***Group 2***	***Group 3***	
McMonnies score		**0-3**	**4-6**	**7-9**	**≥10**
N of patients		**14**	**14**	**10**	**2**
Age		**33.1±7.1**	**30.8±6.7**	**34.7±9.1**	**52**
Inter blink interval		**5.1±1.1 sec.**	**5.3±2.1sec.**	**6.1±3.1sec.**	**9.1±0.45sec.**
Mean TBUT		**10.1±4.1sec.**	**9.4±3.1sec.**	**7.9±2.3sec.**	**5.1±0.3sec.**
Ocular protection index		**2.02**	**1,77**	**1.3**	**0.58**
TFST	***all***	**(1,1,1,2,1,1,1,1, 1,1,1,2,1,1)**	**(1,2,1,2,1,1,1,2, 1,2,1,2,1,1)**	**(1,3,2,2,1,3,1, 1,1,3)**	**(4, 5)**
	mean	**1,14**	**1,36**	**1,8**	**4,5**

Table 2. Results for the group of subjects wearing contact lenses (N=40, n=80) including McMonnies questionnaire score, TBUT, TFST.

Group		***Normal***			***"Dry eye" suspect***
		Група 1	***Група 2***	***Група 3***	
McMonnies score		**0-3**	**4-6**	**7-9**	**≥10**
N of patients		**8**	**13**	**14**	**5**
Age		**28.1±6.1**	**30.0±3.7**	**32.1±2.1**	**37±5.1**
Inter blink interval		**6.4±1.1 sec.**	**6.7±1.9sec.**	**7.5±2.9sec.**	**9.8±0.95sec.**
Mean TBUT		**12.1±2.1**	**10.4±3.1**	**8.4±3.3**	**6.2±1.8**
Ocular protection index		**1,9**	**1,55**	**1.12**	**0.63**
TFST	***all***	**(1,1,1,2,1,2,2,1)**	**(1,2,1,3,1,3,1,2, 1,2,3,2,1)**	**(1,3,2,2,2,3,1, 2,3,3, 2, 2, 3, 3)**	**(4,3 , 4, 2, 5)**
	mean	**1,38**	**1,77**	**2,29**	**3,6**

Table 3. Results for the "dry eye" subjects group (N=20, n=40) including McMonnies questionnaire score, TBUT, TFST.

Group		***Normal***	***"Dry eye" suspect***	***"Dry eye"***
McMonnies score		**≥10**	**11 - 19**	**≥20**
N of patients		**2**	**13**	**5**
Age		**38.1±2.1**	**47.0±3.7**	**51±5.1**
Inter blink interval		**7.1±0.9 sec.**	**8.1±2.4sec.**	**8.9±3.9sec.**
Mean TBUT		**7.1±1.1**	**4.4±3.3**	**3.6±2.8**
Ocular protection index		**1.0**	**0.54**	**0.4**
TFST	***all***	**(2,3)**	**(2,3,3,3,4,3,3,4, 4,3,3,3,2)**	**(3,4,4,5,4)**
	mean	**1,13**	**3,08**	**4**

Table 4. Results for the tear film test dynamics following instillation of two artificial tear film preparation, evaluating 40 patients (n=80 eyes).

Group		*Normal*			*"Dry eye" suspect*
		Group 1	*Group 2*	*Group 3*	
McMonnies score		**0-3**	**4-6**	**7-9**	**≥10**
Patient number		**14**	**14**	**10**	**2**
Mean age		**33.1±7.1**	**30.8±6.7**	**34.7±9.1**	**52**
Mean TBUT for eye receiving medication 1		**9.2±5.3 sec.**	**6.9±2.5sec.**	**8.2±3.3sec.**	**5.2±0.1sec.**
Mean TBUT for eye receiving medication 2		**8.8±2.7sec.**	**8.3±4.4sec.**	**7.8±5.0sec.**	**5.9±0.1sec.**
Mean TBUT 60min after application of medication 1		**10.8±5.4sec.**	**11.9±6.2sec.**	**10±6.4sec.**	**9.2±0.9sec.**
Mean TBUT 60min after application of medication 2		**12.0±8.3sec.**	**9.4±3.1sec.**	**10.3±8.5sec.**	**11.1±0.3sec.**
Mean improvement	*Medication 1*	**1.6 sec.**	**5.0 sec.**	**1.8 sec.**	**4.0 sec.**
	Medication 2	**3.2 sec.**	**1.1 sec.**	**2.5 sec.**	**5.1 sec.**
	T test	**p=0.21**	**p=0.09**	**p=0.3**	**p=0.29**

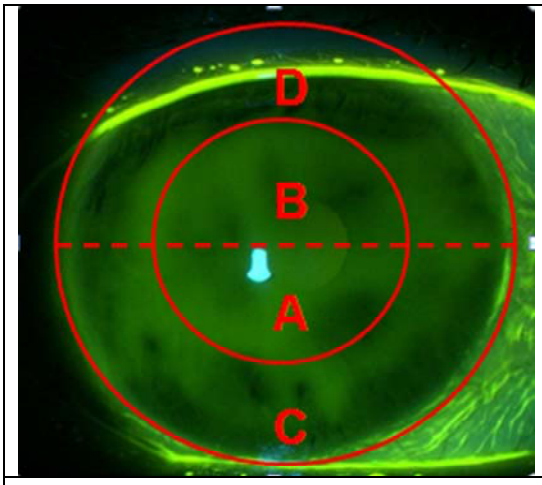

Figure 1. Corneal division into 4 different zones in order to quantify tear film stability.

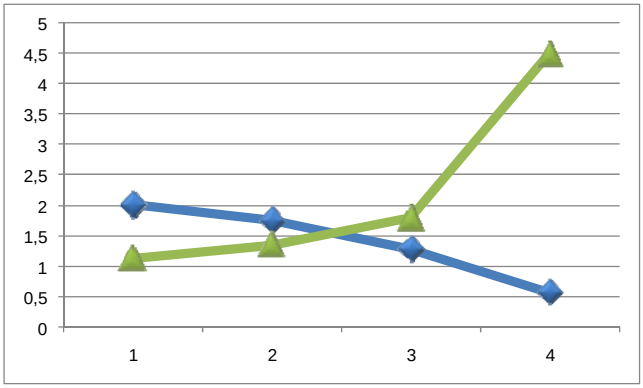

Figure 2. Graphic presentation of ocular protection index (blue) and tear film stability test of 40 normal subjects (n=80 eyes), classified on the basis of McMonnies questionnaires into 4 different groups.

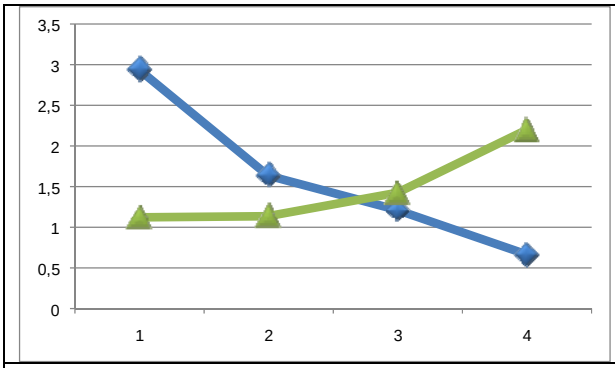

Figure 3. Graphic presentation of ocular protection index (blue) and tear film stability test of 40 subjects wearing contact lenses (n=80 eyes), classified on the basis of McMonnies questionnaires into 4 different groups.

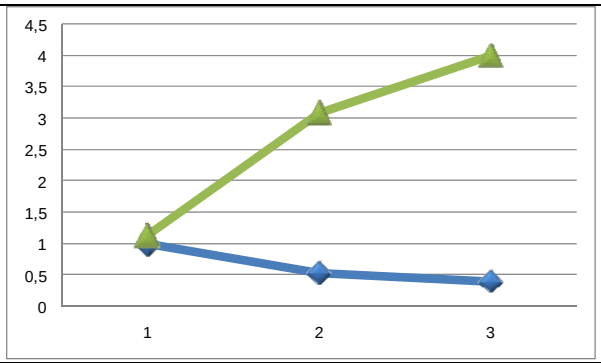

Figure 4. Graphic presentation of ocular protection index (blue) and tear film stability test of 20 "dry eye" subjects (n=40 eyes), classified on the basis of McMonnies questionnaires into 3 different groups.

SUCCESSFUL PHARMACOLOGICAL TREATMENT FOR OCULAR NEOVASCULARIZATION (AGE-RELATED MACULAR DEGENERATION AND CORNEAL NEOVESSELS)

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Introduction: For many years, ophthalmologists have been trying to solve the problem of ocular neovascularization. Development of pathologic vessels in eye tissues, as a result of different pathological processes, had caused irreversible blindness and no treatment was available.
Exudative age-related macular degeneration (AMD) with development of choroidal neovascular membrane (CNV) is the most common cause of severe vision loss in persons over 55 years of age in developed countries, affecting millions of people and their number is expected to increase because of population growth and aging. The treatment of CNV is based on the interrupting of angiogenic cascade involved in the growth of new blood vessels.
Vascular endothelial growth factor (VEGF) plays a major role in ocular neovascularization and the pathogenesis of AMD. VEGF - a homodimeric glycoprotein - has been shown to be an endothelial cell specific mitogen in vitro and an angiogenic inducer in a variety of in vivo models. It increases vascular permeability, induces vascular endothelial cell proliferation, and promotes endothelial cell survival. It also serves as a chemotactic factor for leukocytes[1]. VEGF is up-regulated by hypoxia. Inhibition of VEGF and, thereby, inhibition of angiogenesis and vascular permeability can be an effective treatment for a variety of ocular diseases including neovascular AMD[2] and corneal opacification with neovessels.
Bevacizumab (Avastin®; Roche) is a full-length humanized antibody that binds to all subtypes of VEGF and is successfully used in tumour therapy as a systemic drug.
In July 2005 at ASCRS meeting Rosenfeld and colleagues reported favorable visual acuity responses to systemic bevacizumab[3], and disclosed that they had administered intravitreal bevacizumab in a small number of patients with no serious adverse events and good visual responses[4]. The favourable short-term clinical efficacy was so apparent that use of intravitreal bevacizumab spread rapidly among the retina community.
Uncontrolled prospective and retrospective case series studies including large number of patients in different countries have shown the short-term success of anti-vascular endothelial growth factor (VEGF) therapy with intravitreal bevacizumab for choroidal neovascular membrane regression and visual acuity improvement in patients with neovascular AMD[5, 6] and other pathological processes[7-11].
Recently subconjunctival application of Avastin has been discussed as efficient approach to reduce corneal neovascularization[12].
Meanwhile other anti-VEGF drugs have been introduced for selective AMD treatment. Even though bevacizumab is still used as an off-label therapy, the significant cost difference between it and the other pharmacological treatments for neovascular AMD (pegaptanib, verteporfin and ranibizumab) is an important advantage and motivation for its wide acceptance[13].
Purpose: The purpose of this study was to investigate the therapeutic effect of bevacizumab in patients with neovascular AMD with classic and occult CNV as well as in cases with corneal neovascularization– eyes with penetrating keratoplasty and recurrent pterygium.
Methods: For the period of 29 months (December 2006-April 2009), 115 patients were included in prospective interventional clinical study for evaluation of the efficacy of treatment with intravitreal and subconjunctival Avastin depending on indication. The study was approved by the Institutional Review Board. Examination of AMD patients included: visual acuity testing, intraocular pressure (IOP) measurement, biomicroscopy, ophthalmoscopy, Amsler grid, fluorescein angiography (FA), macular perimetry testing with the Humphrey Automated Perimeter, B-scan and OCT with Stratus OCT (Carl Zeiss Meditec) in selected cases.
In patients with corneal neovascularization, full eye exam included anterior segment photo documentation for monitoring the morphologic changes of corneal vessels.

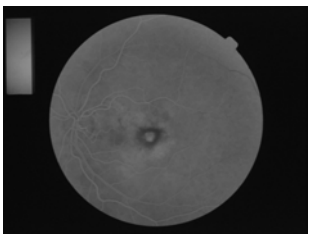
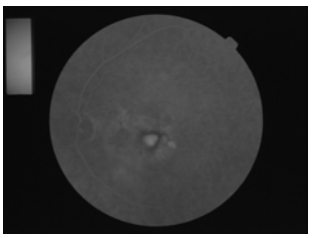
All patients have signed informed consent for the off-label use of bevacizumab after discussing the risks, benefits, and the other possible alternatives for treatment.
Intravitreal and subconjunctival bevacizumab injections were performed in the operating theatre under strict aseptic technique using topical anaesthesia.
Patients who were treated with intravitreal application of the medication received 2 to 6 consecutive 1.25 mg/0.05 ml bevacizumab injections in 30 to 45 days interval. Indirect ophthalmoscopy and tonometry were performed after the procedure in all cases. Topical antibiotic for 7 days was prescribed to all patients. Strict instructions for immediate report on possible complications were given to patients and in many cases to their relatives also. The follow up visits were arranged on the 7^{th} and 30^{th} day after the injection and then every month. In cases with visual acuity deterioration due to active disease (based on FA and/or OCT), another intravitreal injection was performed.
Patients with corneal neovascularization received 2.5 mg/0.1 ml of bevacizumab per affected quadrant at the site of neovascularization at the time of operation (penetrating keratoplasty or excision of the recurrent pterygium with limbal stem cell autotransplantation) or at follow up visits. Indication for re-treatment was the presence of new vessels on the corneal graft or corneal neovascularization after the operation for recurrent pterygium. Effect on vascularization was accessed based on photo documentation and the number, size and centricity of neovessels.
Results: Patients were divided into two groups according to the way of application of the medication as shown in Table 1.

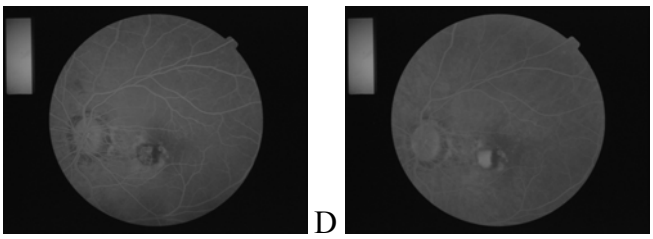
Table 1. Indication for treatment with bevacizumab (Avastin), way of application and patient population

	Group 1	Group 2
Indication	No (%) of patients with ivt. bevacizumab	No (%) of patients with s.c bevacizumab
Neovascular AMD	95, (82.6%)	
Corneal neovasculaization in PKP		15, (13.1%)
Corneal neovasculaization in recurrent pterygium		5, (4.3%)

Ivt.=intravitreal; s.c.=subconjunctival; PKP=penetrating keratoplasty

A total of 95 patients with a mean age of 73 years (range 50-85) were included in the invtravitreal bevacizumab study-group. Occult CNV associated with AMD was the most frequent indication. Preoperative visual acuity in the intravitreal bevacizumab study-group varied from 0.05 to 0.6. Improvement in visual acuity by one, two or more lines was achieved in 65 patients (68.4%) of group 1. Twenty patients (21%) had no change and in 5 cases (10.6%) visual acuity deteriorated despite treatment.
Very encouraging results in terms of visual acuity improvement were obtained in patients with neovascular AMD up to 65 years of age. An example of angiographic changes in a patient with neovascular AMD is shown in Figure 1. The patient is 64 years old and his visual acuity has increased from 0.3 to 0.7 after two intravitreal injections of 1.25mg/0.05 ml of bevacizumab.

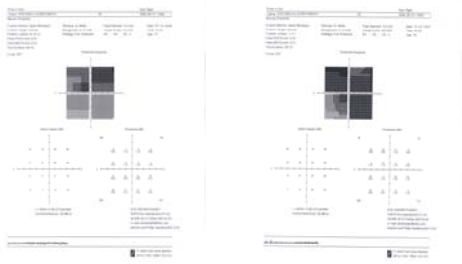
A  B 



C D

Figure 1. A. Pre-treatment early-phase fluorescein angiogram showing a classic subfoveal CNV with (B) intense leakage in the late phase. Early phase (C) and late phase (D) fluorescein angiograms showing staining of the CNV with no evidence of leakage after treatment.

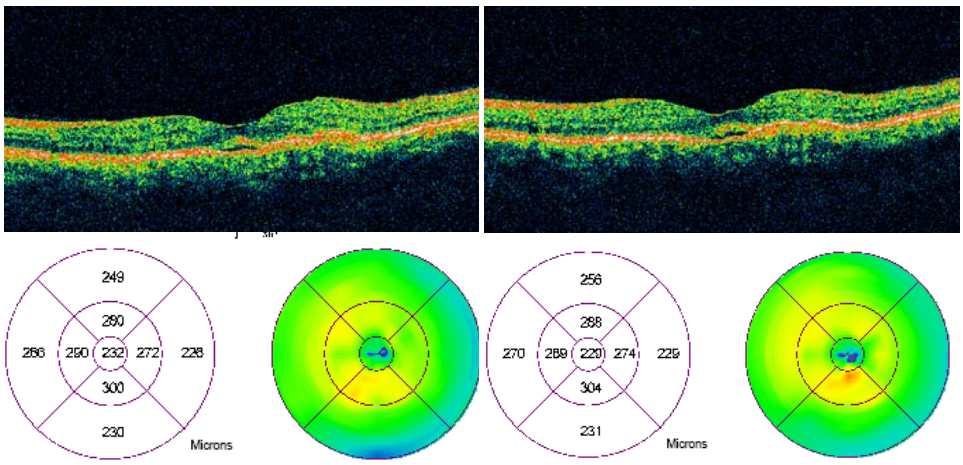
Micro-perimetry test (performed on selected patients) showed slight deterioration of central visual field despite visual acuity improvement (Figure 2).



A B

Figure 2. A Macular perimetry testing with the Humphrey Automated Perimeter before intravitreal administration of bevacizumab and (B) two months later.

CNV documented on FA and OCT demonstrated minor changes following first Avastin application in contrast with rapid and dramatic improvement of visual acuity (Figure 3).



A B

Figure 3. OCT image before (A) and 15 days after (B) intravitreal bevacizumab

None of the patients in the intraocular bevacizumab study group experienced systemic complications associated with the treatment. Transient intraocular pressure (IOP) elevations were detected in 16 patients (24.6%) in group 1 and this was the most common ocular complication. Other complications included subconjunctival haemorrhage, foreign body sensation, and tearing.

Fifteen patients with penetrating keratoplasty with a mean age of 63 years (range 30-83) and 5 with recurrent pterygium with a mean age of 46.8 (range 32-55) have received subconjunctival injection of bevacizumab because of corneal neovascularization (group 2). Figure 4 demonstrates one of the patients in this group. Decrease and even disappearance of the new vessels were observed in 14 patients (82.4 %) from subconjunctival bevacizumab study-group. Adverse reactions included redness, tearing, and foreign body sensation.

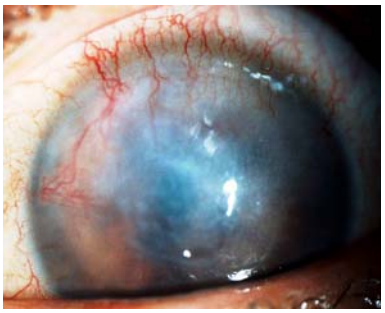
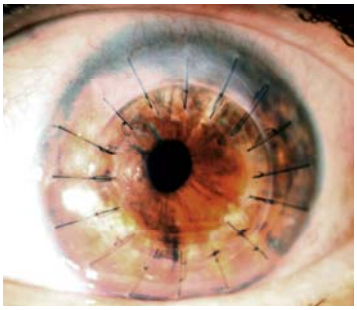
A   B

Figure 4. Patient before (A) and 8 months after (B) after corneal transplantation and subconjunctival bevacizumab application.

Discussion: Studies based on psychophysical (visual fields, colour vision) and electrophysiological (ERG/EOG) tests have approved Avastin as safe and well-tolerated treatment option[14, 15]. Major local complications of intravitreal application of bevacizumab include: endophthalmitis, tears of the retinal pigment epithelium, retinal detachment, traumatic lens injury, and immune reaction to repetitive doses. Systemic complications reported in literature include: hypertension, myocardial infarction, and stroke. None of our patients has experienced significant ocular or systemic complications from this treatment.

There is no consensus yet about the optimal number of intravitreal injections needed for stabilization of results concerning visual acuity, central retinal thickness and flurescein angiography changes. Many studies recently discuss the synergic effect of two or more medications and conclude that combined therapy increases the effectiveness of treatment and delays the necessity of new intraviteal injection of anti-VEGF drugs.

The main limitations of this study are the lack of control groups and the short follow up period. Lack of central visual field improvement may be associated with another aspect of the VEGF-neuroprotective effect. Despite these, our results show that bevacizumab is effective and safe treatment option for AMD and a number of ocular diseases associated with CNV and VEGF overproduction: pathologic myopia, angioid streaks, central or branch retinal vein occlusion, proliferative diabetic retinopathy with or without macular oedema.

Positive effect on corneal neovascularization has also been observed in the majority of patients with subconjunctival application of the medication.

Conclusions: Our results show favourable effect of intravitreal bevacizumab in properly selected patients with wet form of AMD. Subconjunctival Avastin may be used as additional treatment option in cases with corneal neovascularization.

References:

1. Arevalo JF. AAO 2007, Retina Subspecialty day, pp 24-28
2. Krzystolik MG, Afshari MA, Adamis AP, et al. Arch Ophthalmol. 2002; 120:338-346
3. Michels S, Rosenfeld PJ, Puliafito CA, et al. Ophthalmology 2005;112:1035-1047
4. Rosenfeld PJ, Moshfehgi AA. Puliafito CA. Ophthalmic Surg Lasers Imaging 2005; 36:331-335
5. Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Guist MJ. Ophthalmology 2006; 113: 363-372
6. Spaide RF, Laud K, Fine HF et al. Retina 2006; 26: 383-390
7. Yamamoto I, Rogers AH, Reichel E, et al. Br J Ophthalmol 2007; 91: 157-160
8. Chan WM, Lai TY, Liu DT, Lam DS. Ophthalmology 2007; 114: 2190-2196
9. Priglinger SG, Wolf AH, Kreutzer TC, Kook D, Hofer A, Strauss RW, Alge CS, Kunze C, Haritoglou C, Kampik A. Retina 2007; 27(8):1004-1012
10. Bhatnagar P, Freund KB, Spaide RF, Klancnik JM Jr, Cooney MJ, Ho I, Fine HF, Yannuzzi LA Retina 2007; 27(7): 897-902
11. Arevalo JF, Fromow-Guerra J, Quiroz-Mercado H, Sanchez JG, Wu L, Maia M, Berrocal MH, Solis-Vivanco A, Farah ME; Pan-American Collaborative Retina Study Group Ophthalmology 2007; 114(4): 743-50
12. Erdurmus M, Totan Y. Graefes Arch Clin Exp Ophthalmol. 2007; 245(10): 1577-1579
13. Raftery G, Clegg A, Jones J et al. Br J Ophthalmol. 2007;91:1244-1246
14. Wu L, Martínez-Castellanos MA, et al. Graefes Arch Clin Exp Ophthalmol. 2008; 246(1):81-87
15. Ziemssen F et al. Int Ophthalmol. 2008; 28(2):101-109

NEWLY SYNTHESIZED 2-STYRYL-8-HYDROXYQUINOLINES (SQS) AND THEIR DERIVATIVES: INHIBITION OF HUMAN IMMUNODEFICIENCY VIRUS-1 (HIV-1) REPLICATION IN CELL CULTURE

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Introduction

During the last years, the efforts against HIV/AIDS pandemic were directed to find substances blocking viral replication and slow down the progress of infection. The life cycle of HIV is comprised of different steps that are adequate targets for chemotherapeutical interventions. Nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs, NNRTIs), protease inhibitors (PIs) and entry inhibitors are components of routine treatment regimens (4). A very important step of HIV replication is the integration of proviral DNA into cellular genome so ensuring a stable maintenance of the viral genome and persistence of the virus in the host. Integrase (IN), the enzyme responsible for integration and coded for by *pol* gene is therefore an attractive target for novel drugs because of its central role in the life cycle of HIV.

A number of IN inhibitors have been characterized in vitro – synthetic (Diketo acids- DKAs (L-708,906), Diketo aryls (5CITEP), Pyranodipyrimidines, Styrilquinolins - SQs, etc.), and natural (L-Chicoric acid, Coumarins, Cucurmin, etc.) compounds. They can be divided into two groups: those that inhibit the 3'-processing reaction and those that preferentially inhibit the strand transfer reactions. SQs – novel potent integrase inhibitors, inhibit the 3' processing. Studies showed that the subdivision of IN inhibitors into two distinct groups was not so clear (5, 6). In 2007 the first integrase inhibitor – Raltegravire **(Isentress™)** (a strand transfer blocker) was licensed after successful passing of all clinical trials (7).

SQs are synthetic compounds like DKAs, designed (8) to chelate the divalent metal (Mg^{2+}) in the integrase core domain. The SQs explore a competitive mechanism inhibiting with higher affinity the 3'-processing than the strand transfer. The reason for that is their 5-10 times higher affinity to the donor than to the acceptor site of IN. Once the DNA-IN complex is built, the SQs cannot inhibit the 3'-processing because they cannot destroy the complex integrity (2, 8). Essential for the inhibition activity are 7-COOH and 8-OH groups probably participating in the chelation reaction. In current paper data are presented on inhibition of HIV-1 replication in cell culture by six newly synthesized 2-styryl-8-hydroxyquinolinyl acetates (designated as initial substances) (Table 1) and five derivatives of them – salts, bases and hydrochlorides, synthesized after evaluation of anti-HIV activity of the initial substances (Table 2).

Materials and Methods

The chemical synthesis of all SQs was performed in the Veterinary Faculty of Trakia University, Stara Zagora, Bulgaria by A. Pavlov and S.Chervenkov.***MT-2*** cells usually used as a classical model for acute infecion with HIV-1 (X4 strain) and testing for inhibitory effect (9). Cells were grown in RPMI medium with 10% FCS.

HeLaP$_4$ monolayer epithelial cells, engineered to produce CD4 receptors, a kind gift from Prof. J.-L Darlix - Ecole Normale Superieure – Lyon, France, were grown in DMEM supplemented with 10% FCS.

As a source of HIV-1, the supernatant of H9/HTLV III B cell line was used. The supernatants were stocked with known p24 antigen content, RT activity and infectivity.

The following parameters were studied: cytotoxic concentration 50 – CC50 (preventing death of 50% on MT-2/HeLaP$_4$ cells), maximal nontoxic concentration – MNC (the highest concentration causing no cytotoxicity on both cell lines), and inhibitory concentration 50 – IC_{50} (concentration inhibiting by 50% the viral replication). CC50 and MNC were detected by MTT – uptake assay (9).

IC_{50} was studied only on MT-2 cells by microtiter infection assay exploring the cytopathic effect of HIV, using MTT test (9). Experiments under conditions of acute infection were performed in 96-well microplates with 6-8 parallels/experiment. Cell controls (MT-2 cells with medium only) and viral control (virus infected MT-2 cells without treatment with the substances tested) were run with every experiment. For anti-virus assay 50 µl HIV (undiluted or diluted to obtain multiplicity of infection about 0.1) was added to each well except the cell controls. Virus was allowed to attach the cells for an hour at 37ºC/5% CO_2. All the compounds were prepared in 10× dilutions (one dilution/column of plate). The plates were incubated for 72 hours at 37ºC/5% CO_2. After that, MTT test was performed as described (9) and absorbance of viable cells was measured colourimetrically at A540 nm. For all experiments, the mean value of each column was calculated. The 50% cytotoxic concentration (CC_{50}) of the test compound was defined as the concentration reducing the absorbance (A540) of mock-infected cells to 50% compared to the cell controls. IC_{50} was expressed as the concentration where 50% protection of virus-infected and substance treated cells was achieved. The cell survival (% protection) was calculated according to the following formula:

$$\% \text{ protection} = \frac{A540X - A540 \text{ Control HIV}}{A540 \text{Cell Control} - A540 \text{Control HIV}} \times 100,$$

where X is the mean value of A540 of HIV-infected cells, treated with appropriate concentration of the substance studied;

control HIV is the mean value of A540 of HIV-infected cells;
cell control is the mean value of A540 of un-infected and un-treated cells.
As a referent substance, ABC (abacavir - well known NRTI) was used (3).
Endogenous RT activity of supernatants of HIV-1 infected/uninfected MT-2 cells treated/untreated with SQLs was tested by HS-Lenti Kit-RT assay (Cavidi, Sweden). The kit contains recombinant RT (rRT) as a standard, which makes possible RT quantitation (11). Also, the direct effect of the compounds on rRT activity was measured to prove RT as a target of antiviral action.

Results and Discussion

Table 3 shows CC_{50} and MNC of newly synthesized SQs measured through MTT-test in MT-2 and $HeLaP_4$ cell lines. In MT-2 cells, CC_{50} values were so close to MNC that practically could not be detected. Results show the compounds were more toxic for $HeLaP_4$ compared to MT-2 cells by MNC. Table 4 shows MNC of the derivatives of newly synthesized SQs in MT-2 cells – they are more toxic than the initial ones. It is known that free phenol group in the molecule of 8-OH quinoline and its derivatives increase their toxicity. Esterification of –OH group to 8-C of the quinoline ring declines this unfavourable effect. All initial SQs (Table 1) have acetylated hydroxyl group and are less toxic in comparison with their derivatives. The initial SQs having only one halogen substitute (201, 205) in the phenyl ring are more cytotoxic than those with additional halogen substitute (101, 105). A halogen substitute linked to 3'-C of the phenyl ring (101) confers higher cytotoxicity than that linked to 2'-C (100).

The hydrochloride derivatives are more cytotoxic than the initial substances.

Derivative 304B containing indol ring added to the phenyl ring of 105 and 205 is also more cytotoxic than the initial substances.

301S - the salt derivative of 205 does not show any changes in cytotoxicity compared to the initial substance.Tables 5 and 6 show the results from antiviral assays for the initial substances and their derivatives. It is seen that the derivatives are more active than initial SQs (comparison of their IC_{50}). Probably, esterification of -OH group declines the chelating potency conferring the weaker inhibitory effect of initial substances.

Substances 100 and 101 show almost 100% protection of MT-2 cells infected with HIV-1 (table 5), and correlating to inhibition in RT activity in supernatants - 92.96% and 83.59%, respectively, and rRT - 89.90% and 83.94%, respectively (Tables 5 and 6). It is concluded that compounds **100 and 101 explore RT of HIV-1 as a target for their inhibitory effect**.

Substance 103 shows no inhibition of HIV-1 infectivity, correlating well to lack of inhibition of RT in supernatants. At the same time 103 inhibits directly rRT in MNC (73.36%, Table 6). The possible explanation could be that the RT in the virion is somehow "protected" by an unknown mechanism.
Substances 105 and 205 protect cells ~ 90% (table 5) but this effect can only partially (esp. for 205) be explained by RT and rRT inhibition (Tables 5 and 6). In 105, Cl_2 substitute is introduced in the quinolone ring (no such substitute in 205). The phenyl rings of both substances show no difference, but 205 is more cytotoxic than 105, which has a quinoline ring identical to 100 and 101 (also with low cytotoxicity).
Substance 201 protect cells ~ 30%; the RT activity in supernatants is inhibited in 33.91% while the rRT is 78.7% inhibited. This effect needs a further explanation.
Substance 105H (hydrochloride deacetilated derivative of 105) protects MT-2 cells (34.83%) but shows no RT inhibiting effect – Table 7. It is more cytotoxic than 105).
301S, 302H, 303H and 304B in MNC moderately protect MT-2 cells, but demonstrate inhibition of RT activity in supernatants 54-75% with higher inhibition in lower concentrations (Table 7). Except 303H, all of them show higher inhibitory effect on HIV compared to the initial substances (201 and 205).At the same time no effect on rRT was observed that clearly shows that RT is not the target of the inhibiting effect. This is not inconsistent to RT results in supernatants, because RT measures also the virus replication, not only the effect on the enzyme. As far as the SQs are designed as IN inhibitors it seems possible that the derivatives inhibit the HIV-1 replication by impact on IN. Additionally, a combined effect on both RT and/or PR and IN is not excluded because of overlapping of coding regions in *pol*-gene. Further studies are planned to clarify the exact mechanism and target/s of action of the newly synthesized SQs and their derivatives. In conclusion, the derivatives of SQs described here demonstrate increased anti-HIV effect but higher cytotoxicity.

TABLE 1. NEWLY SYNTHESIZED SQS

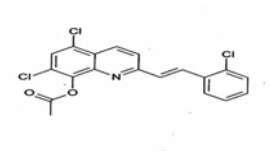
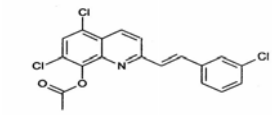
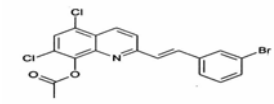
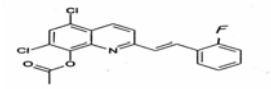
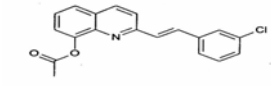
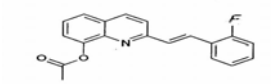
Formula	Code №	Molecular mass (M)
	100	392.7
	101	392.7
	103	437.1
	105	376.2
	201	323.8
	205	307.3

TABLE 2. DERIVATIVES OF THE INITIAL COMPOUNDS

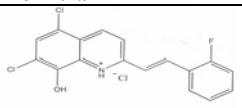
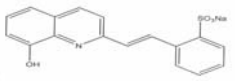
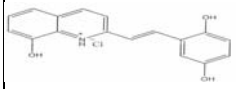
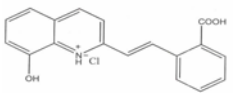
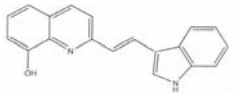
Formula	Code №	Molecular mass (M)
	105H Derivative of 105	370.63
	301S Derivative of 205	349.34
	302H Derivative of 205	315.75
	303H Derivative of 205	327.76
	*304B* *302.37*Derivative of 205	

TABLE 3. MNC (M) AND CC_{50} (M) OF THE INITIAL SUBSTANCES TESTED IN MT-2 AND HELAP$_4$

	100	101	103	105	201	205
MT-2	$2,5 \times 10^{-3}$	$2,5 \times 10^{-3}$	$2,5 \times 10^{-4}$	$2,5 \times 10^{-4}$	$1,25 \times 10^{-4}$	$1,25 \times 10^{-4}$

HeLaP$_4$*	***6,3 x10^{-13}***	***1,25 x10^{-10}***	***2,5 x10^{-9}***	***2,5 x10^{-14}***	***1,25 x10^{-7}***	***1,25 x10^{-11}***
HeLaP$_4$**	***6,25 x10^{-7}***	***6,87 x10^{-6}***	***6,87 x10^{-5}***	***1,25 x10^{-5}***	***2,5 x10^{-5}***	***2,5 x10^{-8}***

***MNC **CC$_{50}$**

TABLE 4. MNC (M) OF THE DERIVATIVES OF THE INITIAL SUBSTANCES IN MT-2 CELLS

105H	*301S*	*302H*	*303H*	*304B*
2.5 x 10^{-6}	***2.5 x 10^{-4}***	***2.5 x 10^{-6}***	***2.5 x 10^{-6}***	***2.5 x 10^{-6}***

TABLE 5. IC$_{50}$(MM) AND THE EFFECT OF THE INITIAL SUBSTANCES ON HIV-1 REPLICATION IN MT-2 CELL, MEASURED BY CELL PROTECTION (%) AND INHIBITION OF *RT* ACTIVITY(%) OF SUPERNATANTS

	100	**101**	**103**	**105**	**201**	**205**	**Ref.ABC**
IC$_{50}$	***750***	***1 585***	***0***	***3***	***38***	***12.5***	***5***
% cell protection	***100****	***100****	***0***	***88.99^{+}***	***28.84****	***98****	***100***
Inhibition of *RT* in supernatants	***92.96***	***83.59***	***ND***	***81.70***	***33.91***	***27.13***	***99***

***MNC^{-3} **MNC^{-2} $^{+}$MNC^{-4} ND – not done**

TABLE 6. INHIBITION (%) OF RRT BY INITIAL SQS (IN MNC)

	100	**101**	**103**	**105**	**201**	**205**
Inhibition (%)	**89.90**	***83.94***	***72.36***	***63.05***	***78.70***	***61.47***

TABLE 7. IC$_{50}$(MM) AND THE EFFECT OF THE DERIVATIVES OF THE INITIAL SUBSTANCES (IN MNC) ON HIV-1 REPLICATION IN MT-2 CELL, MEASURED BY CELL PROTECTION (%) AND INHIBITION OF *RT* ACTIVITY (%) OF SUPERNATANTS

	105H	**301S**	**302H**	**303H**	**304B**
IC$_{50}$	***> 0.001***	***0.00225***	***0.0005***	***0.0001***	***0.0005***
% cell Protection	***34.83***	***54.77****	***78.51****	***54.75***	***69.87***
Inhibition of *RT* in supernatants (%)	***0***	***78.31***	***74.89***	***54.43***	***73.21***

***MNC x 10^{-3} **MNC x 10^{-1}**

REFERENCES:

1. **Craigie R.** (2001), J. Biol. Chem., **276,** 23213–23216.
2. **Deprez E.** et al. (2004), Mol Pharmacol, **65**, 85–98.
3. **Harrigan**, **P**. et al. (2000), J. Inf. Dis., 181, 912-920.
4. **Hazuda D.** et al. (2000) Science, **287,** 646–650.
5. **Hoffmann C., Mulcahy F**. (2007), In: HIV Medicine (Hoffmann, Rockstroh, Kamps eds.) - Flying Publisher, 93-105.
6. **Marchand C.** et al. (2003) Mol. Pharmacol., **64**, 600–609.
7. **Marchand C.** et al. (2002) J. Biol Chem, **277,** 12596–12603.
8. **Markowitz M.** et al. (2007), *JAIDS,* **46** (2), 125–33.
9. **Mekouar K.** et al. (1998), J. Med. Chem., **41**, 2846–2857.
10. **Montefiori D.C.** et al. (1988), J. Clin. Microbiol., **26(2**), 231–235.
11. **Shao X.** et al. (1997), Antiviral Chemistry & Chemotherapy, **8 (2),** 149–159.
12. **Zhuang L** et al. (2003), J. Med. Chem., **46**, 453–456.

MORBUS LYELL - THE MOST SEVERE AND DIFFICULT FOR TREATMENT ADVERSE DRUG REACTION

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Lyell disease (toxic epidermal necrolysis) is severe acute skin disorder, described for the first time in 1956 by Alan Lyell. This condition is most often drug induced (NSAID, barbiturates, some antibiotics, etc) and is characterized by generalized erythema, confluent macules with subsequent generalized epidermal sloughing, mucous membrane involvement, persistent fevers, positive Nikolsky sign. Although rare (average incidence of toxic epidermal necrolysis is 0.5-1.4 cases per million populations per year), this condition has bad prognosis - with estimated mortality rate of 10-70%, depending on the quality of care and the rapidity with which treatment is initiated. The pathophysiology of toxic epidermal necrolysis has not yet been fully elucidated; however, various theories have received wide acceptance. Toxic epidermal necrolysis is believed to be an immune-related cytotoxic reaction aimed at destroying keratinocytes that express a foreign antigen.
We present a case of severe toxic epidermal necrolysis in an 18 years old male with favourable outcome. The initial complaints include fever up to 39ºC and headache. Treatment with antipyretics and augmentin was started, but immediately after the first dose of antibiotic, a skin rash appeared, followed by macules and skin sloughing. Detailed medical history revealed data about various allergic reactions until the age of 6 years. Data about the diagnostic procedures, laboratory examinations including immunological tests, clinical course, complications and treatment of the patient are presented, as well as reach photographic illustration of the patient's condition. Special attention is paid to the standard treatment protocol at the Toxicology Clinic, UMHAT "Pirogov" as it is well known that mortality rate is highly dependent on the aggressiveness of the treatment strategy, quality of care and rapidity with which treatment is initiated.

INTRAVENOUS IMMUNOGLOBULIN THERAPY IN REPRODUCTIVE FAILURE

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Intravenous Immunoglobulins (IVIG) are widely used off label in the treatment of reproductive failure. Intravenous Immunoglobulin**s**, first given to recurrent aborters with anti-phospholipid syndrome (APS), have been administered to unexplained aborters since 1986. In APS-associated reproductive loss, there is encouraging Level II and III evidence and a pilot Level I trial. More recently, it has been suggested that IVIG may improve the success rate of in vitro fertilization and embryo transfer (IVF) in patients with prior IVF failures. A rationale for use of IVIG is provided by a review of mechanisms of IVIG action and a two-year own experience of IVIG treatment of women with recurrent miscarriage and implantation failure in IVF.

ONCOPHARMACOLOGICAL EXPERIMENTAL STUDIES – 15 YEARS BULGARIAN-GERMAN COOPERATION

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A chronological overview of the established long lasting research co-operation will be presented. Ether lipid derived alkylphosphocholines (APCs) and their antineoplastic and antiparasitic activity formed the first and main investigational focus. Miltefosine showed moderate activity in terms of increased life expectancy in a mouse model of African trypanosomiasis. The most effective APCs were found to induce apoptosis in leukemic and bladder carcinoma cells. This class of compounds showed no toxicity against normal hematopoietic progenitors and even stimulated their proliferation. APCs showed fewer efficacies against bcr-abl expressing CML-derived cells that could be inverted by gene silencing. APCs were shown to induce Rb expression and fragmentation and to act by membrane lipid raft reorganization. Members of the APC family synergistically interacted with antimetabolites like cytosine arabinoside and gemcitabine. Erufosine was chosen as lead compound because of the lack of hemolytic properties, which rendered it i.v. applicable. This APC was found to activate caspases and to modulate signal transduction. Natural products such as curcumin had additive to synergistic interactions in combinations with bendamustine and idarubicine. Curcumin was found to produce anticlastogenic and signal-transduction inhibiting effects. Preclinical study of the unusual alkylator bendamustine was made, too. Bendamustine was shown to induce apoptosis in lymphoid cells, but was found to be ineffective against myeloid cells. Bendamustine has a substantially lower clastogenicity in comparison to lomustine. With regard to the malignant disease, the investigations were devoted to CML, AML, MM and breast cancer. Modern techniques for gene silencing such as antisense oligonucleotide and siRNA transfection were successfully used. A special method for lipid raft isolation and immunoblot analysis has being implemented in Heidelberg and few years later in Sofia also. The co-operation resulted in more than 10 diploma theses for Bulgarian students who performed their experimental work in Heidelberg and 3 PhD students and two postdocs in pharmacology who worked or are working in Heidelberg using DAAD and Alexander von Humboldt fellowships.

PHARMACOTHERAPY OF NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases are chronic, slowly progressive diseases that cause loss of specific nerve cells. They are a heterogeneous group of diseases as regards to the specific type of involved nerve cells and the predominating clinical symptoms. The common unifying point is the vulnerability of specific nerve cells, the common pathophysiological mechanisms and therapeutic strategies.

The neurodegenerative diseases are classified pathomorphologically according to the dominating protein deposited in the cells. Sinucleinopathies are a group of neurodegenerative proteinopathies with common pathological lesions, expressed as aggregation of modified alpha-sinuclein protein in some populations of neurons and glia. The abnormal filamentous aggregations of modified alpha-sinuclein protein are the main component of Lewy bodies, dystrophic neuritis (of Lewy), and filaments of Papp-Lantos in the olygodendroglia and neurons of patients with multiple system atrophy. They are associated with degeneration of the involved brain regions. Sinucleinopathies include the Lewy body disease, dementia with Lewy bodies, Parkinson's disease, multiple system atrophy, Hallervorden-Spatz' disease, the motoneurone disease and the group of taupathies. Most common and most successfully treated is the Parkinson's disease.

The nowadays treatment of Parkinson's disease is symptomatic, neuroprotective and restorative. The aim of the symptomatic treatment is to restore the dopamine deficit, to improve the everyday activities and the quality of life. The neuroprotective treatment has effect on the pathophysiological mechanisms of the disease and is able to delay the progress of the disease. The restorative treatment uses different surgical methods to improve the functions.

The symptomatic treatment is connected with drugs that increase the synthesis of brain dopamine, stimulate postsynaptic dopamine receptors in the striatum, inhibit the reuptake of dopamine in the presynaptic structures and inhibit some enzymes connected with the dopamine metabolism. There are some data that levodopa may accelerate the progression of the disease. The increased dopamine turnover causes oxidative stress generating free radicals and thus accelerates the progression of the disease. Patients enter in the phase of complicated Parkinson's disease 5 years after the onset of levodopa therapy. That's why the modern treatment demands applying of levodopa spare therapy. This means that treatment should be started with non levodopa drugs that influence parkinsonian symptoms and after they have no more effect, low-doses levodopa are added to the treatment. Some of these drugs (rasagiline, selegiline, amantadine and dopamine agonists) are supposed to have some neuroprotective effects and to delay the progression of the disease.

Starting treatment with neuroprotective drugs from the onset of the disease, when the symptoms are mild is supposed to delay the progression of the disease and patient's disability and to postpone the onset of levodopa treatment. However there are still no proved data that any of the drugs have neuroprotective effect.

The main parkinsonian symptoms – rigidity and bradikinesia are well influence by the dopaminergic treatment, while the effect on tremor is poor. Moreover the disturbances of speech, swallowing, postural stability and freezing phenomena are also not well affected by the therapy. The same is true about the neuropsychological, autonomic and sleep disturbances.

The most important point in the decision for the treatment is the presence of cognitive disturbances. If the patient has no cognitive impairment and the parkinsonian symptoms are mild without disability, it is better to start treatment with rasagiline or selegiline in order to delay the progression of the disease. If the patient has cognitive deficit and parkinsonian symptoms, it is better to start treatment directly with levodopa as other drugs may deteriorate the cognitive impairment. Patients with onset of the disease over 70 years should be treated directly with levodopa, as the side effects from long term levodopa treatment are not pronounced in this age.

The neurologist has often the difficult task how to choose a strategy for the patient – to try to improve the symptoms as well as possible or to thing about the future of the patient and to treat only

partially his symptoms. It is a difficult decision to start mono- or poly- therapy, to start with the most effective levodopa drugs or to postpone levodopa having in mind the long term side effects. Having in mind that Parkinson's disease is a chronic one, and the patient needs treatment for a long period of time, the neurologist should consider the duration of the treatment efficacy and the complications from the treatment. However in the modern treatment there are a lot of possibilities aside from levodopa.

One of the disadvantages of standard levodopa formulations is the short plasma half-life and the consequent fluctuations of the serum concentration. This is the main reason for the movement complications following long term levodopa treatment. This problem is partially solved with the controlled released levodopa formulations (Madopar HBS, Sinemet CR).

The COMT (cathehol-ortho-methyl transferase) inhibitors are used combined with levodopa in order to increase the bioavailability and prolong the clinical efficacy. These drugs block the degradation of levodopa without increasing the maximal plasma concentrations. Entacapone is a potent and selective inhibitor of COMT already used 10 years in the clinical practice. The drug has effect on the parkinsonian symptoms and gives the possibility for decreasing the amount of levodopa with 25%.

Rasagiline is a selective, non reversible MAO-B inhibitor of second generation. It may be used once daily, without titration and has no amphetamine metabolites that deteriorate the cognitive functions. Thus contrary to selegiline it causes no hallucination and dyskinesias. There are some data that the drug has a neuroprotective effect by influence on the oxidative stress and apoptosis and may delay the progression of the disease.

The glutamate antagonists – amantadines are antagonists of N-methyl D-aspartate receptors (NMDA) and thus tend to normalize the proportion of dopamine and glutamate, decreasing the level of glutamate. The glutamatergic transmission between the subthalamic nucleus and internal globus pallidus is inhibited, as well as the excessive activity by the indirect extrapyramidal pathway. Amantadine as a glutamate antagonist is a neuroprotector and delays the progression of the disease.

Dopamine agonists have common properties to stimulate directly the dopamine receptors (D_2 и D_3), as the molecular configuration is similar to that of dopamine. These drugs have neuroprotective properties and do not cause movement complications. The drugs are first choice in the combined treatment of movement complications due to the longer half-life and the lower needed doses of levodopa. Pramipexole is a nonergotamine dopamine agonist, which is very effective for patients with early and late Parkinson's disease. It has a good effect on all parkinsonian symptoms and mainly on the tremor. Rotigotine is the first dopamine agonist applied through a transdermal system. It is a nonergotamine dopamine agonist that activates predominantly the D_2 receptors. The drug has advantages for patients with swallowing difficulties. The plasma drug concentrations are more stable for 24 hours and it is applied only once daily. The plasma concentration of the drug is not dependent from the food and stomach evacuation.

Duodopa is a stable gel suspension of levodopa/carbidopa, applied through duodenal tube using computerized pump. This mode of application stabilizes the drug serum levels, there is not a pulsate stimulation of the dopamine receptors and thus the motor fluctuations disappear.

The antagonists of A2A adenosine receptors are new and perspective drugs for symptomatic treatment of Parkinson's disease. They are xanthine derivates, synthesized from the natural methylxanthines as caffeine and theophylline. They have effect on all parkinsonian symptoms including tremor. It is supposed that the antagonists of adenosine receptors have also neuroprotective properties, as they inhibit the glutamatergic transmission and the excitotoxicity. Blocking the activity of adenosine receptors causes increased activity of the dopamine receptors.

Despite of the numerous drugs that have effect on the parkinsonian symptoms the modern medicine is still not able to suspend the progression of the disease or to prolong considerably the neurodegenerative process that slowly but steadily ends with disability and death of the patients.

Modern Diagnosis and Treatment of Tuberculosis (TB)

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Tuberculosis (TB) is a chronic and immunologically complicated disease. It affects all organs and systems of organism, so having multiform clinical characteristics.
Diagnosis is based on isolation of the causative agent – Mycobacterium Tuberculosis. This is achieved in 80% at adults and in 15% at children.
The modern methods for aetiological diagnosis are based on amplification techniques (PCR, Real-time PCR), BACTEK – TB technologies. Activity of the TB process is assayed by IGRA-tests – TSPOT – TB and QFT-gold and in tube.
Modern tuberculosis treatment is underlined by a combined therapy, determined by the pharmacokinetic parameters of anti-TB medicines. A difference should be made between Latent TB Infection (LTI) and TB disease. Multi-drug resistant (MDR) and extensively drug-resistant (X-DR-TB) patients are a global problem.
TB is a disease, which cannot be eliminated from the list of infectious diseases, but could be under a direct and persisting control.

ADVERSE DRUG REACTIONS: EXPERIMENTAL STUDIES ON METABOLIC–MEDIATED TOXICITY

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The used terminology of this area contains many different terms, which described an Undesired Reaction, Adverse Drug Reaction, Adverse Event, Adverse Effects and Side Effects. The common definition of Adverse Drug Reaction is: a response to a drug, which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or the modification of physiological function. The importance of adverse drug reaction (ADR) is often underestimated. It is very important to understand the role of metabolism of drug toxicity and ADR. Different authors classify these effects in various ways. Along WHO, ADR are classified into 6 types: dose-related (Augmented); non-dose related (Bizarre); dose- and time-related (Chronic); time-related (Delayed); withdraw (End of use); failure of therapy (Failure).

It is very convenient to resolve all drug adverse effects as either reversible (Type A) or irreversible (Type B). Type A ADR are reversible but not related with toxicity. These effects can be caused by intensification of pharmacodynamic effect. These effects, also known generally as "side effects", are mostly an augmentation of the "main effects" and are to be cause of more than 80% of patients, problems with drug therapy. If the drug is interacting reversible, it cannot "damage" a tissue, it has not changed its structure or its functions irreversibly. Reversible ADR can be received during the metabolism of any given drug. It is possible that one or other of metabolites may disrupt cellular function in a way that is unrelated to the pharmacological effect of the drug, but the disruption is reversible and predictable. Type B ADR are irreversible effects. The drug must somehow change cellular structure. This can happen if the drug can form and unstable and reactive toxic metabolite, which can react covalently with the cellular structures. A variety of therapeutic drugs can undergo biotransformation via Phase I and Phase II enzymes to reactive metabolites, which have reactivity toward to proteins and cause potential toxicity.

Factors, predisposing to ADR include: dose, pharmaceutical variation in drug formulation, kinetic and dynamics abnormalities and drug-drug interactions (DDI). DDI occurs when therapeutic agent either alters the concentration (pharmacokinetics interactions) or the biological effect of another agent (pharmacodynamic interactions). Pharmacokinetics DDI can occur at the level of absorption, distribution or clearance of the affected agent. Many drugs are eliminated by metabolism. The microsomal reactions involved cytochrome P450 family of enzymes (CYPs), of which a few are responsible of the majority of metabolic reactions, involving drugs. This includes the isoforms: CYP1A2, 2C9, 2C19 (15%), 2D6 (20%), 3A4 (50%) (Venkatakrishman K et al., 2001). The cytochrome P450 family of heme-monooxygenases comprises the most important group of Phase I enzymes. Many drug interactions are result of inhibition or induction of CYP's enzymes. For that in particular at the level on the liver metabolizing system, DDI can result both in toxicity or loss of efficacy.

Drug metabolism can be a key determinant of drug toxicity. A non-toxic parent drug may be transformated by drug metabolizing enzymes to toxic metabolites (metabolic activation, bioactivation). Conversely a toxic drug may be transformated to non-toxic metabolites (detoxication). A clear understanding of the role of drug metabolism in toxicity can add the identification of risk factors that may potentiate drug toxicity and may provide key information for the development of safe drugs.

Our scientific studies included a group of medicines and perspective bioactive substances (BAS), from natural and synthetic origin, with a certain pharmacological activity and proved or supposed hepatic biotransformation. The compounds have been investigated for cytotoxicity and in some cases for antioxidant and protective effect. These studies were performed in cellular and subcellular models of toxicity, both *in vitro* and *in vivo*. The effects of the examined compounds have been

compared to the effects of referent compounds. Elucidating of these effects might contribute to enrichment of their characteristics, linked to their metabolism and to prevent possible metabolic interactions. These experiments are part of the pre-clinical studies of novel compounds. In many cases they could explain some of the future serious clinical problems, as well as to elucidate the main causes for the failure of certain molecules, as candidate-drug.

The experimental systems, mostly used for investigating drug metabolism, cytotoxicity and DDI, are liver microsomes and isolated hepatocytes. Isolated hepatocytes have been widely employed for studying the biotransformation of chemicals, their cytotoxicity and hepatotoxicity, including their mechanisms. The most important parameters that assessed the functional-metabolic status of the hepatocytes, recommended and implemented by ECVAM are: cell viability; activity of lactatedehydrogenase (LDH) – in cases, when the membrane integrity is affected; level of cell glutathione (GSH) and quantity of malondialdehyde (MDA). The alteration in the cells at different level, as a result of biotransformation and/or bioactivation of the examined compounds, is evaluated by the changes in the level of reduced glutathione – the most important factor of the cell's defence. At the same time, malondialdehyde is determined as a biomarker of the process of lipid peroxidation. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts. Along with these parameters, in our studies, the quantity of cytochrome P 450, the activity of some drug metabolising enzymes, as well as changes in mitochondrial potential, levels of Ca^{2+} and ROS, were measured, under the influence of the some perspective compounds.

In view of the expected protective properties of some of the tested BAS by natural origin, their effects were studied on a appropriate experimental models of toxicity, provoke by different agents - CCl_4, Chlorpromazine, Metoprolol, Paracetamol, t-BuOOH, which possess different toxic mechanisms.

On the basis of a part of our results, we could formulate the following conclusions (Mitcheva M., 2008):

- 14 derivatives of benzimidasole, with a putative hepatic metabolism, have been screened for hepatotoxicity. 4 of them, with antihelmint activity, similar with those of Albendazole and less toxicity, have been selected (Mavrova A. et al., 2005; Mavrova A. et al., 2006).

- 5 bensofenones and Gentisein, isolated from Hypericum annulatum, have been characterized *in vitro*. For 3 of the compunds, a cytoprotective and antioxidant effects, studied in models of cytochrome P 450 mediated toxicity - CCl_4, Chlorpromazine, Metoprolol – were observed (Mitcheva M et al., 2006). These effects were similar with the effects of Silymarin, well known hepatoprotector and antioxidant. The selected compounds have been tested in Chang Liver cells, where they changed the parameters, linked to the mitochondrial function. These results correlate with the studies of Prabhakar BT et al. (2006) that discuss proapoptotic activity of some synthetic analogues of bensofenones.

- Studies of BAS, with known pharmacological activity

Diosgenin, isolated from Asparagus officinalis

Diosgenin is a steroidal saponin, with an established pharmacological activity, mainly antihypercholesterolemic. Using a model of lipid peroxidation (LPO) - enzyme-induced and non-enzyme-induced, our studies proved an antioxidant effect of Diosgenin, similar to the effect of the scavenger Promethazine. We discussed a possible membrane stabilized effect of the compound. On cell level – in isolated rat hepatocytes, in a model of cytochrome P 450-mediated toxicity - CCl_4, Chlorpromazine, Metoprolol, and in a model of oxidative stress - t-BuOOH, Diosgenin exerted cytoprotective effects, similar with the effects of the Silymarin. In Chang liver cells, Diosgenin showed pro-apoptotic effect, however the ROS levels remained unchanged.

In vivo, after multiple administrations, Diosgenin is performed as an inducer, similar to Phenobarbital. The results from the Western blot analysis, showed that Diosgenin caused an expression of CYP 3A, similar to those, caused by Phenobarbital. On the basis of our results, we suggest that the antihypercholesterolemic activity of Diosgenin might be due to its influence on cholesterol metabolism and kinetics in the hepatocyte.

- Studies on some psychoactive compounds, undergo hepatic biotransformation

Besides potential risk of tolerance and dependence development, multiple administration of psychoactive compounds, is associated with neurotoxicity and hepatocellular damage. The majority of the psychoactive substances undergo extensive hepatic biotransformation, mediated by cytochrome P450, to active and reactive metabolites. The metabolism of Morphine, Cocaine and Amphetamine is mediated mainly by CYP3A and CYP2D6 (Sun L&Lau CE, 2001; Projean D et al. 2003; Carvalho F et al., 1996).

Preincubation of the hepatocytes with inhibitor of CYP3A – Amiodaron, and inhibitor of CYP 2B - Chloramphenicol, resulted in reduction of Cocaine hepatotoxicity *in vitro*. These data suggest an involvement of Cocaine's metabolism in its toxicity.

In vitro, Amphetamine showed cytotoxic effect, which was diminished after preincubation with inhibitors of its biotransformation – Quinidine, inhibitor of CYP2D and Amiodarone, inhibitor of CYP3A. On the basis of this study we suggested the involvement of CYP3A in Amphetamine hepatotoxicity (Vitcheva V at al., 2009).

The involvement of CYP3A in metabolism of Morphine, Cocaine and Amphetamine, implies possible metabolic interactions with other substrates, inducers or inhibitors of this isoform that might lead to changes in their metabolism and toxicity.

Ca-channel blockers, such as Nifedipine, have been reported to modulate tolerance and dependence development. At the same time, Nifedipine is known to be a substrate and an inducer of some isophorms of cytochrom P450, includes CYP3A (Drocourt L et al. 2001).

After multiple administrations, Cocaine *in vivo* changed some parameters of drug metabolism and toxicity. In combination with Nifedipine, Cocaine could not exert its own effect on the drug-metabolizing enzymes. Since both Cocaine and Nifedipine are substrates of one and the same isoform CYP3A, these results are probably due to a metabolic interaction between both compounds (Vitcheva V&Mitcheva M, 2007).

Multiple co-administration of Nifedipine and Amphetamine resulted in changes in some parameters of drug metabolism that differ from those, observed after their alone administration. Regarding the metabolic pathways of Amphetamine and Nifedipine, we suggest a metabolic interaction, involved several cytochrome P450 isoforms.

Our results show that Morphine and Nifedipine, per se, increased the activity of drug metabolizing enzyme systems, while their co-administration resulted in reduction of values of the examined parameters (Vitcheva V&Mitcheva M, 2004). Regarding the metabolic pathways, namely N-demethylation, of both compounds, these results might be due to a possible metabolic interaction of the two drugs. At the same time it is important to note that among the rats, which Morphine and Nifedipine were co-administered, an increased toxicity, manifested by respiratory depression, cyanosis and even death (4/7), was observed.

- In vivo interaction on the metabolic level

Interactions of Paracetamol

The hepatotoxic agent Naphthalene undergoes metabolic activation to dyol-epoxides – reactive intermediate metabolites responsible for a toxic stress in the cell. As a result of our study, based on the investigation of Paracetamol and Naphthalene interaction, we found out that in co-administration of Paracetamol and Naphthalene, Paracetamol decreased the toxicity of Naphthalene. This effect is probably due to a competition for one and the same cytochrome P 450 isoforms.

Multiple co-administration of Grapefruit juice and Paracetamol, led to changes in some parameters, connected with drug metabolism, compared to their alone application. These changes correlate with the observed increased plasma level of Paracetamol.

Conclusions: The results obtained, confirmed the necessity to have a knowledge of the possible interactions involved different compounds – substrates and/or inhibitors of one and the same cytochrome P450 isoforms, with a view to prevent the adverse drug-drug interactions, which can have serious clinical consequences, as well as to avoid the discontinuation of needed pharmacotherapy. *In vitro* based experimental system used in combination with *in vivo* animal system, represent the best approach to assess this important drug properties before clinical trials.

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References

1. Carvalho F, Remião F, Amado F, Domingues P, Correia AJ, Bastos ML. D-Amphetamine interaction with glutathione in freshly isolated rat hepatocytes. Chem Res Toxicol, 9(6), 1996: 1031-6.
2. Drocourt L, Pascussi JM, Assenat E, Fabre JM, Maurel P, Vilarem MJ. Calcium channel modulators of the dihydropyridine family are human pregnane X receptor activators and inducers of CYP 3A, CYP 2B and CYP 2C in human hepatocytes. Drug Metab Dispos, 29(10), 2001: 1325-1331.
3. Mavrova A., Anichina K., Vuchev D., Tsenov J., Kondeva M., Micheva M. Synthesis and antitrichinellosis activity of some 2-substituted-[1,3]thiazolo[3,2-a]benzimidazol-3(2H)-ones. Bioorg Med Chem, 13, 2005: 5550-5559.
4. Mavrova A., Anichina K., Vuchev D., Tsenov J., Denkova P., Kondeva M., Micheva M. Antihelminthic activity of some newly synthesized 5(6)-(un)substituted-1H-benzimidazol-2-yl thioacetylpiperazine derivatives. Eur J Med Chem, 41, 2006: 1412-1420.
5. Mitcheva M. Mechanisms of toxicity and possibility for protection. Ed. by Eco Ring Rumena, 2008.
6. Mitcheva M., Kondeva M., Vitcheva V., Nedialkov P., Kitanov K. Effect of benzophenones from *Hypericum annulatum* on carbon tetrachloride-induced toxicity in freshly isolated rat hepatocytes. Redox Report, 11(1), 2006: 1-8.
7. Prabhakar BT., Khanum SA., Jayashree K., Salimath BP., Shashikanth S. Anti-tumor and proapoptotic effect of novel synthetic benzophenone analogues in Ehrich ascites tumor cells. Bioorg Med Chem, 14(2), 2006: 435-446.
8. Projean D., Morin, PE, Tu, TM., Ducharme J. Identification of CYP 3A4 and CYP 2C8 as the major cytochrome P450s responsible for morphine N-demetilation in human liver microsomes. Xenobiotica, 33,,2003: 841-54.
9. Sun L & Lau CE. Simultaneous pharmacokinetic modeling of cocaine and its metabolites, norcocaine and benzoylecgonine, after intravenous and oral administration in rats. Drug Metab Dispos, 29(9), 2001: 1183-1189.
10. Venkatakrishman K., von Moltke L., Greenblatt G. Human drug metabolism and the cytochrome P450 application and relevance of *in vitro* models. J Clin Pharmacol, 41, 2001: 1149-1179.
11. Vitcheva V, Mitcheva M. Effect of nifedipine on behavioral and biochemical parameters in rats after multiple morphine administration. Methods Find Exp Clin Pharmacol**,** 26, 2004: 631-4.
12. Vitcheva V, Mitcheva M. **C**hanges in liver and brain cytochrome P450 after multiple cocaine administration, alone and in combination with nifedipine. Arh Hig Ind Toxicol, 58(3), 2007: 287-91.
13. V. Vitcheva**,** M. Kondeva-Burdina, M. Mitcheva. D-amphetamine toxicity in freshly isolated rat hepatocytes: a possible role of CYP 3A. Arh Hig Ind Toxicol 2009; 60 (2) *in press*.

EFFECTS OF THE HORMONES OF HYPOTHALAMIC-PITUITARY-THYROID AXIS ON TISSUE FACTOR AND TISSUE FACTOR PATHWAY INHIBITOR PLASMA LEVELS IN RATS

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Introduction

Tissue factor (TF) is a critical initiator of the physiologic and pathologic coagulation that binds FVIIa to form TF/ FVIIa complex, which triggers the coagulation protease cascade (1,2). TF originates mainly from vascular endothelium (and all tissues) (3,4). Tissue factor pathway inhibitor (TFPI) is the major physiological inhibitor to TF-mediated coagulation process (5,6,7). TFPI binds to factor Xa and inhibits TF/ FVIIa (8,9,10). TFPI originates mainly from vascular endothelium (8,11).
The **Aim** of the present study was to examine the effects of the hormones of the hypothalamic - pituitary - thyroid axis on plasma levels of the tissue factor and the tissue factor pathway inhibitor.

Material and Methods

The study was carried out on 65 male Wistar rats, weighing 200-220 g. The animals were fed by standard briquette food and received water ad libitum. Procedures involving animals and their care were conducted in conformity with the requirements of the European Convention for the Protection of Experimental Animals (Protection of animals used for experimental purposes, Council Directive 86/609/EEC of November 1986). The animals were divided into five identical groups – one control group (injected with saline) and four experimental groups, injected as follows: the first group was injected by Thyrotropin releasing hormone (TRH), acetate salt (Sigma, minimum 97 %), in a dose of 0.06 mg/kg b.w.; the second group - by Thyroid stimulating hormone (TSH), bovine (in a substance with activity of 2 MU/mg, Sigma), in a dose of 1 MU/kg b.w.; the third and the fourth group, respectively by Liothyroninum (Trijodthyroninum – T_3) and Levothyroxinum (Thyroxin – T_4) (substances produced by VEB Berlin – Chemie, Germany), in a dose of 0.08 mg/kg b.w. each.
The animals were injected s.c. once daily for three consecutive days.
The necessary blood volume was acquired by a cardiac puncture under ether narcosis. Sodium citrate (0.11 mol) was used as anticoagulant (blood/citrate ratio 9:1). The following parameters were determined: tissue factor (TF) in pg/ml, free tissue-factor pathway inhibitor (free TFPI) in ng/ml, tissue-factor pathway inhibitor activity (activity TFPI) in % and prothrombin time (PT) in sec. All parameters examined were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits of Diagnostica Stago (France) and America Diagnostica inc. (USA).
All data obtained have been analyzed by variation analysis using Student-Fisher's t-test.

Results

1. Influence of TRH, TSH, T3, and T4 on TF level (Fig. 1).
TRH decreased TF level by 29.11 % ($p < 0.001$), TSH – by 65.42 % ($p < 0.001$), T_3 – by 25.14 % ($p < 0.001$), and T_4 – by 56.68 % ($p < 0.001$).
2. Influence of TRH, TSH, T3, and T4 on free TFPI level (Fig. 2).
TRH increased free TFPI level by 83.10 % ($p < 0.001$), TSH – by 148.68 % ($p < 0.001$), T_3 – by 64.84 % ($p<0.001$), and T_4 – by 134.00 % ($p< 0.001$).
3. Influence of TRH, TSH, T3, and T4 on activity TFPI level (Fig. 3).
TRH increased activity TFPI level by 37.97 % ($p < 0.001$), TSH – by 64.99 % ($p < 0.001$), T_3 – by 37.41 % ($p<0.001$), and T_4 – by 48.51 % ($p< 0.001$).
4. Influence of TRH, TSH, T3, and T4 on PT (Fig. 4).
TRH increased PT by 94.19 % ($p < 0.001$), TSH – by 192.19 % ($p < 0.001$), T_3 – by 105.95 % ($p<0.001$), and T_4 – by 162.10 % ($p< 0.001$).

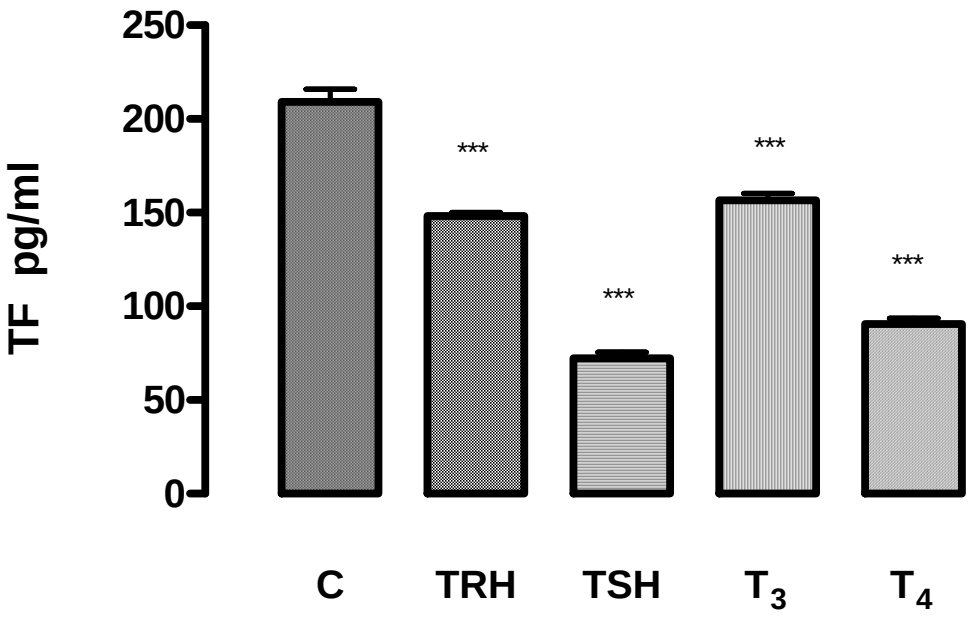

Fig. 1. Effects of TRH (0.06 mg/kg b.w.), TSH (1 MU/kg b.w.), T_3 (0.08 mg/kg b.w.), and T_4 (0.08 mg/kg b.w.), applied s.c. to male Wistar rats once daily for three consecutive days on TF level (pg/ml).
TF - Tissue factor; TRH – thyrotropin-releasing hormone; TSH – thyroid-stimulating hormone; T_3 – Trijodthyroninum, T_4 – Thyroxin, C – control saline-injected rats
***** - $p < 0.001$**

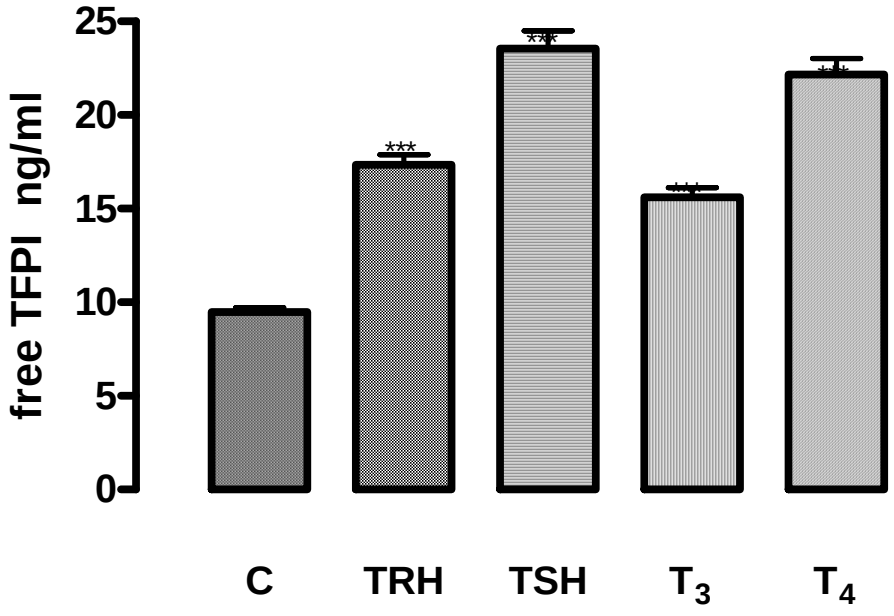

Fig. 2. Effects of TRH (0.06 mg/kg b.w.), TSH (1 MU/kg b.w.), T_3 (0.08 mg/kg b.w.), and T_4 (0.08 mg/kg b.w.), applied s.c. to male Wistar rats once daily for three consecutive days on free TFPI (ng/ml).
free TFPI – free Tissue factor pathway inhibitor; TRH – thyrotropin-releasing hormone; TSH – thyroid-stimulating hormone; T_3 – Trijodthyroninum, T_4 – Thyroxin, C – control group rats, injected with saline
***** - $p < 0.001$**

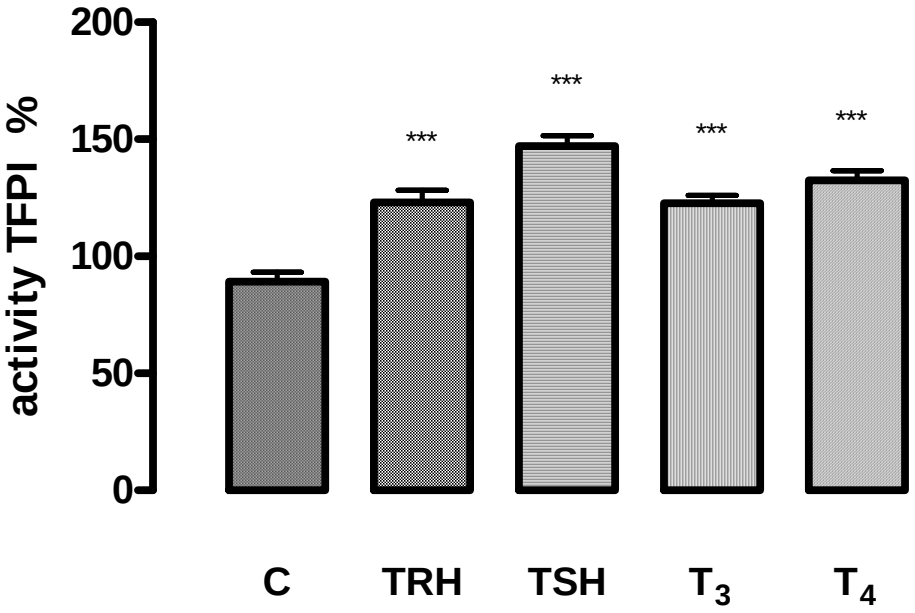

Fig. 3. Effects of TRH (0.06 mg/kg b.w.), TSH (1 MU/kg b.w.), T_3 (0.08 mg/kg b.w.), and T_4 (0.08 mg/kg b.w.), applied s.c. to male Wistar rats once daily for three consecutive days on activity TFPI (ng/ml).
activity TFPI – Tissue factor pathway inhibitor activity; TRH – thyrotropin-releasing hormone; TSH – thyroid-stimulating hormone; T_3 – Trijodthyroninum, T_4 – Thyroxin, C – control group rats, injected with saline
***** - $p < 0.001$.**

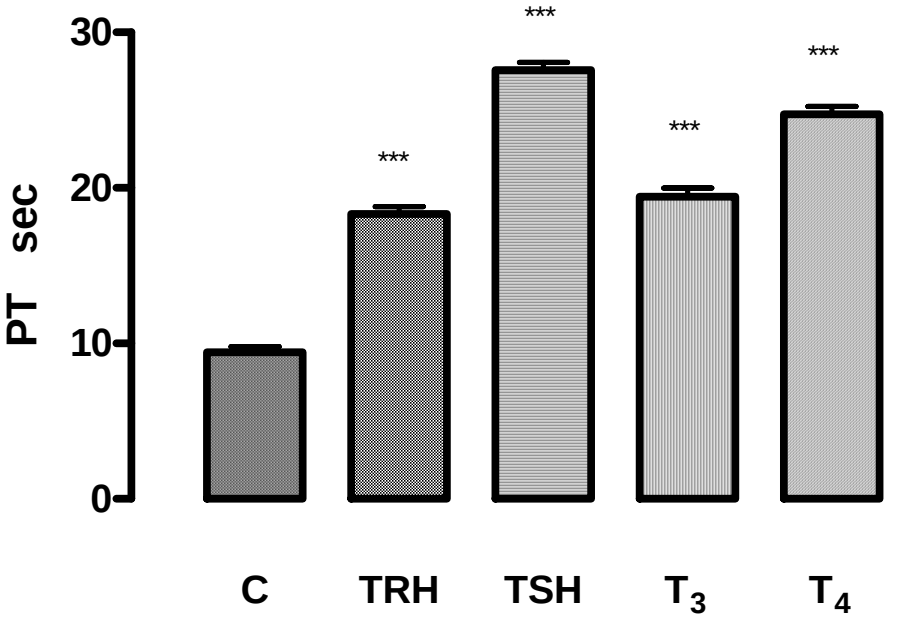

Fig. 4. Effects of TRH (0.06 mg/kg b.w.), TSH (1 MU/kg b.w.), T_3 (0.08 mg/kg b.w.), and T_4 (0.08 mg/kg b.w.), applied s.c. to male Wistar rats once daily for three consecutive days on PT (sec).
PT- prothrombin time; TRH – thyrotropin-releasing hormone; TSH – thyroid-stimulating hormone; T_3 – Trijodthyroninum, T_4 – Thyroxin, C – control group rats, injected with saline
***** - $p < 0.001$.**

Discussion

The analysis of the effect of the hypothalamic-pituitary-thyroid (HPT) axis hormones on plasma level of TF, presented on Fig. 1, shows a significant decrease of TF plasma level under the influence of TRH, TSH, T_3, and T_4 as compared to the control group of rats. As far as TF is known to trigger the extrinsic pathway of coagulation (7), it could be suggested that the hormones used can cause hypocoagulability. It is noticeable that TRH and T_3 similarly decreased TF, while TSH and T4 also showed similar and even more pronounced effects. Taking into consideration that T_4 has a stronger effect compared to T_3, and the fact that TSH evoked the most significant effect on TF level, it is conceivable that TSH exerts its effects both trough the thyroid axis and directly, influencing plasma TF level. The results presented on Fig. 2 and Fig..3 reveal a hypocoagulability, too. TRH, TSH, T_3, and T_4 exerted pronounced unidirectional effect on free TFPI and activity TFPI – parameters, which objectively point to TFPI involvement in hemocoagulation balance. Having in mind that TFPI is the principle inhibitor of TF-initiated coagulation (5) it is reasonable to state that the significant increase of free TFPI (Fig. 2) and activity TFPI (Fig. 3) leads to a misbalance and hindered coagulation without bleeding. Considering the TFPI mechanism of action (6) it might be assumed that the increased levels of free TFPI and activity TFPI are probably of major importance for the observed decrease of TF (Fig. 1). The changes of prothrombin time (Fig. 4), influenced by TRH, TSH, T_3, and T_4, also point to hypocoagulability.

In **conclusion**, the hormones of HPT axis TRH, TSH, T_3, and T_4, produced a pronounced hypocoagilability in rats by significantly reducing TF and increasing the plasma levels of free TFPI and activity TFPI.

References

1. Tilley, R., Mackman, N. TF in hemostasis and thrombosis. Semin.Thromb.Hemost. 2006, 32,5-10.
2. Steffal, J., Luscher, T. F., Tanner, F. C.TF in cardiovascular diseases: molecular mechanisms and clinical implications. Circulation. 2006,113, 722-731.
3. Erez O, Espinoza J, Chaiworapongsa T, Gotsch F, Kusanovic JP, Than NG, Mazaki-Tovi S, Vaisbuch E, Papp Z, Yoon BH, Han YM, Hoppensteadt D, Fareed J, Hassan SS, Romero R. A link between a hemostatic disorder and preterm PROM: a role for tissue factorand tissue factor pathway inhibitor. J Matern Fetal Neonatal Med. 2008, 21,732-744.
4. Morel O, Morel N, Freyssinet JM, Toti F. Platelet microparticles and vascular cells interactions: a checkpoint between the haemostatic and thrombotic responses. Platelets. 2008, 19, 9-23.
5. Al-Mugeiren, M. M., Al-Rasheed, S. A., Al-Salloum, A. A. Tissue factor pathway inhibitor in childhood nephritic syndrome. Pediatr Nephrol. 2006, 21, 771-777.
6. Golino P, Ravera A, Ragni M, Cirillo P, Piro O, Chiariello M. Involvement of tissue factor pathway inhibitor in the coronary circulation of patients with acute coronary syndromes. Circulation. 2003, 108,2864-2869.
7. Hedner U, Ezban M. Tissue factor and factor VIIa as therapeutic targets in disorders of hemostasis. Annu Rev Med. 2008, 59, 29-41.
8. Bajaj MS, Birktoft JJ, Steer SA, Bajaj SP. Structure and biology of tissue factor pathway inhibitor. Thromb Haemost. 2001, 86,959-972.
9. Rosing J, Maurissen LF, Tchaikovski SN, Tans G, Hackeng TM. Protein S is a cofactor for tissue factor pathway inhibitor. Thromb Res. 2008,122,60-63.
10. De Smedt E, Wagenvoord R, Coen Hemker H. The technique of measuring thrombin generation with fluorogenic substrates:The effects of sample dilution. Thromb Haemost. 2009,101,165-170.
11. Kothari H, Kaur G, Sahoo S, Idell S, Rao LV, Pendurthi U. Plasmin enhances cell surface tissue factor activity in mesothelial and endothelial cells. J Thromb Haemost. 2009,7,121-131.

MEDICAL THERAPY IN ACROMEGALY

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Introduction

Acromegaly is a serious endocrinopathy that is associated with considerable morbidity and increased mortality ([1-3]). Although the pituitary tumours associated with acromegaly are usually benign, the elevated levels of growth hormone (GH) and insulin-like growth factor (IGF)-1 lead to a wide range of cardiovascular, respiratory, endocrine and metabolic alterations. Thus, number of co-morbidities are present in patients with acromegaly, including arthropathy, hypertension, sleep apnoea, diabetes, dyslipidaemia, cardiomyopathy, colon polyps, goiter, and headache ([4-7]).
In a recent meta-analysis, the weighted mean of the standardized mortality ratio (SMR) from 16 published studies of patients with acromegaly was 1.72 ([8]). In the more recent studies improved survival is reported, presumably due to modern treatment modalities and strictly defined cure criteria, but nevertheless there was a 32% increased risk for all-cause mortality acromegaly ([8]). Patients with random serum GH levels <2.5 ng/ml after treatment, mostly measured by standard radio-immuno assay (RIA), had mortality close to expected levels (SMR 1.1) compared with a SMR of 1.9 for those with a level >2.5 ng/ml. Similarly, a normal serum IGF-1 level for age and sex was associated with a SMR of 1.1 compared with a SMR of 2.5 for those with elevated IGF-1 levels ([9]).

Treatment goals and approaches

Currently, treatment options in acromegaly include surgical removal of the tumour, medical therapy, and radiation therapy of the pituitary. Each treatment modality has specific advantages and disadvantages but the optimal use of these treatments and their combination should result in a reduction of mortality in the acromegaly patient population to that of general population.
Goals of treatment are to:

- Inhibit GH hypersecretion and normalize IGF-1 levels;
- Control tumour growth and relieve the pressure that the growing pituitary tumour may be exerting on the surrounding brain areas;
- Preserve normal pituitary function or treat hormone deficiencies;
- Improve the symptoms of acromegaly.

Based on the fact that basal GH levels >2.5 ng/ml ([3,10]), elevated IGF-1 ([3,11,12]), age and disease duration ([3, 12]), hypertension (3) diabetes and cardiac diseases are the main determinants of mortality, biochemical goals to control mortality are a GH<2.5 ng/ml or a normal range and sex adjusted IGF-1 levels. Measurement of GH during an oral glucose tolerance test (OGTT) may be preferred to a random GH measurement, and a biochemical control is defined as a nadir of GH<1.0 ng/ml during the OGTT. Successful treatment of GH/IGF-1 hypersecretion is expected to improve co-morbidities in patients with acromegaly to varying degrees. Nevertheless, some may persist even after successful control of acromegaly, and some may improve even if control is not achieved ([13]). So, all co-morbidities should be actively diagnosed and treated irrespective of GH and IGF-1 levels, and therapeutic decisions should be made according to both clinical and biochemical assessment.

Medical therapy

Currently, there are 3 drug classes available for the treatment of acromegaly: *dopamine agonists (DAs)*, *somatostatin analogues* or *somatostatin receptor ligands (SRLs)*, and a *GH receptor antagonist (GHRA)*. After transsphenoidal surgery, SRLs are generally the first line of treatment, followed by GHRA or DAs.

Somatostatin receptor ligands

The SRLs act primarily via somatostatin receptor subtypes 2 and 5 leading to a decrease in adenoma GH secretion. They have a multitude of other endocrine and non-endocrine effects, including inhibition of glucagon, VIP, and gastrointestinal peptides. Periodically GH/IGF-I levels should be monitored to assess response. The use of SRLs is most appropriate ([14]):

- After surgery has failed to achieve biochemical control;

- To provide disease control, or partial control, in the time between administration of radiation therapy and the onset of maximum benefit attained from radiation therapy;
- As first-line therapy when there is a low probability of a surgical cure (for example, large extrasellar tumours with no evidence of central compressive effects);
- Before surgery to improve severe co-morbidities that prevent or could complicate immediate surgery ([15]).

Long-term studies indicate that approximately 70% of patients receiving SRLs have GH levels <2.5 ng/ml and normalized IGF-1 and maximal benefit may be achieved after 10 years of therapy ([16]). However, these studies often include patients pre-selected for GH responsivity. In unselected populations, SRLs reduce GH to <2.5 ng/ml and normalize IGF-1 in 44% and 34% of patients, respectively ([17]). Tumour shrinkage of >20% occurs in approximately 75% of acromegaly patients receiving these drugs (mean 50% reduction in tumour volume) ([18]). These peptide analogues have a proven safety record. Common side effects include altered gastrointestinal motility with a reduction over the first few months of treatment. Multiple small gallstones and gallbladder sludge commonly occur, but rarely cause cholecystitis. Because of alteration in counterregulatory hormones (eg, insulin, glucagon, GH), hypoglycaemia or hyperglycaemia can occur. Bradycardia, cardiac conduction abnormalities, and arrhythmias have been reported. Thus, patients on insulin, oral hypoglycaemias, beta-blockers, and calcium channel blockers may need dosage adjustments. Secondary hypothyroidism can also occur because of parallel inhibition of thyrotropin secretion. Patients should remain on the same dose for 3 months (assuming medication tolerance) to properly assess adequacy of treatment and the need for dose titration.

In well-designed trials, the long-acting formulations appear to be equivalent in the control of symptoms and biochemical markers in patients with acromegaly ([19]).

Octreotide (Sandostatin) - adult dose: initial: 50 µg SC tid; can increase to 500 µg tid; doses of 300-600 µg/d or higher seldom result in additional benefit. Adverse effects include nausea, abdominal pain, diarrhoea, and increased incidence of gallstones and biliary sludge. Caution is needed in renal impairment. It is usually safe in pregnancy but benefits must outweigh the risks (category B).

Octreotide LAR (Sandostatin LAR)

The long-acting somatostatin analogue is administered every 4 weeks (adult dose 10-30 mg i.m. q28d). Similar improvements occur in GH/IGF-I levels compared to octreotide but are associated with fewer adverse effects. Safety for use during pregnancy has not been established (category C).

Lanreotide (Somatuline Depot)

This octapeptide analogue of natural somatostatin elicits high affinity for human somatostatin receptors 2, 3, and 5. Inhibits a variety of endocrine, neuroendocrine, exocrine, and paracrine functions, including basal secretion of motilin, gastric inhibitory peptide, and pancreatic polypeptide. Markedly inhibits meal-induced increases in superior mesenteric artery blood flow and portal venous blood flow. Also significantly decreases prostaglandin E1-stimulated jejunal secretion of water, sodium, potassium, and chloride. Reduces prolactin levels in acromegalic patients, when treated long term. Fetal risk is revealed in studies in animals but is not established or not studied in humans (category C in pregnancy). Lanreotide is indicated for long-term treatment of acromegaly in patients who experience inadequate response to other therapies. Adult dose - 90 mg s.c. q4wk for 3 months initially; dosage range 60-120 mg q4wk. In moderate to severe renal or hepatic impairment: 60 mg s.c. q4wk for 3 months. The dose is adjusted according to GH/IGF-1.

Dopamine agonists

DAs are the only oral medication available for acromegaly. These agents are usually added to SRLs if complete remission has not been achieved. They have modest effects if used as a single agent and are less potent than SRLs in decreasing both GH (42.4% vs 62.8%, p<0.008) and IGF-1 (8% vs. 40.4%, p=0.05) ([21]). Clinical situations where DAs may be useful include (14):

- Preferred oral medication;
- After surgery (very occasionally as first-line therapy) in selected patients, such as those with markedly elevated prolactin and/or modestly elevated GH and IGF-1 levels;
- As additive therapy to SRLs in patients partially responsive to a maximum SRLs dose - about 50% of such patients may achieve control of GH and IGF-1 with combination therapy ([20]).

Bromocriptine (Parlodel)

Acts on central dopamine receptors. It is considered to be more effective in tumours that co-secrete prolactin and the dose used to treat acromegaly is usually much higher than that used for hyperprolactinemia. Initial adult dose is 1.25 mg p.o. and is increased gradually; maintenance dose is 20-30 mg p.o. Adverse effects include nausea, vomiting, headaches, nasal congestion, orthostatic hypotension, and digital vasospasm. Patients tend to develop tolerance to adverse effects. Caution is needed in renal or hepatic disease. Bromocriptine is usually safe in pregnancy but benefits must outweigh the risks (category B).

Cabergoline (Dostinex)

Of the two DAs, the long acting one - cabergoline is considered to be more effective in acromegaly, and this is limited - monotherapy is effective in less than 10% of patients ([22]). The weekly dose is 1.0-2.0 mg. High doses of cabergoline in patients with Parkinson's disease (higher than doses used in acromegaly), and a prolonged duration of therapy, are associated with the development of cardiac valvular abnormalities. Valvular disease has not been found in patients receiving the conventional doses used for pituitary tumours ([23]). Patients receiving higher than conventional doses of cabergoline for prolonged periods of time should be monitored by performing echocardiography.

Growth hormone receptor antagonist

Pegvisomant (Somavert) is a recombinant DNA analog of GH that is structurally altered to act as a GHRA. It selectively binds to GH receptors on cell surfaces, thereby blocking endogenous GH binding. This action interferes with GH signal transduction, resulting in decreased IGF-I, IGF binding protein-3 (IGFBP-3), and acid-labile subunit. The indications for its use are (14):

- In patients that have persistently elevated IGF-1 levels despite maximal therapy with other treatment modalities.
- *Possibly as monotherapy or in combination with a SRLs in other patients.*

Pegvisomant is highly effective in acromegaly and significantly improves the quality of life in patients that require both SRLs and pegvisomant to achieve biochemical control ([24]). Loading adult dose is 40 mg s.c., and maintenance dose is 10 mg s.c.qd initially. It may increase or decrease q4-6wk by 5-mg increments as determined by IGF-I levels. The dose may not exceed 30 mg/d. Safety issues with GHRA include liver function abnormalities and tumour growth. Tumour growth is infrequent (<2%) ([25]) and approximately 25% of patients have liver function abnormalities, but these appear to be transient in most patients without changing the GHRA dose ([26]). Whether the tumour growth is due to the GHRA or merely reflects ongoing tumour growth when there is no therapy directed specifically at the tumour has not been established definitively. Pegvisomant improves insulin sensitivity without affecting insulin secretion and may increase insulin or oral hypoglycemic effect. Pegvisomant treatment decreased fasting insulin, fasting glucose and HbA_{1C} and improved metabolic control in acromegalic patients with and without diabetes (26). Recent publications suggest that GHRA may be useful in combination therapy with a SRL ([27, 28]), but there are no direct comparisons between combination therapy and monotherapy with GHRA. The combination of a SRL and a GHRA may be useful for acromegaly that is resistant to other treatment modalities, for patients who have not achieved biochemical control after surgery, or to improve cost-effectiveness in patients that would otherwise require high-dose GHRA monotherapy.

Conclusion

No single treatment is effective for all patients. Treatment should be individualized, and often combined, depending on patient characteristics such as age, duration of the disease, tumour size, complications and co-morbidities. There are certain areas where more data are needed on the use of medical therapies. No head-to-head studies of the different SRLs of adequate design and power are available to recommend one drug over the other. More data on the potential use of GHRA as a first-line treatment or in combination with SRLs are needed. The relative cost-effectiveness of all medical therapies as monotherapy, or in the various combination options, requires evaluation. In addition to medical therapy for GH/IGF-1 hypersecretion, treatment of co-morbidities has an important impact on quality of life and mortality.

References:

1. Holdaway IM, Rajasoorya C. Epidemiology of acromegaly. *Pituitary* 1998; 2:29-41.

2. Shimatsu A, Yokogoshi Y, Satio S, et al. Long-term survival and cardiovascular complications in patients with acromegaly and pituitary gigantism. *J Endocrinol Invest* 1998; 21: 55-7.
3. Holdaway IM, Rajasoorya C, Gamble GD. Factors influencing mortality in acromegaly. *J Clin Endocrinol Metab* 2004; 89; 667-674.
4. Orbetzova M. Carbohydrate and lipid disturbances in patients with acromegaly. *Endocrinologia* 2000, 2: 13-21.
5. Melmed S. Medical progress: Acromegaly. *N Engl J Med* 2006; 355:2558-73.
6. Colao A, Ferone D, Marzullo P, Lombardi G, et al. Systemic complications of acromegaly: epidemiology, pathogenesis, and management. *Endocr Rev* 2004; 25:102-152.
7. Webb SM, Badia X. Quality of life in growth hormone deficiency and acromegaly. *Endocrinol Metab Clin North Am* 2007; 36:221-232.
8. Dekkers OM, Biermasz NR, Pereira AM, et al. Mortality in acromegaly: a metaanalysis. *J Clin Endocrinol Metab* 2008; 93; 61-7.
9. Holdaway IM, Rajasoorya C, Gamble GD. A meta-analysis of the effect of lowering serum levels of GH and IGF-1 on mortality in acromegaly. *Eur J Endocrinol* 2008; 159: 89-95.
10. Kauppinen-Makelin R, Sane T, Reunanen A, et al. A nationwide survey of mortality in acromegaly. *J Clin Endocrinol Metab* 2005; 90: 4081-6.
11. Swearingen B, Barker FG, Katznelson L, et al. Long-term mortality after transspenoidal surgery and adjunctive therapy for acromegaly. *J Clin Endocrinol Metab* 1998; 83: 3419-26.
12. Biermasz NR, Dekker FW, Pereira AM, et al. Determinants of survival in treated acromegaly in a single center: predictive value of serial IGF-1 measurements. *J Clin Endocrinol Metab* 2004; 89: 2789-96.
13. Maison P, Tropeano AI, Macquin-Mavier I, et al. Impact of somatostatin analogs on the heart in acromegaly : a metaanalysis. *J Clin Endocrinol Metab* 2007; 92: 1743-7
14. Melmed S, Colao A, Barkan A, et al. Guidelines for acromegaly management: an update. *J Clin Endocrinol Metab* 2009; 10.1210/jc.2008-421.
15. Carlsen SM, Lund-Johansen M, Schreiner T, et al.Preoperative octreotide treatment in newly diagnosed acromegalic patients with macroadenomas increases cure short-term postoperative rates: a prospective, randomised trial. *J Clin Endocrinol Metab* 2008;93: 2984-90.
16. Maiza JC, Vezzosi D, Matta M, et al. Long-term (up to 18 ys) effects on GH/IGF-1 hypersecretion and tumor size of primary somatostatin analogue therapy in patients with GH-secreting pituitary adenoma responsive to SSTa. *Clin Endocrinol (Oxf)* 2007; 67:282-9.
17. Mercado M, Borges F, Bouterfa H, et al. A prospective multicenter study to investigate the efficacy, safety and tolerability of octreotide LAR in the primary therapy of patients with acromegaly. *Clin Endocrinol (Oxf)* 2007; 66:859-68.
18. Bevan JS. Clinical review.The antitumoral effects of somatostatin analog therapy in acromegaly. *J Clin Endocrinol Metab* 2005; 90: 1856-63.
19. Murray RD, Melmed S. A critical analysis of clinically available somatostatin analog formulations for therapy of acromegaly. *J Clin Endocrinol Metab* 2008; 93: 2957-68.
20. Selvarajah D, Webster J, Ross R, Newell-Price J. Effectiveness of adding dopamine agonist therapy to long-acting somatostatin analogues in the management of acromegaly. *Eur J Endocrinol* 2005; 152: 569-74.
21. Sherlock M, Fernandez-Rodriguez E, Aragon Alonso A, et al. Medical Therapy in Patients with Acromegaly: Predictors of Response and Comparison of Efficacy of Dopamine Agonists and Somatostatin Analogues. *J Clin Endocrinol Metab* 2009; 94:1255-63.
22. Cozzi R, Attanasio R, Barausse M, et al. Cabergoline in acromegaly: a renewed role for dopamine agonist treatment? *Eur J Endocrinol* 1998; 139: 516-21.
23. Kars M, Delgado V, Holman ER, et al. Aortic valve calcification and mild tricuspid regurgitation but no clinical heart disease after 8 ys of dopamine agonist therapy for prolactinoma. *J Clin Endocrinol Metab* 2008; 93:3348-56.
24. Neggers SJ, van Aken MO, de Herder WW, et al. Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. *J Clin Endocrinol Metab* 2008; 93:3853-9.
25. Frohman LA. Bonert V. Pituitary tumor enlargement in two patients with acromegaly during pegvisomant therapy. *Pituitary* 2007; 10: 283-9.
26. Schreiber I, Buchfelder M, Droste M, et al. Treatment of acromegaly with the GH receptor antagonist pegvisomant in clinical practive: safety and efficacy evaluation from the German Pegvisomant Observational Study. *Eur J Endocrinol* 2007; 156; 75-82.
27. Neggers SJ, van Aken MO, Janssen JA, et al. Long-term efficacy and safety of combined treatment of somatostatin analog and pegvisomant in acromegaly. *J Clin Endocrinol Metab* 2007; 92:4598-601.
28. Feenstra J, de Herder WW ten Have SM, et al. Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. *Lancet* 2005; 365: 1644-6.

THE PLACE OF POLYBACTERIAL IMMUNOSTIMULANTS IN MEDICAL PRACTICE

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Medical practice faces serious problems due to the constantly increasing bacterial poly-resistance to antibiotics, and the side effects of the latter, often including allergic and immunosuppressive reactions. One way to resolve this problem is the application of immunomodulators that increase resistance to bacterial and viral infections by stimulating non-specific immunity mechanisms. In the course of more than 20 years. a number of oral immunomodulators have been developed and investigated in the NCIPD, Sofia: Respivax, Urostim and Dentavax are widely and successfully applied in clinical practice for immunotherapy and immunomodulation. Data about the effects of polybacterial immunomodulators demonstrate a pronounced stimulation of phagocytosis, synthesis of secretory IgA, surfactant, interferon and a number of Th1 type of cytokines. Numerous investigations, including double-blind studies, have proved their efficacy in the prevention and treatment of non-specific respiratory, uro-genital, and periodontal infections as well as in the complex therapy of AIDS. Based on this, polybacterial immunostimulators seem a very promising tool for immunotherapy and immunoprevention, allowing the modulation of immune responses in a most beneficial way.

Key words: polybacterial immunostimulator, immune system, non-specific immunotherapy and immunoprophylaxis, immunostimulation

REFERENCES

1. Dobrev P., Nikolova P., Stankova S. Prevention of respiratory infections with Bulgarian immunostimulator Respivax, Pneumonology and Phthisiatry, 1989, 27:27-31.
7. Iliev I., Radanova V., Georgieva P. Clinical and immunological results after treatment with Respivax of children with different pulmonary diseases, Pediatry, 1990, 29: 55-61.
3. Kojouharova M., Petrunova S., Panova N. The influence of Respivax on the level of endogenous alfa-interferon. Infectology. 1998, 35: 30-32.
8. Kojouharova M., Gacheva N., Vladimirova N. The effect of immunostimolator Respivax on the immunogeneses after Td vaccination. Infectology. 1999, 36: 25-27.
4. Petrunov B., Nenkov P., Cvetanov I. Study the effect of the polybacterial immunostimulator Respivax in the course of immunoprophylaxis and immunotherapy non-specific respiratory infections. JMEI, 1991, 4: 34-39.
6. Stoichkov I. et al. Immunomodulators and cancer. Sofia, Medicina and Phiscultura Ed., 1990.
9. Ahrens J., Bronhovaxom in clinical practice. Scientific Proceedings OM Laboratories. Geneva, 1984, 29-39.
10. Cvetanov J., Petrunov B., Nenkov P., Dragulev B. Immunomorphological changes in the lymphnodes and parenchim organs of mice treated with Respivax. Problems of Infect. Parasitic Dis. 1989**,** 26: 81-89.
11. Ho, D. Long term non-progressors. Proceedings of X Inter. Conference on AIDS, 1994, Yokohama, Japan.
12. Kassabov K., Stoichkov J. Inhibitory effect of the oral preparation Respivax on pulmonary metastasis of Lewis lung carcinoma in mice. C.R. Acad. Bulg. Sci. 1990, 43:117-119.
13. Jossifov J., Bakalova S., Stankova S. et al. Use of Respivax for prophylaxis of bronchopulmonary infections in children. Pneumology. 1989, 9:20-23.
14. Kisyova K., Zlatanov Z., Peneva M. et al. Evaluation of immunostimulants used in pulmologic practice. Inter. Diseases. 1989, 21:85- 90.
15. Klausen B., Evans R., Ramamurthy N., Golub N. et al. Periodontal bone level and gingival proteinase activity in gnotobiotic rats immunized with Bacterioides gingivalis. Oral Microbial. Immunol. 1991, 6: 193-201.

16. Levy, J. Research findings and future prospects in AIDS. Proc. X Inter. Conference on AIDS. 1994, Yokohama, Japan.
17. Maestrony C., Losa G. Polybacterial immunostimulants in respiratory diseases. Scientific Proceedings OM Laboratories, Geneva, 1984, 56-65.
18. Marinova S.,Tchorbadjiiska L. et al. Immunostimulating and protective effects of an oral polybacterial immunomodeulator in rabbit experimental model. Int. J. Immunopharmac. 2000, 22:843-854.
19. Markova R., Marinova S., Petrunov B., Cvetanov J. et al. Stimulating effect of an oral polybacterial immunostimulator on the proliferation activity of guinea pig lymphocytes. Int. J. Immunopharmac. 1997, 19: 205-214.
20. Morikawa K., Oseko F., Morikawa S. Immunomodulatory effect of Fosfomycin on human B-lymphocyte function. Antimicrob. Agents Chemother. 1993, 37:270-275.
21. Page R. Vaccine development for periodontal diseases. Mucosal Immunology Update.1995, 3: 9-11.
22. Persson G., Engel L.,Whiney C. et al. Macaca fascicularis as a model in which to assess the safety and efficacy of a vaccine for periodontitis. Oral Microbiol. Immunol. 1994, 9**:**104-111.
23. Petrunov B., Nenkov P., Marinova S. et al. New per oral immunostimulator for immunotherapy and immunoprophylaxis of non-specific infections in the mouth and parodont. Infectology. 1993, XX: 24-31.
24. Petrunov B., Nenkov P., Wright D., Raines B., Williams I. Evaluation of polybacterial immunostimulant Factor-R on patients with HIV-infection. Probl. Inf. Parasit. Dis. 1996, 23: 25-28.
25. Petrunov B., Nenkov P.The role of immunostimulants in immunotherapy and immunoprophylaxis of newly immerging or intentionally spread / bioterrorism / infectious diseases. In: A.Monov (Ed.). Boiterrorism. Bruxell/NATO, 2004 (in press).
26. Petrunov B. Study the effect of the polybacterial immunostimulant Respivax on some non-specific immune mechanisms. C.R. Acad. Bulg. Sci. 2004, 57: 37-41.

HOMEOPATIC TREATMENT OF ATOPIC DERMATITIS IN THE CHILDHOOD – RETROSPECTIVE SUDY

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Introduction

Atopic dermatitis (AD) is an itching dermatosis with outset in sucklings and early childhood and affects 15% of the children. It is quite often associated with other atopic diseases, such as asthma, allergic rhinitis, allergic conjunctivitis, urticaria and high IgE levels[1, 2, 3]. For the last few years many patients have been looking for relief in homeopathy after series of unsuccessful trials with methods of medical treatment. Very little has been studied the place of homeopathy in the treatment of AD.

The aim of this study is to analyse the therapeutic effect of homeopathy in the treatment of AD in children and teenagers.

Material and methods

A multi-centre, retrospective study of the homeopathic records of 55 children and teenagers with AD aged up to 18 was conducted in the course of 1 year. We analyzed 624 homeopathic prescriptions with symptomatic and terrene drugs for AD.

Criteria for including patients in the investigation:

1/ Age: from 0 to 18 years. 2/ Sex: both boys and girls included. 3/ The diagnose AD was determined in advance by dermatologist. 4/ Presence of concomitant atopic and non-atopic diseases was allowed. 5/ Therapy till the moment – only with local medications: corticosteroids, antibiotics emollients, hydratants etc.

Criteria for excluding patients from the investigation:

1/ Patients not answering the criteria for including. 2/ Presence of congenital anomalies, metabolic and endocrine diseases. 3/ Patients conducting treatment with immunosuppressors – oral or parenteral therapy.

Demographic and clinical indices of the kids included in the investigation before the treatment with homeopathy:

The investigation included 32 (58%) boys and 28 (42%) girls, divided in three age groups: from 0 to 6 years – 28 (51%); from 7 to 12 years – 15 (27%) and from 13 to 18 years – 12 (22%).

Distribution according to the phase of AD: 1 (2%) child in remission, 33 (60%) children with weak manifestation of AD and 21 (38%) kids in fit. The average duration of AD is 4.8±2.3 years (x±SD). Twenty-nine (53%) children were hereditary defective for atopic diseases. Distribution according to the place of residence: 8 (15%) of the kids live along the seaside, 33 (60%) – in the plane far from the sea and 14 (25%) – in mountainous regions. Alongside with AD 20 (36%) of the patients suffered other atopic disease - allergic conjunctivitis, pollen fever, asthma, food allergy etc.

Thirteen (24%) kids were with chronic no allergic disease: adenoid vegetations, frequent catarrhal inflammations, etc. All kids were treated with local conventional medications before they went to homeopathic doctor.

Statistics

The data was processed by a statistical program *SPSS.11* for medical and sociological investigations by ANOVA and alternative analysis.

Results and discussion

I. Changes in the clinical symptoms

A year after of the beginning of treatment with homeopathy the main clinical symptoms were significantly reduced. At the first medical check 29 (53%) of the patients complaint of itching, whereas on the 12th month it was reduced to 8 (15%) kids ($p<0.05$). Thirty-one (60%) of the patients complained of dry skin, and on the 12th month their number was lessened to 13 (24%) kids ($p<0.05$).

A reverse effect of the typical AD skin lesions was observed too. On the 6th month they were registered in 45 (82%) of the patients and on the 12th month they were 23 (44%). Erythema was

observed in 33 (60%) of the patients in the beginning of the investigation and was reduced to 9 (17%) kids in the end of the first year.

II. Changes in the acuteness of AD

As a result of the treatment with homeopathy in the course of 12 months the number of patients in remission increased considerably – 25 (48%) compared to their number in the beginning - 1 (2%), ($p<0.001$). The number of patients in active phase decreased too.

III. Changes in the choice of the therapeutic approach

At the end of the first year 37 (72%) of the patients were treated only with homeopathic drugs, 18 (28%) of them were treated both with homeopathic and allopathic means. The treatment with allopathic means was reduced to 1 (2%) patient. The necessity of local corticosteroids in the course of one year was significantly reduced from 32 (58%) patients to 10 (18%), $p<0.05$. Approximately 3 times was reduced the treatment with anti-histamine medications, emollients, local antibiotics, etc.

IV. Changes in the homeopathic therapy

The average number of the visits in the course of one year was 3.1±0,7. Six hundred twenty-four prescriptions, containing 39 symptomatic and 24 terrene medications were written. Homeopathic dilutions from 9 CH to 30 CH were used. The average number of medications prescribed per patient ware respectively – 2.3 symptomatic and 1.7 terrene medications.

Symptomatic medications

From 415 AD oriented prescriptions with symptomatic medications most often were prescribed: Apis mellifica 70 (16,9%), followed by Lycopodium clavatum 67 (16,1 %) and Histaminum 58 (14,0 %). Natrum muriaticum, Poumon histamine and Arsenicum iodatum were used very often too. Various authors mention the combination of Apis mellifica with Histaminum or with Poumon histamine as quickly suppressing the symptoms of acute AD – oedema, rash and itching, whereas Arsenicum iodatum has a beneficial effect on the dry, husking skin([4, 5]**)**. The present investigation shows that it is necessary to combine the above medications with Lycopodium clavatum or Natrum muriaticum to maintain the good results. Other authors quote these two medications as used very often in the chronic phase of dermatitis([6, 7, 8]).

Apis mellifica was most frequently used in early childhood from 0 to 6 years - 45 (21.4%) prescriptions in comparison to teenagers ($p<0.02$, Tabl. 1.). The high percent of prescriptions with Poumon histamine 10 (23,8%) and Arsenicum album 3 (7,1%) in the seaside regions was probably due to the fact that the patients in those towns suffered often from atopic bronchial asthma as a concomitant disease (Tabl. 1.). There weren't statistically significant differences in the prescribed symptomatic medications for both boys and girls.

Tabl. 1. Significant differences in the number of prescriptions of symptomatic drugs depending on age and place of residence.

Homeo-pathic drugs (dilution from 9CH to 30CH)	Age groups (in years)			Distribution according to the place of residence		
	0-6 * nb.(**%**)	**6-12** ** nb.(**%**)	**12-18** *** nb.(**%**)	The * seaside nb.(**%**)	Far ** from the sea nb.(**%**)	*** Mountainous region nb.(**%**)
Apis mellifica χ^2, p * acc. ** χ^2, p * acc. ***	45 (**21,4**) n.s. 5.1, <0.02	19 (**14,2**)	6 (**8,5**)	n.s.	n.s.	n.s.
Arsenicum alb χ^2, p * acc. ** χ^2, p * acc. ***	n.s.	n.s.	n.s.	3 (**7,1**) 8.8, <0.003 n.s.	1 (**0,4**)	4 (**4,3**)
Poumon hist. χ^2, p * acc. ** χ^2, p * acc. ***	n.s.	n.s.	n.s.	10 (**23,8**) 22.4, <0.001 8.0, <0.005	10 (**3,6**)	5 (**5,4**)
Total	210(**51**)	134(**32**)	71(**17**)	42(**10**)	281(**68**)	92(**22**)
	415 (**100**)			415 (**100**)		

Note: **acc.** – according to**; n.s.** – no statistically significant difference.

Terrene medications

Two hundred and nine prescriptions of terrene medications were prescribed in the course of 1 year most frequently of which were: Lycopodium clavatum 37 (17,7%) prescriptions, Sulfur 28 (13,4%)

and Silicea 23 (11,0%). They were used independently, or in combination with Tuberculinum, Calcarea carbonica or Natrum muriaticum.
Calcarea carbonica was prescribed 6 times more frequently to boys from 0 to 6 years in the seaside regions compared to girls ($p<0.01$, Tabl. 2.). Other two terrene medicines Sulfur and Tuberculinum were prescribed more frequently to sucking children and children aged up to 12 (Tabl. 2.). In the teenage period the number of prescriptions with Natrum muriaticum rose.
J. Jouanny et al.[9] recommend Lycopodium clavatum as a basic terrene medication for urticaria induced by alimentary allergens and renal and hepatic lithiasis. Despite of that this medication plays a leading role in this study both as a symptomatic and terrene choice for the treatment of AD. The results of the investigation show the benefit of the complex therapy in the cases with AD. The therapeutic approach was in correspondence with the therapeutic concepts of AD of other authors ([10, 11, 12]). Other authors recommend a combination of several homeopathic groups – medications specifically used for atopic diseases, on one hand (Apis mellifica, Histaminum), and on the other – medications with affinity to skin lesions and drugs, affecting kidneys and liver (Lycopodium clavatum). The use of Silicea in the overall therapeutic plan is important in case we want to cope with the concomitant inflammatory diseases.

Tabl. 2. Significant differences in the number of prescriptions of terrene drugs depending on sex, age and place of residence.

Homeo-pathic drugs (dilution from 9CH to 30CH)	Sex		Age groups (in years)			Distribution according to the place of residence		
	M * nb.(**%**)	**F** ** nb.(**%**)	**0– 6** * nb.(**%**)	**6– 12** ** nb.(**%**)	**12–18** *** nb.(**%**)	The * seaside nb.(**%**)	Far ** from the sea nb.(**%**)	*** Mountainous region nb.(**%**)
Calc. carb χ^2, p * acc. ** χ^2, p *acc. ***	14(**12,3**) 6.2, <0.01	2(**2,1**)	13(**13,4**) 4.2, <0.05 5.4, <0.05	2(**3,4**)	1(**1,9**)	9(**23,7**) 12.1, <0.001 5.1, <0.02	5(**4**)	2(**4,4**)
Na muriat χ^2, p * acc. ** χ^2, p *acc. ***	n.s.	n.s.	0(**0**) 11.7, <0.001 19.2, <0.001	7 (**11,9**)	8(**15,1**)	n.s.	n.s.	n.s.
Pulsatilla χ^2, p * acc. **	1(**0,9**) 4.3, <0.05	7(**7,4**)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sulfur χ^2, p * acc. ** χ^2, p *acc. ***	n.s.	n.s.	17(**17,5**) n.s 7.9, <0.01	7 (**11,9**)	4(**7,5**)	n.s.	n.s.	n.s.
Tuberculin χ^2, p * acc. ** χ^2, p *acc. ***	n.s.	n.s.	12 (**12,4**) n.s. 4.8, <0.05	5 (**8,5**)	1(**1,9**)	n.s.	n.s.	n.s.
Total	114(**54**)	95(**46**)	97 (**46,4**)	59 (**28**)	53 (**25**)	38 (**18**)	126 (**60**)	45(**22**)
	209 (**100**)		209 (**100**)			209 (**100**)		

Note: **acc.** – according to**; n.s.** – no statistically significant difference.

Conclusion

Homeopathy is a beneficial, effective method in the treatment of AD in children. It is necessary to apply it for a long time if a long-term remission is to be achieved. The best results were observed after a year of treatment. A considerable number of patients achieved full or partial remission of their disease, their physical condition as a whole was improved. The use of corticosteroids and anti-histamine drugs was reduced considerably.

References

1. Sibbald B. Epidemiology of allergic rhinitis. In: ML B, ed. Epidemiology of Clinical Allergy. Monographs in Allergy. Basel, Switzerland: Karger; 1993:61-69.
2. Hurwitz S. Eczematous eruptions in childhood. In: Clinical Pediatric Dermatology: A Textbook of Skin Disorders of Childhood and Adolescence. 2nd ed. Philadelphia, Pa: WB Saunders Co; 1993: 45-60.
3. Beltrani VS. Atopic dermatitis: An update. J Allergy Clin Immunol 1999 Sep; 104(3 Pt 2): S85-6.
4. Demarque D, Jouanny J, Poitevin B, Sen-Jan I. Pharmacologie et Matiere Medicale homéopathique. CEDH France, 2003.

5. Guermonrez M et al. Matiere Medicale Homéopathique. 2 ed. Boiron Lyon, 2000.
6. Boguniewicz M. Advances in the understanding and treatment of atopic dermatitis. Curr Opin Pediatr 1997 Dec; 9(6): 577-81.
7. Hoffjan S, Epplen J. The genetics of atopic dermatitis and recent findings and future options. J Mol Med (2005) 83: 682-692.
8. Boulet J. Homeopathie. L'enfant. Marabout, 2003, 33-41.
9. Jouanny J, Crapanne J, Dancer H, Masson J. Thérapeutique homéopathique. Possibilités en pathologie chronique.BOIRON 2000, 374-412.
10. Halbert AR, Weston WL, Morelli JG. Atopic dermatitis: is it an allergic disease? J Am Acad Dermatol 1995 Dec; 33(6): 1008-18.
11. Chefdeville F, Poncet J.E. Pratique homéopathique en allergologie, Editions du CEDH, 1995, 182-190.
12. Boulet J. Therapeutique homéopathique. Schemas & Protocoles. CEDH, 2007, 33-41.

PHARMACOVIGILANCE IN THE POST-MARKETING PERIOD

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One of the important forms of the pharmacovigilance activities in the post-marketing period is sending of Individual Case Safety Reports (ICSRs) for suspected adverse drug reactions (ADRs) from healthcare professionals and collecting them into a single national database. Speaking about Bulgaria, this is the suspected adverse drug reaction's database within the Bulgarian Drug Agency (BDA). Collecting of data on ADRs was officially started in 1971 and was done by the institutions preceding BDA. By now, the national suspected adverse reaction's database includes about 7 700 cases. In the last years, the annual input of ADR's numbers about 160 reports.

Reports on ADRs from healthcare professionals are validated, assessed for seriousness, expectedness and causal relationship. The drug database is ground for signal generation; the signal is further investigated, evaluated and, as a result of this evaluation, a regulatory change with important clinical impact could be put in to effect.

Since 1974, all ICSRs received in the Bulgarian drug agency have been forwarded to the WHO ADR's Monitoring center in Uppsala, Sweden. After the accession of Bulgaria to the European Union on 1st January 2007, ICSRs are sent also to the European Drug Data Base – EUDRA VIGILANCE, located in the European medicines Agency (EMEA). Eudra Vigilance has started as a pilot project on 01.01.2002 and since November 2007 became mandatory as a single point of collection of ICSRs for all medicinal products, authorized in the countries of the European Union. This database currently numbers more than 1 300 000 cases and it grows monthly by about 10-15 000 reports. Quantitative methods of analyses within Eudra Vigilance are based on the statistical methodology of detection of Signals of Disproportionate reporting (SDRs) and the specific disproportionality measure implemented in Eudra Vigilance Data analyses system is the proportional reporting ratio (PRR). As a consequence of our input for the fulfilment of the Eudra Vigilance, any ICSR sent by healthcare professional from Bulgaria has a much fold-increased chance for contributing in the signal detection process.

Referring to data from EMEA, the most common reasons for withdrawals of Marketing authorizations on the ground of safety concerns are: hepatobilliary disorders (26%); hlood disorders (10%), cardio-vascular disorders (9%); skin disorders (6%), malignancies (6%). Most commonly withdrawn medicinal products for safety reasons belong to the following groups: medicinal products affecting the nervous system (31%); medicinal products acting on the musculoskeletal system (16%); cardio-vascular drugs (15%); analgesics (8%); antidepressants (7%); vasodilatators (6%); antipsychotics (4%); others (13%).

Important changes in safety data that recently have affected therapeutic behaviour are: the discovery of nephrogenic systemic fibrosis and its relationship to the gadolinium containing MRI contrast mediums; genetic predispositions for severe adverse reactions to carbamazepin, abacavir, allopurinol; emerging of suicide ideations under treatment with antidepressants and antiepileptics; myocardial ischemia under treatment with long acting beta agonists; influence of NSAIDs on the cardio-vascular system and skin disorders etc.

In conclusion: Several examples could demonstrate the importance of the pharmacovigilance for the clinical practice. The role of health care professionals in this respect is essential. Regardless of the possibilities given to us by the access to Eudra Vigilance, the number of ICSRs originating from Bulgaria is too low. Means for increasing the adverse reaction reporting activities of healthcare professionals is needed at all levels: medical universities; specialized medical associations; professional unions; health insurance funds; drug regulatory authority.

PHYTOTHERAPY – PAST AND PRESENT
(Review)

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The phytotherapy in its different forms has always been an important part of the culture and the living of the people. Usage of medicine herbs origins from the dawn of the human history and develops through the early civilizations of Assyrio- Babylon, Egypt, Greece, Rome, The Arabs and Renaissance to become part of the present pharmacotherapeutic methods and means. Many of the important names in the medicine like Hippocrates, Dioscorid, Clavdii Galen, Avicenna, Paracels and others have left their substantial part in the phytotherapy, describing and applying medicinal plants in their practice.

It is assumed, that around 300 000 to 500 000 different plant types exist on earth. Almost 10 percent of that is used as food and less- around 5% is studied for the presence of pharmacologically active substances (1). According to different authors, currently from 25 to 30% of all the medications are acquired from plant origin, 55- 60% are synthetically synthesized, and the rest are from microbial (12%), mineral (7%), animal or biotechnological origin. However medicines from plants are extracted from around 0.1% from the known plant types, which shows that there is a vast area for research work in this field. According to information from WHO 75% of the world population depends on medicines produced from plants, due to cultural, economical, and other reasons (3).

Bulgaria is a country with a great variety of plant types, from which 650 are medicinal (6). The pharmacological action of many of them are studied and applied and the chemical composition established (2). Because of the great variety of soils and climate conditions, the Bulgarian medicinal plants contain high percentage biologically active substances, including alkaloids, glycosides, tannins, saponins, polishaharides, flavonoids, essential oils, and may others. The rich experience of the Bulgarian traditional medicine has often been used for the reveling of the full potential of action of the medicinal plants. The treatment of Parkinson disease offered by the healer Ivan Raev in the beginning of the last century, based on the tropan alkaloids in Atropa Belladonna L., has become world known. Also, the background of the Bulgarian traditional medicine gave a clue for the antiholin esterase effect of one of the types of snowdrop (Galantus nivalis L.), which is currently used in treatment of poliomyelitis, neuritis, radiculitis, and in poisoning with curare as the drug Nivalin.

Phytotherapy is a science and method for treatment of different by using of chemically non-treated extracts of plant, known as Galenic preparates. Pure phytochemical components of the plants prepared as a medical products are not included into the term phytotherapeutic medicines (5). This is the reason why these medicines are infusions, teas, decocts or total extracts or plant drugs. In some cases is possible to use the plant parts in their native condition, mechanically treated only.

Plants through the process of photosynthesis from water, CO_2, minerals and solar energy synthesize organic substances (primary and secondary organic compounds), and secure oxygen for existence of other living organisms, including humans. During the phylogenesis, plants have formed some biologically active substances that help them to adapt to the environment and protect from their enemies, like bacteria, viruses, pathogenic fungi and others.

Polysaccharides and proteins are related to the **primary organic compounds** (2). Polysaccharides are macromolecules constructed of monosaccharide units. They are intrinsic for the high plants, algae and lichens. They are divided into homopolysaccharides (cellulose, amylopectin, glycogen, inolin and others) and heteropolysaccharides (pectin, rubbers, mucous substances, starch and others) and are localized mainly in the roots and the seeds of the plants (Radix Altheae, Semen Lini, Radix Glycyrrhiciae, Semen Cydoniae). They are used as softening means in acute or chronic inflammatory diseases of the respiratory and digestive system, as emulsifiers, and binding substances in the

production of drugs and others. Proteins as simple or compound peptides are mainly used for food, and as a source for a certain substances as glutamine acid, methionin and others.

The **secondary natural biologically active compounds** include alkaloids, glycosides, saponins, tannins, iridoids, essential oils, vitamins and others (2). Most of them have well expressed pharmacological action and are used for the treatment of different diseases.

Alkaloids are compounds with alkali reaction, containing nitrogen in its molecule. Most of them have strong action and toxicity and only very small doses are used. They are mostly used as substrates for production of drugs, and rarely as medicinal plants for direct application. The main groups of alkaloids are: derived from the opium poppy (Papaver somniferum) (fenantren- morphine, codeine, tebain; and isochinolinic – papaverin, narcotin and narcein), which have strong analgesic action and are used as spasmolytics; N-cholinolytics (tubocurarin – peripheral myorelaxant of concurent type); non direct adrenomimetics (ephedrine- alkaloid from the plant Ephedra officinalis, with psychostimulating and bronchodilatating action); M-cholinomimetics with direct and indirect action (pilocarpine, galantamin, physiostigmine, muscarin and others); tropan alkaloids (M-cholinolytics - atropine, scopolamine, hyoscyamin); alkaloids with local anesthetic action (cocaine, derivate from the plant Erythroxilon coca); chinoline alkaloids, extracted from the cortex of the quinine tree - quinine, quinidine, etc.

Glycosides are compounds with variable structure and wide spreading in the plants. Depending of the structure of their aglycon part of the molecule they are divided into phenolic glycosides, flavonoid glycosides, cyanogenic glycosides, anthraquinone glycosides, coumarin glycosides and cardiac glycosides. Cardiac glycosides are called cerdenolides (digoxin, digitoxin, convalarotoxin and other). They have cardio stimulating effect in the chronic heart failure. Flavonoid glycosides (flavonic, flavanolic, isoflavonic) are biologically active substances with a wide pharmacological application with antihaemorrhagic, spasmolytic, capillary protective, antioxidant, diuretic, anti-inflammatory, anti microbial and antiviral activity. Rutin, hesperidin, naringin, quercitrin and other are included in this group. Their application is as phytotherapeutic preparations of drugs in early stages of atherosclerosis, frequent haemorrhages, hearth neurosis, dermatitis, vitamin PP insufficiency and other.

Cyanogenic glycosides have a similar activity to the flavonoid ones. They have anti inflammatory activity; reduce the capillary permeability and increase the vision sharpness (Cyanogenic glycosides in the fruits of the blackberry – Vaccinum myrtilus). Anthraquinone glycosides are knows as laxatives for the treatment of chronic constipation. They are contained in the roots of Rheum palmatum L. (Radix Rhei), cortex of glossy buckthorn (Cortex Frangulae), fruits and leaves of senna (Cassia senna) and other. Phenolic glycosides, containing as aglycon phenols or phenolic acids are widely spread amongst the Willow family (Silicacae) and Erica herbacea (Fricacae/Ericacea). In this group are the glycosides arbutin and methylarbutin. They are contained in the leaves of the bearberry (Arctostaphylos uva ursi) and the lingonberry (Vaccinium vitis-idaea) and have strong disinfective action on the urogenital system. Coumarin glycosides are expressed in the Rutaceae and ***Apiaceae/Umbelliferae*** family and have photoprotective, diuretic, spasmolytic and anticoagulant activity.

Tannins are polymeric phenolic compounds, widely distributed in the plant world. Drugs from the cortex of oak (Cortex Querci), leaves from smoke bush (Rhus cotinus), blades of common agrimony (Agremonia eupatoria) are the most often used. Because of containing of katehins, galotannins, galic and elagalic acids, tannins have coagulating effect on the skin and mucosa as well as adstringent, haemostatic, adsorbing ananti-inflammatory activity.

Essential oils contain mono- and hexaterpens, alcohols, aldehides, ketons, acids and phenols. They are typical for the Pinus, Cupressaceae, Lauraceae, ***Apiaceae families and others. They have wide spectrum of pharmacological activities. Oils containing azulen possess antiinflammatory action (Matricaria chamomilla), oil from*** yarrow ***(***Achillea millefolium) and the rest oils from anise - Oleum Anisi (Pimpinella anisum), thyme – Oleum Thymi (Thymus vulgaris), eucalypt oil are known for their action as expectorants. However, essential oils possess diuretic, spasmolytic, antiseptic, improving the bile circulation, antihelminthic, hyperemic and heating action.

Medicinal plants contain other biologically active substances like saponins, iridoids, mucouse substances and other.

Modern phytotherapy has the following options:

- to be applied simultaneously with synthetic medicines in the subacute or chronic phase of the diseases
- it has milder, longer and with less side effects action
- it is cheaper and accessible
- many years of practical experience, shows better therapeutic effect of the total extract of the medicinal plants, compared to the purified extracts and chemically treated plant product
- phytotherapy not only treats, but has a stimulating effect for the defence systems in the body, improves functional condition of the organs and the physiological systems and optimizes the metabolism. Herbs and medicinal plants could be used in many different conditions, but especially in the internal medicine.

A renaissance in the phytotherapy has been observed in the last few decades, nevertheless theachievements of the modern chemistry and application of synthetic drugs. However, scepticism about the rationality and the capabilities of the phytotherapy is present in some of the physicians and medical staff. It is necessary to mention, that herbalism is not very appropriate for some disease, the healing effect is unsure and individual, it is not always possible to supply herbs with the same concentration of biologically active substances, inaccuracy in dosage.

One of the modern forms of the phytotherapy is **food supplements**. They are newly appeared products on the food and pharmaceutical market, with a beginning in the 80^{-th} of the last century. The theoretical base for the appearing, production and application of food supplements are the principals of orthomolecular medicine and valeology -health science. According to the founder of the orthomolecular medicine Linus Pauling its main principle is the "containing of good health and healing of the diseases by altering the concentrations of the substances in the human body, which are normally present and necessary for its good health condition" (4). The concept of the valeology is the same and learns that health is a condition of optimal proportion of the ingredients of the human body.

In about 80% of the **food supplements** medicinal plants have been included as extracts or in natural form. Directive 2002/46/EC of the European Union, food supplements are determined as "*all the essential nutritive substances, that are or not normal ingredients of the foods, which aim to preserve from or correct a demonstrated deficit of one or more nutrition substances in the society or in some specific groups*". It is obvious, that the food supplements, containing only or mainly medicinal plants are out of the meaning of this directive. Our and European current legislation is not precise enough in respect to food supplements containing medicinal plants or other biologically active substances. On some experts opinion they should be separated in a new group- "parapharmaceutical products" with a different regiment of production and application compared to the foods.

The modern pharmacotherapy is too much leaning towards the chemistry. It is necessary a new evaluation of some therapeutic methods to understanding the risks of polypharmacy and drug dependence, which could lead to unwanted results. Phytotherapy is an alternative of the synthetic drugs and more and more people wishing the lead close to nature way of life are choosing it (5).

References:

1. *** Selected medicinal plants. Vol. 1, 2. WHO monographs, Geneva, 2002
2. Asenov, Iv. et al. Pharmacognosy. State Publishing House "Medicina i Fizkultura", Sofia, 1989 (in Bulgarian)
3. Mills, Simon (Ed.) ESCOP monographs. (vol. I-VI) Thieme publishing group, Germany, 2003
4. Pauling, L. The Future of Orthomolecular Medicine. The Orthomolecular Medical Society Conference. May 8, 1983, San Francisco
5. Petkov, V. (Ed.) Modern phytotherapy. State Publishing House "Medicina i Fizkultura", Sofia, 1982 (in Bulgarian)
6. Stoyanov, N. et al. Flora of Bulgaria (vol 1,2), State Publishing House "Nauka i Izkustvo", Sofia, 1966 (in Bulgarian)

REFLEX PATHWAYS CONTROLING RECTO-ANAL EVACUATORY MOTILITY

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Because of the clinical and social impact of diseases in the large intestine (tumours, inflammation, nerve degeneration) the motor activity of the recto-anal part of the gut is a matter of increased interest. The recto-anal evacuatory mechanism is a complex process involving the voluntary control of excretion as well as the myogenic properties of smooth muscles in the recto-anal region and the innervation of the rectum and internal and external anal sphincters.

The main injury in the recto-anal evacuatory process is the anal incontinence which is a status of inability of anal sphincters to restrain the discharge of the rectal content. Anal incontinence affects people of all ages but it is not an obligatory accompanying part of aging. This disorder is common in women than in men because of trauma of the anal muscles and nerves that can occur during childbirth and more in older adults than in younger adults as the muscles that control bowel movement (anal sphincters) weaken. Subjects related to anal incontinence are often beset by feeling of shame, refuse to seek medical help and instead attempt to self-manage the problem which can lead to social withdrawal and isolation. Many people resort to altering their physical and social activities, even their employment, to cope with the problem. Anal incontinence affects about 10 % of the U.S. population. Such effects may be reduced by undergoing prescribed treatment, taking prescribed medicine and making dietary changes, expedient drug treatments and social procedures and by determining the mechanisms underlying the changes in the physiological balance of the recto-anal evacuatory motility[1].

The normal control of bowel movement depends on proper functioning of the smooth muscle layers of the colon and rectum, the muscles surrounding the anal aperture (internal and external anal sphincters), the brain centres of voluntary control and the body nervous system. Obviously, the physiological evacuatory process depends mainly on the properties of colo-recto-anal muscles and on the reflex pathways underlying the motor events.

The colo-recto-anal region is regulated by a dual nerve supply, somatic and autonomic[2]. Mechanographic and electrophysiological observations demonstrate that the motor activity of the mammalian large intestine occurs also in isolated preparations, thus indicating that reflex pathways underlying the motility of the distal part of the gut are contained within the gut wall[3-6].

Despite of the analysis of gut motility has advanced considerable the interaction of activity in longitudinal and circular muscles is not fully understood and is a matter of studying. The functional coordination of the movements, contraction or relaxation, in both layers is not clear. It is believed that the spontaneous smooth muscle activity depends on the functional purposes of the gut region. In the small intestine, the food bolus moves in a step-wise fashion while in the large intestine the content can be transmitted for long distances by giant propagated contractions. The migrating motor complex and spontaneous giant contractions in the distal colon have been attributed to the contractile activity of circular muscle. The circular muscle showed spontaneous activity composed of two types of contractions: low-frequency and high amplitude contractions with superimposed high-frequency and small-amplitude contractions. The coordination of motor activity of the longitudinal and circular muscles in the distal part of the gut and anal sphincters is not clear and the specific role of both muscles in the spontaneous and evoked motor events characterizing the evacuatory motility has not been identified.

We have re-examined colonic and recto-anal motility using a rat large intestine model-preparation consisting of colonic and recto-anal isolated segments mounted in flat partitioned organ bath[7]. In particular, we were interested in evaluating the spontaneous and induced contraction and/or relaxation activity to display functional coordination between the colonic and rectal longitudinal and circular muscles and the anal sphincters.

The smooth muscles of colon, rectum and anal canal produced spontaneous high-amplitude contractions. The colonic contractions appeared synchronously in the longitudinal and circular

muscles suggesting co-activation of nervous pathways supplying both muscles. The contractions arose in the proximal part and propagated to the distal part of the preparation indicating the involvement of descending excitatory pathways. Similar to the colon, the spontaneous activity of rectum demonstrated synchronization of high-amplitude contractions in both muscles, probably due to activation of descending colo-rectal excitatory pathways. The giant migrating contractions of the colon can propagate to the rectum and anal sphincters[8]. The rectal contractions were followed by contractions of anal canal thus demonstrating a descending recto-anal excitatory reflex. It is likely that activation of the rectum can elicit recto-anal neuronal circuitry to organize coordinated descending motor activity in the longitudinal and circular axis.

The application of electrical field stimulation either to the proximal or to the distal part of colonic or recto-anal preparation elicited TTX-sensitive, i.e. neurogenic by nature, motor responses of the stimulated part. The responses were considered as result of local excitation of modular nervous structures[9]. Local response of the longitudinal muscles of colon and rectum was contraction while the circular muscle of colon responded with relaxation followed by contraction. There were no differences between the relaxations of circular muscle in proximal or distal part of colonic preparation. The contractile component of the responses in the circular muscle was considerably less pronounced than that in the longitudinal muscle suggesting predominant excitatory activity of longitudinal muscle in colonic motility.

The local response of the internal anal sphincter was a short-lasting contraction followed by a relaxation while the anal canal responded with contraction thus suggesting that a modular neural circuit subserving the sphincter region controls the sphincter contraction/relaxation mechanism. The contractile responses of longitudinal muscle and the contractile components in the responses of circular muscle increased from colon to rectum while the relaxation responses were relatively uniform indicating a higher contractile potency than relaxation ability in colo-recto-anal tube[10].

Recently we evaluated the ascending motor responses of the longitudinal and circular muscle of rectum and the descending motor responses of anal sphincters as a display of functional characterization of reflex motor pathways subserving recto-anal evacuation[11]. Electrical stimulation applied either to the anal or to the rectal part of the rat recto-anal segment elicited local motor responses of the stimulated part of preparation. At the same time ascending or descending motor responses of the contra-lateral, non-stimulated part were obtained, indicating that locally induced nerve activation propagated via intrinsic ascending or descending pathways. Contractile ascending motor responses of the longitudinal and circular muscle of rectum and contractile descending motor responses of the internal anal sphincter or the anal canal were observed demonstrating that neuronal and neuromuscular communications provided excitatory responses in both oral and anal directions in recto-anal tube. An ano-rectal excitatory reflex which produced rectal contraction upon stimulation of anal stretch receptors has been recently described in human and was suggested to be a second defecation reflex whereas the recto-anal inhibitory reflex was the primary reflex[12]. We found that the ascending contractile motor responses were more pronounced than the descending motor responses when compared to the respective local responses thus suggesting that the nerve activation was expressed more in the ascending than in the descending recto-anal reflex pathways. Our data obtained in large intestine are in accordance with experiments performed in small intestine showing that the contractions of longitudinal and circular muscles induced by electrical or mucosal brush stimulation applied anally to the recording point were higher as compared to the responses to orally applied activation. The finding that the magnitude of the ascending responses of rectal longitudinal and circular muscles and the descending response of anal canal were less expressed as compared to the local responses supported the view that the propagation of excitation in nerve structures declined depending on the distance from the application of stimulation.

Both local and ascending contractile responses were more pronounced in the longitudinal muscle than those in the circular muscle, probably related to the functional purposes. Whereas the colon is considered as a site of faecal storage, there is controversy regarding the function of the rectum as a conduit or as a reservoir. The prevalence of contraction or relaxation events in the rectal motor activity is not clear. Our findings showing a dominant excitatory activity and efficacy of longitudinal muscle in the regional rectal motility suggest an essential role of this muscle layer in the contractile evacuatory mechanisms[13].

The internal anal sphincter responded to locally applied electrical stimulation with an initial contraction followed by a relaxation. The local response of the anal canal was a high-amplitude contraction. Atropine inhibited but not prevented the contractile responses suggesting that excitatory neurotransmissions except for the cholinergic one are involved in the sphincters motor activity. Contractile responses of internal anal sphincter elicited by electrical field stimulation or induced by adrenergic agonists were more recently described in dog, monkey and human and Substance P was proposed as exciting mediator of rat internal anal sphincter.

Surprisingly, a descending relaxation as a component of recto-anal inhibitory reflex was not observed of the internal anal sphincter response in drug-untreated preparations, probably due to the fact that electrical stimulation was applied to nutrient solution filled rectum without solid pellets. It is likely that recto-anal inhibitory reflex, i.e. descending relaxation of the internal anal sphincter could be activated in response to rectal distension when afferent neurons sensitive to mechanical stimuli were involved.

It is believed that the physiological significance of the recto-anal evacuatory activity could be mainly attributed to the autonomic recto-anal inhibitory reflex underlying the functional nature of rectal discrimination. The recto-anal inhibitory reflex consists of distension-evoked rectal reflex contraction and a synchronous internal anal sphincter reflex relaxation showing the importance of the propulsive capacity of the internal anal sphincter.

The contribution of recto-anal neurotransmission to the contractile and/or relaxant activity of internal and external anal sphincters requires further elucidation. According to Bharucha[2] both anal sphincters are responsible for maintenance of the neurogenic recto-anal motility. We failed to find experimental data demonstrating coordination of the rectal autonomic nerve pathways with the motor responses of the anal canal in the presence of preserved anatomical and functional integrity of internal and external anal sphincters. What is why we re-examined reflex evacuatory activity in the recto-anal region using balloon inflation-induced local distension of the rectal wall at a different distance from the anal canal as a display of the topography of descending motor reflex pathways controlling the evacuatory motility of anal sphincters integrity[14].

We observed that the motor response of the anal canal was dependent on the localization of the rectal wall distension, which induced contraction or relaxation when applied far off or nearby the anal canal, respectively. This finding demonstrates locality-dependence of anal sphincters reflex motility. The differences in the pattern of responses suggest that distension-activated mechanoceptors located along the rectal wall communicate with different neurotransmission(s) consecutively acting during the evacuatory process. It could be assumed that physiological topography of the recto-anal descending motor reflex involves topical distribution of longer excitatory cholinergic and non-cholinergic pathways situated along the rectum providing contraction and shorter inhibitory mainly nitric oxide-dependent pathways located predominantly in the anal area underlying relaxation of the anal canal. The balloon inflation used imitates the distension evoked by stools in physiological conditions and it could be suggested that depending on its position along the rectum the rectal content initiates different phases of the recto-anal evacuatory mechanism.

References

1. NIH Medline Plus – Bowel incontinence.
2. Bharucha AE. Neurogastroenterol Motil 2006; 18: 258-260.
3. Grider JR, Makhlouf GM. Am J Physiol Gastrointest Liver Physiol 1986; 51: 40-45.
4. Smith TK, McCarron SL. J. Physiol. 1998; 512: 893-906.
5. Matsufuji H, Yokoyama J. J. Smooth Muscle Res 2003; 39: 11-20.
6. Bian XC, Heffer LF, Gwynne RM, Bornstein JC, Bertrand PP. Am Gastrointest Liver Physiol 2004; 287: 1017-1027.
7. Ivancheva C, Radomirov R. Meth Find Exp Clin Pharmacol 2001; 23: 223-9.
8. Bassotti G, de Roberto G, Castellani D, Sediari L, Morelli A. World J Gastroenterol 2005; 11: 2691-2696.
9. Costa M, Brookes SJ, Hennig GW. Gut 2000; 47 (Suppl 4): 115-119.
10. Brading AF, Ivancheva C, Radomirov R. Meth Find Exp Clin Pharmacol 2008; 30: 201-207.

[11.] Radomirov R, Ivancheva C, Brading AF, Itzev D, Rakovska A, Negrev N. Brain Res Bull 2009; 79: 147-155.
[12.] A. Shafik A, Shafik A, Ahmed I. J. Spinal Cord Med 2003; 26: 380-383.
[13.] Radomirov R, Brading AF, Ivancheva C. Autonomic Autacoid Pharmacol 2006; 26: 67-69.
[14.] Radomirov R, Ivancheva C, Itzev D, Petkova-Kirova P. APS (in press).

BEVACIZUMAB FOR THE TREATMENT OF MACULAR OEDEMA IN PATIENTS WITH DIABETIC RETINOPATHY AND RETINAL VASCULAR OCCLUSIVE DISORDERS

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Introduction

Although of differing aetiologies, both diabetic retinopathy (DR) and retinal vein occlusion (RVO) are associated with visual impairment due to retinal ischemia, macular oedema, non-resolving vitreous hemorrhage, and possibly retinal neovascularization [1, 2]. The ischemia of RVO commonly results from narrowing of arteriovenous crossings and consequent venous compression [2], while in DR retinal vascular leukostasis, leading to capillary blockage and damage to the retinal vasculature, is believed to be a major contributing factor [1]. GRID laser photocoagulation is an-evidence-based therapeutic option to reduce the macular oedema in patients with DR, branch retinal vein occlusion (BRVO) and central retinal vein occlusion (CRVO) [1, 2, 3]. However, over the past 15 years investigations into the underlying molecular and cellular mechanisms of ischemia-related vision loss have clarified the essential role of vascular endothelial growth factor (VEGF) in promoting both macular oedema and retinal neovascularization [4]. VEGF increases retinal permeability, causes breakdown of the blood-retinal barrier and leads to macular oedema. Moreover, VEGF synthesis is unregulated by the hypoxia that accompanies retinal ischemia [5]. As a result, ocular levels of VEGF are significantly increased in patients with RVO [6] and DR [4]. According to these findings, anti-VEGF medications may have a critical role in prevention and/or treatment of macular oedema and iris neovascularization.VEGF-A is a prototype member of the VEGF family, which includes six principal isoforms. Bevacizumab (Avastin, Genentech, San Francisco, CA) is a humanized recombinant antibody that binds to all isoforms of VEGF. Intravitreal Bevacizumab (IVB) has been recently reported as a treatment modality for macular oedema in patients with diabetes and after RVO [6, 7].
In this study, we sought to evaluate the efficacy of IVB in reduction of the macular oedema in patients with DR or RVO.

Patients and methods

In a prospective study were included 107 eyes of 96 patients (55 male and 41 female) with proliferative DR, type 2 diabetes and 31 eyes of 31 patients (13 male and 17 female) with RVO (16 with BRVO, 15 with CRVO) occurred maximum 1 month ago. All of them were with central macular oedema (CME). The mean age of patients with DR was 59.7 years (range, 41-74 years) and 68 years (range, 51-79 years) with RVO respectively. All patients gave their informed consent prior to their inclusion in the study with specific emphasis on the off-label character and possible side effects of Bevacizumab.
Inclusion criteria were: fundoscopically and angiographically diagnosed DR, BRVO or CRVO with CME of more than 250 μm (measured by OCT/SLO), best corrected decimal visual acuity ≤ 0.5, patient able to give informed consent.
Exclusion criteria were: pregnancy, cardiac or apoplexies incidence during the last 3 months, patient note able to give informed consent.

Intravitreal injection of Bevacizumab

Three consecutive injections were performed at 4-weeks intervals. Injections were done under sterile conditions with topical anesthesia. For both group of patients 1.25 mg (0.05 cc) Avastin was injected intravitreally with a 30-gauge needle through the pars plana in inferotemporal quadrant of the eye. All eyes underwent an ophthalmic examination, checking 1 and 7 days after each injection for intraocular inflammation and intraocular pressure rise. Complete ophthalmic examination, optical coherence tomography (OCT) and fluorescein angiography were performed before the first, second and third injections and 4 weeks after the last intervention. We also documented the development of best-corrected visual acuity (BCVA), central macular thickness, and complication rate (i.e. inflammation, endophthalmitis, increased intraocular pressure, progression in lens opacity,

vitreous hemorrhage, retinal tears, retinal detachment, and thromboembolic events). Statistical analysis was performed with SPSS software (Statistical Package for Social Science version 13.0, SPSS). T-test and chi-square test were used.

Results

Diabetic retinopathy

At the baseline examination, mean BCVA was 0.08±0.84 (range 0.05-0.2). BCVA improved to 0.2±0.75 (range 0.08-0.6) at the 4-week follow-up, 0.3 ±0.55 (range (0.09-0.4) at week 8, 0.3±0.85 (range 0.09-0.5) at week 12, and 0.3±0.90 (range (0.1-0.7) at week 16 (Table l).

Table1. Visual and central macular thickness outcomes (mean ± SD) in patients with proliferative DR during follow-up examinations

Time	BCVA (decimal)	CMT (micron)
Baseline	0.08 ± 0.85	482 ± 158
Week 4	0.2 ± 0.75	374 ± 129
Week 8	0.3 ± 0.55	291 ± 114
Week 12	0.3 ± 0.85	263 ± 142
Week 16	0.3 ± 0.90	249 ± 106

BCVA=Best-corrected visual acuity, CMT=Central macular thickness

These BCVA readings were all statistically significant improved compared to the baseline ($p<0.001$). However, BCVA values at week 8 versus week 12 and week 16 did not show statistically significant increase ($p>0.05$). The dynamics of the central retinal thickness changes is presented on the same table (Table1). At the baseline examination, mean CMT was 482±158 μm (range 514-396). In addition, the difference between the follow-up changes in CMT versus the baseline appeared 4 weeks after the first injection and persists until 16 weeks ($p<0.001$). There was a progressive decreased of the CMT with every one injection. CMT at week 8 was statistically significant lower than those at week 4 ($p<0.01$), and CMT at week 8 was significantly lower than those at week 12 ($p<0.01$).

Retinal vein occlusion

In patients with BRVO mean visual acuity increased by more than 3 lines compared to baseline at week 6 ($p<0.001$) and was stable up to 16 weeks. These findings were comparable with the changes in the CME. In contrast, in patients with CRVO the visual acuity fluctuated between 0.03 and 0.04 and remained 0.02 at end of the study. However, CME decreased significantly at week 4 ($p<0.001$) and was relatively stable up to the week 16 (Table 2).

There were no significant side effects of IVB application in both groups of patients during the follow-up period. One eye with DR (0.93%) showed an intraocular pressure rise to 24 mmHg at 1 day after the injection. The elevated intraocular pressure was controlled with an anti-glaucoma drop (Cosopt).

Table 2. Visual and central macular thickness outcomes (mean ± SD) in patients with BRVO and CRVO during follow-up examinations

Time	**BRVO**		**CRVO**	
	BCVA(decimal)	CMT(micron)	BCVA(decimal)	CMT(micron)
Baseline	0.09±0.45	407±126	0.02±0.85	617±214
Week 4	0.3±0.65	301±147	0.03±0.65	318±117
Week 8	0.4±0.35	254±102	0.04±0.45	294±128
Week 12	0.4±0.75	242±94	0.03±0.25	278±142
Week 16	0.4±0.55	248±106	0.02±0.75	306±114

BCVA=Best-corrected visual acuity, CMT=Central macular thickness, BRVO=Branch retinal vein occlusion, CRVO=Central retinal vein occlusion

Discussion

With bevacizumab a new treatment option has been introduced for early intervention against the formation of CME [1, 6]. Considering the severity of CME in patients with proliferative DR and RVO and in the attempt to maximize the potential effect of bevacizumab, we decided to perform three consecutive injections as a loading dose in our treatment strategy. In the group of patients with DR, the treatment effect was achieved at week 8 and remained relatively stable to the end of the study [Table 1]. Our results are comparable with those of Haritoglu et al. [8]. In diabetes, VEFG is present in high concentrations and produced continuously. Is there a problem with attacking macular oedema with a relatively short-acting anti-VEGF agent? There was reported that primary IVB at doses of 1.25 to 2.5 mg seems to provide stability or improvement in visual acuity and CMT in eyes with diabetic macular oedema at 6 months [1].

Although the exact pathological sequence of RVO is unknown, visual acuity seems to be only depending on macular ischemia, but mainly on CME and photoreceptor damage in the early period of the disease. Both groups of patients with BRVO and CRVO with low as well as with high baseline CME benefited from bevacizumab injections. There was reported that the lowest mean level of CMT was achieved at week 12 [9]. The evaluation of functional results showed differences in the positive impact of early treatment on visual acuity [9]. In our study, most of the patients with BRVO improved the visual acuity significantly at week 8 after the second injection in comparison with baseline [Table 2]. The functional results remain stable to the end of the study. In the group of patients with CRVO mean CME decreased significantly, but despite the gut anatomical results, the visual acuity did not improve. It can be assume that the degrees of ischemia as well as other individual factors have an impact on treatment response.

In summary, an IVB injection seems to stimulate the reduction of the CME in patients with DR and retinal vascular occlusive disorders within the first 8 to 12 weeks. In general, vision improved or stabilized in patents with DR and BRVO. In contrast, no significant functional improvement was observed in patients with CRVO. These results are encouraging and merit further long-term investigation in larger scale studies.

Key words: Central macular oedema (CME), Visual acuity (VA), Bevacizumab (Avastin), Diabetic retinopathy (DR), Branch retinal vein occlusion (BRVO), Central retinal vein occlusion (CRVO)

References

1. Guardiola AA, Busquets MA. Diabetic macular oedema: treatment updates 2008. Retina today 2008, 3:38-41
2. Heyreh SS. Venous occlusive disease: management 25 years ago. Retina 2006; 26(6 Suppl):51-62
3. The Central Vein Occlusion Study Group. Evaluation of grid pattern photocoagulation for macular oedema in central vein occlusion .Ophthalmology 1995; 102:1425-1433
4. Nguyen QD, Tatlipinar S, Shah SM et al. Vascular endothelial growth factor is a critical stimulus for diabetic macular oedema. Am J Ophthalmol 2006; 142:961-969
5. Aiello LP, Northrup JM, Key BA et al. Hypoxic regulation of vascular endothelial growth factor in retinal cells. Arch Ophthalmol 1995; 113:1538-1544
6. Noma H, Minamoto A, Funatsu H et al. Intravitreal levels of vascular endothelial growth factor and interleukin-6 are correlated with macular oedema in branch retinal vein occlusion. Graefes Arch Clin Exp Ophthalmol 2006; 244:309-315
7. Ahmadieh H, Ramezani A, Shoeibi N et al. Intravitreal bevacizumab with or without triamcinolone for refractory diabetic macular oedema: a placebo controlled, randomized clinical trial. Graefes Arch Clin Exp Ophthalmol 2008; 246:483-489
8. Haritoglu C, Kook D, Neubauer A et al. Intravitreal bevaciuzumab (Avastin) therapy for persistent diffuse diabetic macular oedema. Retina 2006; 26:999-1005
9. Stahl A, Agostini H, Hansen L, Feltgen N. Bevacizumab in retinal vein occlusion-results of prospective case series. Graefes Arch Clin Exp Ophthalmol 2007; 245:1429-1436

INNATE IMMUNITY AND IMMUNOREGULATORY MECHANISMS OF POLYBACTERIAL IMMUNOMODULATORS

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Innate immunity is extremely important as an immediate defense mechanism, but today it's clear it also takes the central stage in activation and regulation of the adaptive immune response. Cellular elements of natural resistance are key participants in this process.

Respivax (BulBio-NCIPD Ltd.) is an oral polybacterial immunomodulator intended for treatment and prevention of non-specific respiratory tract infections (NSRTI). We studied for the first time its effects on the inductive mechanisms of innate immunity, in the course of 3 consecutive cycles of treatment (one tablet (50mg) daily for 20 days, followed by a 10-days pause) in 25 patients with NSRTI by means of flow cytometry.

Our **results** show that chronification of respiratory infections is connected with decreased expression on phagocytes of: CD11b receptor responsible for the transendothelial migration, TLR2 for the gram (+) bacteria recognition, the complex TLR4/CD14 on monocytes, decreased oxidative burst level in Gr in weak stimulation response.

Respivax increased the effectiveness of phagocytes as it restored the ability for: endothelial adhesion and transmigration (increasing the expression of CD62L and CD11b), recognition of Gram (+) and Gram (-) bacteria with high sensitivity. The preparation restored the oxidative processes in response to low-dose stimulations. It increased the resistance to viral infections as: increased the percentage and the resistance of NK cells and the relative share of plasmocytoid dendritic cells. NSRTI are characterized with deficiency of the antigen-presenting (AP) functions of the immune system: decreased expression of HLA-DR and CD86 co-stimulator on monocytes, decreased percentage of precursors of dendritic cells (CD14+16+), disturbance of the maturation of dendritic cells (lower ratio CD86low/CD86hi).

Respivax restored the AP potential of the immune system as it stimulated: phagocytosys, AP capacity of circulating monocytes, differentiation and AP ability of dendritic cells and decreased the portion of the inhibitory regulatory CD4+CD25hiFoxP3+ population.

NSRTI are connected with prevalence of second type cytokines (decreased ratio IFN-g / IL-4) and very low values of IL-10 regulatory cytokine. Respivax restored the flexibility of the immune system as it modulated the extreme deviations in cytokine levels and potentated Th1 immune responses without over stimulation of the pro inflammatory mechanisms.

We conclude that Respivax treatment restores the inductive function of innate immunity at three key levels: antigen recognition and presentation, co-stimulation of naïve T cells, and Th1/Th2 balance. This results, at least in part, from a differential modulation effect on the expression of different pathogen-recognition receptors (TLRs).

MANAGEMENT OF ACUTE GASTROENTERITIS IN CHILDREN

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Diarrhoeal disease is a leading cause of illness and death in children worldwide. Many of the deaths are caused by dehydration resulting from loss of water and electrolytes due to intestinal malabsorption or increased secretion.
We discuss the recommendations given on the indications of drug therapy in infant and child acute diarrhoea, based upon the current knowledge on their effectiveness and tolerance.
Replacement of the water and electrolyte losses by oral
re-hydration solutions is the mainstay of therapy for children with watery diarrhoea. Research during the last 10 years has identified novel accessory secretory and pro-absorptive pathways that may prove to be useful targets for the pharmacological control of secretory diarrhoea. It is hoped that novel antisecretory drugs will complement re-hydration interventions by decreasing the severity of the illness and at the same time reducing re-hydration fluid requirement. The rational use of probiotics derived from *Saccharomyces boulardii* and *Lactobacillus* GG in the treatment of acute diarrhoea is discussed.

RIFAMPICIN IN THE TREATMENT OF PSORIASIS

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Background: The efficacy of traditional systemic therapies for psoriasis is limited by various side effects toxicity, drug-drug interactions, and the need for frequent laboratory monitoring. In animal models, rifampicin causes immunosuppression and in conventional doses is suppresses the T-cell functions.

Materials and methods: A total of 76 patients (34 men and 42 women) aged between 12 and 68 years) with eruptive psoriasis were enrolled in the study. They were divided into two groups. *Objective:* To show that rifampicin has a therapeutic effect in eruptive psoriasis and to try to explain its mode of action, according to the evidence of a concomitant streptococcal infection. Rifampicin was administered orally in a 600 mg daily dosage for at least 60 days. Only emollients were given for topical therapy.

Results: A statistical (chi-squared test) analysis was done and it could be concluded that improvement in the two groups was statistically indistinguishable ($p=0.892$), while comparison with the control group showed a significant difference ($p=0.00082$).

Conclusion: The results express that there is no statistically significant difference between the treating groups and the effect of rifampicin could not be related only to its antimicrobial properties. Its therapeutic effect most probably is due to its immunosuppressive properties.

PHARMACOLOGICAL MANAGEMENT OF PHEOCHROMOCYTOMA

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Pheochromocyomas are rare neuroendocrine tumours, arising from the chromaffin cells of the adrenal medulla and due to the hypersecretion of catecholamines are associated with high cardiovascular morbidity and mortality if left untreated. Currently the only definitive therapy is the surgical resection of the tumour. However, surgery in itself carries a very high risk of evoking a massive release of catecholamines into the circulation, resulting in one or more of serious cardiovascular complications including fatal hypertensive crisis and cardiac arrhythmias. Moreover, postoperatively, the sudden drop in catecholamine levels may result in hypotension and hypoglycaemia. To prevent these problems, patients with pheochromocytoma must undergo pharmacologic blockade of catecholamine synthesis and effects before surgery [(1)]. The introduction of pharmacological pretreatment in the 1950s reduced the perioperative mortality rate from as high as 45% to < 2% [(2-4)].

The main goal of preoperative management of a pheochromocytoma patient is to normalize blood pressure, heart rate and function of other organs; restore volume depletion and prevent a patient from surgery-induced catecholamine storm and its consequences on the cardiovascular system [(5)]. Several drugs have been recommended for this purpose, including selective and non-selective α- and β-adrenoceptor antagonists, calcium channel blockers and drugs that inhibit catecholamine synthesis.

The two most commonly used α-adrenergic antagonists are phenoxybenzamine and doxazosin [(6)]. Phenoxybenzamine is a non-selective, non-competitive α-adrenergic antagonist with a plasma half-life of 24 hours. Starting dosages of 20–40 mg daily are titrated depending on patient response. Non-selective α-adrenergic blockade can result in reflex tachycardia, for which the addition of a β-adrenergic blocker is often required for symptomatic relief from tachycardia or tachyarrhythmias. Selective postsynaptic α_1-receptor antagonists, such as prazosin and doxazosin, have been used to escape from some of the side effects of phenoxybenzamine. Since these drugs leave the presynaptic receptors on the neuronal surface open, they do not produce reflex tachycardia. They also have a shorter duration of action, permitting more rapid adjustment of dosage and a reduced duration of postoperative hypotension. The calcium channel blockers are useful in patients who are normotensive but have paroxysmal episodes of hypertension, because they are less likely to cause significant orthostatic hypotension. α-Methyl-para-tyrosine competitively inhibits tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis [(7)]. Treatment with metyrosine reduces tumour stores of catecholamines, decreases the need for intraoperative medication to control blood pressure, lowers intraoperative fluid requirements, and attenuates blood loss [(8)]. Based on the fact that pheochromocytoma is accompanied by reduced intravascular volume, it is common to increase sodium intake both orally and intravenously simultaneously with the antihypertensive therapy.

In conclusion, the pharmacological treatment of pheochromocytoma has to meet three basic goals: 1) to oppose any catecholamine associated medical problems; 2) to treat hypertension and tachyarrhythmias and 3) to restore intravascular volume. The introduction into practice of the modern treatment methods has lead to significant increase of cure rates and reduction of complications during the last 50 years.

Once the diagnosis of a pheochromocytoma is made, appropriate preoperative medical management is necessary to reduce the risk for perioperative complications. There are currently no randomized prospective clinical trials to establish the optimal preoperative pharmacological management of pheochromocytoma and it is doubtful whether these will ever be performed in view of the low prevalence of this disorder. Therefore, the best available evidence is derived from retrospective studies, patient series and case reports. As a result, there is no clear consensus regarding the drug of choice. The most commonly used drugs are selective and non-selective α-blockers, β-blockers, calcium channel blockers and inhibitors of catecholamine synthesis [(9)].

Non-selective α-antagonists

Since the early 1950s, phenoxybenzamine has been widely used as the main drug for preoperative management of a pheochromocytoma. It is a non-competitive α_1- and α_2-antagonist, with a maximal effect four to six hours after administration and a pharmacological half-life of 24 hours. A regular starting dose is 10 mg twice daily, which can be increased to a daily dose of 80 to 100 mg/day. As the correct dose is approached, paroxysmal hypertensive episodes are brought under control, and when the right dose is achieved, the patient becomes normotensive or mildly hypotensive. Disadvantages of phenoxybenzamine are the occurrence of reflex tachycardia and excessive orthostatic hypotension. Reflex tachycardia is caused by blockade of α_2-receptors localized in the presynaptic membrane. Stimulation of the α_2-receptor inhibits norepinephrine release. Therefore, α_2-receptor blockade will interrupt this negative feedback mechanism thus resulting in increased occurrence of tachyarrhythmias. Other disadvantages of phenoxybenzamine are central sedation and prolonged duration of action. Continuing α-receptor antagonism in combination with the postoperative decrease in catecholamine levels can result in prolonged hypotension after surgery [10]. Furthermore, it should be mentioned that compared with α_1-adrenoceptor blockers, phenoxybenzamine is an expensive drug.

Selective α_1-antagonists

Currently used selective α_1-adrenoceptor blocking agents are prazosin and doxazosin [5,11]. They are specific, competitive and short-acting α_1-adrenergic antagonists. Doxazosin has a half life of 16 to 30 hours and can be given in a single dose varying between 1 to 8 mg. Prazosin has a much shorter half-life of 2 to 3 hours and needs to be ingested three to four times daily. Prazosin is administered in doses of 2 to 5 mg three times a day and doxazosin is given in doses of 2 to 8 mg per day. However, they have the potential for severe postural hypotension immediately after the first dose, especially prazosin, and therefore should be given just as the patient is ready to go to bed. Because of a short half-life of α_1-adrenergic antagonists, these drugs should also be given in the morning before surgery. In some centres, phenoxybenzamine is used first and then replaced by α_1-adrenergic antagonists before surgery to reduce postoperative hypotension [12].

β-blockers

β-Adrenergic blockers should never be used alone and should be commenced only after adequate pretreatment with α-adrenergic blockade, because unopposed β-adrenergic receptor stimulation can induce a catastrophic hypertensive crisis (4,5,13). The main purpose of their administration is to prevent occurrence of catecholamine- or α-blockers – induced tachyarrhythmias[5,10].

Cardioselective β_1-blockers are preferred such as atenolol, given in doses of 12.5 to 25 mg two or three times a day, and metoprolol in doses 25–50 mg three to four times a day [5]). Caution is warranted when administering β-antagonists to patients with severe left ventricular dysfunction, a condition, which is not uncommon with a pheochromocytoma due to cardiomyopathy induced by chronic exposure to high catecholamine levels [14].

Calcium channel blockers

Calcium channel blockers reduce arterial blood pressure by inhibiting the norepinephrine-mediated transmembrane calcium influx in vascular smooth muscle. They are used as an add-on therapy to α-blockers in patients with poor blood pressure control or when there are severe side effects with the α-antagonists [5]. These drugs do not produce hypotension and, therefore, may be used safely in patients who are normotensive but have occasional episodes of paroxysmal hypertension [9].

Amlodipine is given in a dose from 10–20 mg, and nicardipine in a dose from 60–90 mg per day. Nifedipine is given in a dose from 30–90 mg and verapamil in a dose from 180–480 mg per day.

Inhibitors of catecholamine synthesis

α-Methyl-L-tyrosine or metyrosine is an analogue of tyrosine that competitively inhibits tyrosine hydroxylase, which catalyzes the conversion of tyrosine to dihydroxyphenylalanine, the first step of catecholamine synthesis [15]. It significantly but not completely depletes catecholamine stores with maximum effect after about 3 d of treatment. Metyrosine facilitates blood pressure control both before and during surgery, especially during the induction of anaesthesia and surgical manipulation of the tumour when extensive sympathetic activation or catecholamine release occurs [16]. Treatment is started at a dose of 250 mg orally every 8 to 12 h and, thereafter, the dose is increased by 250 to 500 mg every 2 to 3 days or as necessary up to a total dose of 1.5 to 2.0 g per day [5]. It is centrally

acting and therefore can result in sedation, anxiety, psychic disturbance, and extrapyramidal side Patients may also experience severe diarrhea necessitating treatment with anti-diarrhoeal agents [9]. Metyrosine and α-adrenoceptor blockers when used together result in less labile blood pressure during anesthesia and reduced intraoperative blood loss, and reduced volume replacement during surgery compared with the use of α-adrenoceptor blockers alone [5,16,17].
Currently, there is no consensus as to when to start adrenergic blockade while preparing a patient for surgery. In most medical centers, adrenergic blockade usually starts 7–14 days preoperatively to have adequate time to normalize blood pressure and heart rate and to expand the contracted blood volume [5,18,19]. Volume expansion should be optimized preoperatively by the administration of i.v. saline or colloid, because this minimizes the blood pressure fluctuations that can occur intraoperatively with the administration of antihypertensive agents.
In spite the lack of large randomized, prospective, and controlled studies evidence from retrospective studies, suggest that α-adrenoceptor blockade is currently the preferred choice in preoperative management of pheochromocytoma patients (10, 12). Calcium channel blockers and β-adrenoceptor blockers are most often used as co-drugs when blood pressure cannot be well controlled while on α-adrenoceptor blockade or if tachyarrhythmia occurs. At some medical institutions, metyrosine is given to all patients, and at others only to those patients who have highly active tumours associated with difficult-to-treat symptoms and signs of catecholamine excess.

References

1. **Walther MM, Keiser HR, Linehan WM**. Pheochromocytoma: evaluation, diagnosis, and treatment. World J Urol. 1999;17:35-9. [PMID: 0010096149]
2. **Apgar V, Papper EM**. Pheochromocytoma. Anesthetic management during surgical treatment. AMA Arch Surg 1951;62:634–648.
3. **Plouin PF, Duclos JM, Soppelsa F et al**. Factors associated with perioperative morbidity and mortality in patients with pheochromocytoma: Analysis of 165 operations at a single center. J Clin Endocrinol Metab 2001; 86:1480 –1486.
4. **Kinney MA, Narr BJ, Warner MA**. Perioperative management of pheochromocytoma. J Cardiothorac Vasc Anesth 2002;16:359 –369.
5. **Pacak K**. Approach to the patient Preoperative management of the pheochromocytoma patient J Clin Endocrinol Metab 2007 92(11):4069–4079
6. **Lenders JW, Eisenhofer G, Mannelli M et al.** Phaeochromocytoma. Lancet 005; 366:665–675.
7. **Pullerits J, Ein S, Balfe JW.** Anaesthesia for phaeochromocytoma. Can J Anaesth. 1988;35:526-34.
8. **Perry RR, Keiser HR, Norton JA, Wall RT, Robertson CN, Travis W, et al**. Surgical management of pheochromocytoma with the use of metyrosine. Ann Surg. 1990;212:621-8.
9. **J. T. Adler, G. Y. Meyer-Rochow, H. Chen, D. E. Benn, B. G. Robinson, R. S. Sippel, and S. B. Sidhu**; Pheochromocytoma: Current Approaches and Future Directions. Oncologist, July 1, 2008; 13(7): 779 – 793
10. **A.N.A. van der Horst-Schrivers, M.N. Kerstens, B.H.R. Wolffenbuttel.** Preoperative pharmacological management of phaeochromocytoma. Neth J Med - 01-SEP-2006; 64(8): 290-5
11. **Nicholson Jr JP, Vaughn Jr ED, Pickering TG, Resnick LM, Artusio J, Kleinert HD, Lopez-Overjero JA, Laragh JH** 1983 Pheochromocytoma and prazosin. Ann Intern Med 99:477–479
12. **Malchoff CD, MacGillivray D, Shichman S** 2004 Pheochromocytoma treatment. In: Mansoor GA, ed. Secondary hypertension. Totowa, NJ: Humana Press; 235–249
13. **Mannelli M**. Management and treatment of pheochromocytomas and paragangliomas. Ann N Y Acad Sci 2006;1073:405– 416.
14. **Prys-Roberts C**. Phaeochromocytoma--recent progress in its management Br J Anaesth 2000;85:44-57.
15. **Brogden RN, Heel RC, Speight TM, Avery GS** 1981 α-Methyl-p-tyrosine: a review of its pharmacology and clinical use. Drugs 21:81–89

16. **Steinsapir J, Carr AA, Prisant LM, Bransome Jr ED** 1997 Metyrosine and pheochromocytoma. Arch Intern Med 157:901–906
17**. Perry R, Keiser H, Norton J, Wall R, Robertson C, Travis W, Pass H, Walther M, LinehanW**1990 Surgical management of pheochromocytoma with the use of metyrosine. Ann Surg 212:621–628
18. **Williams DT, Dann S, Wheeler MH** 2003 Phaeochromocytoma–views on current management. Eur J Surg Oncol 29:483–490
19. **Eigelberger MS, Duh QY** 2001 Pheochromocytoma. Curr Treat Options Oncol 2:321–329

CANCER PAIN: MECHANISMS BASED PERSONALIZED ANALGESIA

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The International Association for Study of Pain (IASP) has launched Global Year Against Cancer Pain which runs from October 2008 through October 2009. Efforts are centred to educate health-care providers, governments and the public on the pain and suffering experienced by people with cancer (IASP Newsletter (№4), December 2008). More than 10 million people are diagnosed with cancer each year and it is estimated that by 2020 this would increase to 15 million new cases each year (Stewart & Kleihues, World Cancer Report, 2003). Cancer-associated pain can be present at any time during the course of the disease, but the frequency and intensity of cancer pain tend to increase with advancing stages of disease. Between 75 and 90% of patients with metastatic or advanced-stage cancer will experience significant cancer-induced pain (Meuser et al., 2001). Despite the increasing prevalence of cancer, improvement in the detection and treatment (aggressive chemotherapy) of most types of cancer has resulted in a significant increase in survival rates (Eduards et al., 2005). Given the increasing life span of cancer patients, new mechanism-based therapies need to be developed to reduce cancer-related pain.

Until recently the management of cancer pain has been largely empirical (Mantyh, 2006a). The World Health Organisation (WHO) has promoted the three-step analgesic ladder as a framework for the rational use of analgesics in the treatment of cancer pain (Cancer Pain Relief.WHO, Geneva; 1986, 1996). Step I specifies the use of non-opioid analgesics (paracetamol, non-steroidal anti-inflammatory drugs) for the treatment of mild pain; step II recommends weak opioids (codeine, tramadol, etc) with or without non-opioids, for moderate pain; and step III comprises strong opioids with or without non-opioids for strong pain. If needed adjuvant drugs can be used at each step. Despite the general conclusion that the WHO method has been of enormous benefit for the treatment of cancer pain worldwide (Eisenberg et al., 2005) it has been shown that the rate of pain relief in patients with advanced cancer is as low as 50% (Murakawa et al., 2007). Although there are various reasons for this poor improvement of the rate of pain relief, the leading factors are inadequate (relatively low) doses of opioids used, the mechanisms of cancer pain and the genetic determinants of the patients (Riley et al., 2007). Both medical doctors and patients are concerned about the adverse effects of opioid and non-opioid analgesics chronically used. In the case of opioids major concerns are development of tolerance to analgesic activity and withdrawal reaction (physical dependence). With the development of tolerance the dose of opioid is increased in order to achieve analgesia which increases the risks of toxic/adverse (vomiting, constipation, somnolence, pruritus, myoclonus, delirium, blurred vision,etc.) effects from opioids. Long-term side effects are abnormal pain sensitivity, hypogonadism (testosterone or estrogen replacement needed) and immunosuppression. Increased pain sensitivity is observed not only during opioid withdrawal, but new evidence suggests that increased pain sensitivity can also occur during opioid administration. Interestingly, the cellular mechanisms of opioid-induced hyperalgesia have much in common with those of neuropathic pain and opioid tolerance, including glutamatergic mechanisms (Mao, J., 2008). Our results have suggested that NMDA antagonists or/and opioid rotation/switching might be useful to alleviate opioid-induced hyperalgesia (Vlaskovska et al., 1997).

Recently a five-step pain and side effect ladder is proposed (Riley et al., 2007). The proposed 4th step involves "opioid switching" and includes both pain and side effects as criteria for switching analgesics. Interestingly 3 out of 4 factors predicting need to switch to other opioid are adverse effects of analgesics used. Finally, if switching opioids fails, it is proposed that the 5th and final step of WHO analgesic ladder should involve anaesthetic intervention.

Different new formulations of opioids are introduced to cope breakthrough pain in cancer patients. Breakthrough pain is a transient flare-up pain superimposed on an otherwise stable pain pattern in patients treated with opioids. It is normally severe in inensity with a rapid onset and variable

duration. Breakthrough pain is considered a negative prognostic factor (Mercadante, S., 2006). Patients with untreated breakthrough pain have greater levels of anxiety and depression and are less satisfied with their opioid therapy. Use of antidepressants and anxiolytics might be helpful.

In order to optimize analgesic efficacy, to lower adverse effects and to optimize dosing, emphasis shifts to clinical genomics, gender/sex and psychosocial issues (Bernardes et al,. 2008), which play significant role in analgesic and adverse effects of opioids, non-opioid analgesics (NSAID) and psychotropic drugs (anticonvulsants, antidepressants, benzodiazepines) which are used to treat cancer pain. Experimental and clinical data showed that expression polymorphism in CYP2D6 (codeine, tramadol, 5-HT3 antagonists, tricyclic antidepressants, etc.), CYP2C19 (diazepam, omeprazole) and CYP2C9 (NSAID, warfarin) could result in lack or decreased analgesic activity, sedation or increased risk of gastrointestinal bleeding (Gardiner & Begg, 2006). The A118G polymorphism of the μ-opioid receptor has been shown to change the analgesic potency of morphine and morphine-6 glucorunide. The analgesic and toxic doses of opioids differ significantly in patients with polymorphism of the enzyme COMT and melanocortine receptors (MC1R) (Reyes-Gibby et al., 2007). It should be also stressed that experimental, clinical and epidemiological studies have shown that women experience and report experiencing more pain than men. Women suffer more from migraine, rheumatoid arthritis and visceral pains from non-sex specific organs that share at least part of their central sensory projection with the reproductive area like irritable bowel syndrome, pain from the urinary system etc. The analgesic effect of k-agonists is much stronger in women with two inactive variance of MC1R allele. Sex steroid hormones influence not only the sensitivity of peripheral sensory neurons but also development of central sensitization or pain memory, stress, anxiety and motivation (Nashar et al., 2006). The role of sex steroid hormones is very important in cancer pain and analgesia, since hormone sensitive tumours are often treated with drugs affecting the levels of estradiol/testosterone.

The primary goal of preclinical and clinical studies is to provide insight into the mechanisms that drive or mask cancer pain, the mechanisms by which anti-neoplastic agents induce peripheral neuropathy, as well as to study the specific mechanisms of visceral and bone (cancer) pain, the influence of sexual hormones and genetic variants on different types of pain and on the effects of analgesics used. Considering most of the factors mentioned, medical doctors would be able to apply new mechanism-based analgesic therapies for alleviation of cancer pain.

Cancer pain can arise from different processes, either by direct tumour infiltration/involvement, as a result of diagnostic or therapeutic surgical procedures (such as biopsies and resection), or as a side effect of toxicity related to therapies used to treat cancer (chemotherapy and radiation therapy). The pathophysiology of cancer pain comprises of various types of pain: nociceptive/inflammatory hyperalgesia (tumour infiltration, surgical procedures, inflammation etc), neuropathic pain (traumatic and toxic destructions of sensory nerves), visceral pain (colorectal or hollow tube sack organs distension) and psychogenic (disease progression and toxic effects of the tumour and anticancer drugs).

Development of experimental models of cancer pain, revealed that tumour cells produce large amounts of PGs, endothelins, ATP, activating $P2X_3$ purinoceptors as well as bradykinin, interleukins, epidermal growth factor, transforming growth factor etc. (Mantyh, P., 2006b), which activate specific receptors/nociceptors. It is well known that PGs potentiate more than 40 fold the nociceptive and pro-inflammatory effects of the mentioned mediators. Several tumour cells and tumour-associated macrophages express high levels of COX2, which produce PGs involved not only in pain and inflammation, but also in tumour cell growth and metastasis, as they inhibit prostacyclin and angiogenesis. Therefore, COX2 inhibitors (celecoxib, etoricoxib, valdecoxib, parecoxib, lumiracoxib) might be successfully used not only as analgesics in bone and inflammatory pain, but also to suppress tumour growth and metastases. However, the same mechanism of action causes risks from thrombosis, which is a serious adverse effect of coxibes.

Tumour cells become ischemic and undergo apoptosis as the tumour burden exceeds its vascular supply, which results in local acidosis. The two major classes of acid-sensing channels expressed by nociceptors are TRPV1 and ASIC-3 and transmit pain due to low pH and heat. Tumour-induced release of protons and acidosis may be particularly important in the generation of bone cancer pain. Recent work has shown that osteoprotegerin and bisphosphonate, both of which are known to

induce osteoclast apoptosis, are effective in decreasing osteoclast-induced bone cancer pain. TRPV1 and ASIC antagonists (AZD1386) are in phase II clinical trial and may be used to reduce bone cancer pain by blocking acid-sensitive channels. Interestingly the metabolite of paracetamol N-acetylphenolamine interacts with TRPV1 receptors, which might be connected to its analgesic activity.

Another very important aspect of cancer pain is the mechanism of peripheral neuropaty induced by anticancer drugs and mechanisms of central sensitization due to tumour growth and destruction of the sensory nerves. Data are accumulating for the specific changes in DRG by paclitaxel and other anticancer drugs, which lead to typical neuropathic pain. In the cases of neuropathic changes, a better analgesic effect could be observed with pregabalin and other anticonvulsants that specifically block the overexpressed Na^+ or Ca^{2+} channels in sensory ganglia.

Tumour cells release high concentrations of ATP, which is a major sensory transmitter. We showed that purinergic transmission is involved in various mechanisms/types of cancer pain (visceral, inflammatory, neuropathic). Nociceptors are densely supplied with $P2X_3$ and $P2X_{2/3}$ receptors activated by ATP, which is released from the cells due to damage, inflammation, hypoxia or distension/constriction. ATP can also be released from sympathetic nerves, endothelia and tumour cells. In the spinal cord the receptors are localized presynaptically, which activation by ATP released upon the incoming peripheral noxious stimulation facilitates glutamate release or postsynaptically secondary sensory neurons, which evoked excitation. Spinal interneurons releasing ATP as a fast transmitter co-secrete the inhibitory transmitter GABA and vice versa - GABA releasing interneurons co-secrete ATP, which suggests that spinal GABAergic interneurons could be source of synaptic ATP. $P2X_3$ receptors contribute to heat induced pain as they are co-localized with vanilloid $TPVR_1$ receptors.

Acute pain: It is known that exogenous administration of P2X agonists evokes acute nocifensive responses. However, the release of ATP and activation of $P2X_3$ and $P2X_{2/3}$ receptors play a secondary role in the physiological nociception where other algogenic substances are more important for the induction of acute pain. It is plausible to speculate that $P2X_3$ receptors contribute substantially to the acute pain induced by tissue damage, which is often the case in cancer pain.

Inflammatory pain: The sensitivity of P2X receptors augments in the presence of inflammatory mediators (PGE2, bradykinin, substance P, histamine, 5-HT) and/or tissue acidosis and vice versa the inflammation is intensified by ATP.

Visceral pain: The submucous plexuses of most visceral organs contain $P2X_3$ and $P2X_{2/3}$ positive afferents. During distension of the wall of tube or sack viscera ATP is secreted from epithelial cells and diffusing in the vicinity may activate the purinergic receptors of low threshold intrinsic sensory nerves thus contributing to peristaltic reflexes. If excessive distension occurs higher amount of ATP is released from epithelial cells, which activates $P2X_3$ and $P2X_{2/3}$ receptors of high-threshold extrinsic sensory afferents thus exciting the pain related nerve structures (Vlaskovska et al., 2001). Interestingly Metamizole (analgin) was found to suppress sensory nerve firing upon distension of urinary bladder. These data showed that part of analgesic activity of Metamizol in visceral pain due to distension (tumour, gall bladder stones, etc) might be due to interference with purinergic receptors.

Neuropathic pain: $P2X_3$ and $P2X_{2/3}$ receptors up-regulation, resulting in hypersensitivity at the site of nerve injury, is a likely component of the multi-etiological pathogenic mechanisms of neuropathic pain. Another purinergic component of neuropathic pain pathogenesis could be the co-release of ATP from postganglionic sympathetic neurons of dorsal root ganglia.

Pharmacological basis for analgesic drug development: The ubiquity of purinergic transmission and the major role of P2X purinoceptors in nociception necessitate the development of selective P2X antagonists. The invention of potent, non-toxic $P2X_3$ antagonists would pave the way for development of new analgesic drugs. However, this mission is not jet completed. Advancement has been made recently with the introduction of 2, 3'-O-(2', 4' 6')-trinitrophenyl-ATP (TNP-ATP) and pyridoxal-phosphate-6-azophenyl-2', 4'-disulphonate (PPADS) as specific antagonists of $P2X_3$ and $P2X_1$ receptors and 8, 8'-[carbonyl-bis-(imino-3, 1-phenylene-carbonylimino)-bis-(1, 3, 5-naphthalenetrisulfonic acid)] (NF023) as specific antagonist of $P2X_1$ receptors. Together with nicotinic acetylcholinergic and glutamatergic receptor superfamilies P2X purinergic receptors are

the third class of ligand-gated ion channels. Local anesthetics, dizolcipine, phencyclidine, d-tubocurarine and other ion channel blockers could be useful for design of specific P2X antagonists with therapeutic potential.

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REFERENCES

1. Bernandes, S., et al. Bridging the gap between pain and gender research: A selective literature review. Eur. J. Pain, 12, 427-440, 2008.
2. Eduards B., et al. Annual report to the nation on the status of cancer, 1975-2002, featuring population-based trends in cancer treatment. J. Natl. Cancer Inst., 97, 1407-1427, 2005
3. Eisenberg, E. et al. Time to modify the WHO Analgesic Ladder? Pain.Clinical Updates, Vol. XIII, № 5, 2005.
4. Gardiner, S., Begg, E. Pharmacogenetics, Drug-Metabolizing Enzymes, and Clinical Practice. Pharmacol. Rev. 58,521 -590, 2006.
5. Mantyh, P. Cancer pain and its impact on diagnosis, survival and quality of life. Nature Rev. Neurosci., 7, 797-809, 2006a.
6. Mantyh, P. Cancer pain: causes, consequences and therapeutic opportunities. In: Wall and Melzack's Textbook of Pain. Eds.: S. B. McMahon, M. Koltzenburg, pp. 1087-1098, Elsevier, NY, 2006b
7. Mao, J. Opioid-induced hyperalgesia. Pain.Clinical Updates. Vol.XVI (2), 2008.
8. Mercadante, S. Breakthrough pain in cancerpPatients. Pain. Clinical Updates, Vol XIV (1), 2006.
9. Meuser, T., et al. Symptoms during cancer pain treatment following WHO-guidelines: a longitudinal follow-up study of symptom prevalence, severity and etiology. Pain, 93, 247-257, 2001.
10. Murakawa, K., et al. Cancer pain management modalities and positioning of oxycodone. Eur. J. Pain, Suppl.1, 48-52, 2007.
11. Nashar, M., et al. Sex hormone modulation of pain and analgesia. Eur. J. Pain, 10, Suppl. 1, 88-89, 2006.
12. Reyes-Gibby, C., et al.. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. Pain,130, 25-30, 2007.
13. Riley, J., et al. Proposed 5-step World Health Organization analgesic and side effect ladder. Eur. J. Pain., Suppl.1, 23-30, 2007
14. Stewart B., Kleihues, P. World Cancer Report. International Agency for Research on Cancer, Lyon, 2003.
15. Vlaskovska, M**.,** et al. Opioid effects on $^{45}Ca^{2+}$ uptake and glutamate release in rat cerebral cortex in primary culture. J. Neurochem., 68, 517-524, 1997
16. Vlaskovska, M., et al. P2X3 knockout mice reveal a major sensory role for urothelial released ATP. J. Neurosci., 21, 5670-5677, 2001.

ADVERSE DRUG REACTIONS AND PERSONALIZED MEDICINE

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Introduction: Adverse drug reactions (ADR) can be defined as, "an unwanted or harmful reaction experienced following administration of a drug, or combination of drugs, *under normal conditions of use* and is suspected as being related to the drug (or combination)".
In general they can be divide into two main groups: type A (predictable, linked to the main pharmacological effect) and type B (unpredictable, idiosyncratic, non-connected with the main pharmacological effect (**Table 1**).

Table 1. Classification of ADR

	Type A	**Type B**
Pharmacology	**Qualitatively normal** Quantitatively abnormal	Qualitatively abnormal
Predictability	Yes (usually)	No (usually)
Drug-dependence	Yes	No (usually)
Frequency	Common (usually)	Rare (usually)
Seriousness	No (usually)	Yes (often)

We can further divide ADR in **type C** (associated with long-term use, involves dose accumulation; example: phenacetin and interstitial nephritis); **type D** (delayed effects like carcinogenicity, teratogenicity, dose independent; example: fetal hydantoin syndrome); **type E** (withdrawal reactions); **type F** (failure of therapy).
The current classification is defined only by properties of the drug – its known pharmacology and the dose dependence of its effects. A modern view of development of ADR include also properties of the reaction (the time course of its appearance and its severity) and properties of the individual (the genetic, pathological, and other biological differences that confer susceptibility). That is the three dimensional classification system based on dose relatedness, timing, and patient susceptibility (**DoTS**) proposed by J.K.Aronson and R.E.Ferner (BMJ, 2003; 327; 1222-1225).
Adverse drug reactions (ADRs) often arise because of the formation of metabolic intermediates. Our knowledge of the drug metabolizing systems showed that they have a high level of genetic variation. When those variations are present in individuals taking more than one drug the chance of having an adverse drug reaction is greatly increased.
In this presentation, the mechanisms of some adverse drug reactions are described based on different geno-and phenotype of drug metabolizing systems and pharmacological targets (enzymes and receptors).
Discussion: In general, we can divide drugs in two groups, depending of the way by which the reactive metabolites induced ADRs – those of which possessed intrinsic toxicity and those induced metabolic idiosyncrasy. The first group induced impairment of cellular metabolism and calcium homeostasis; oxidative stress and lipid peroxidation; bind covalently to different cellular macromolecules; developed cell apoptosis and necrosis.
Many factors, including genetic, environmental, in uterus exposure, life style, diet, drugs and eating habits leave a "metabolic signature" and contribute to a "metabolic fingerprint map" unique for each individual organism. This fact could explain why after the same drug dose two different persons react different, with development of ADR or some times of drug failure.
I. Genetic polymorphism, drug metabolism and ADR (type A)

From the top 27 drugs frequently cited in ADR reports 59% (16/27) metabolized by at least one enzyme having poor metabolizer (PM) genotype and 38% of them are (11/27) metabolized by CYP 2D6.

Here are some examples of drugs metabolized by enzymes with variant alleles associated with **poor metabolism** and implicated in different ADRs:
- ***CYP1A2*** (typical antipsychotics and tardive dyskinesia);
- ***CYP2C9*** (Warfarin /haemorrhage/, Tolbutamide /hypoglycaemia/, Phenytoin /skin toxicity/);
- ***CYP2C19*** (Mephenytoin /neurotoxicity/, Diazepam /prolonged sedation/); Omeprazole and Lansoprazole /higher therapeutic response/.
- ***CYP2D6*** (Antiarrhythmics /arrhythmias/, β-Blockers /bradycardia/, Tricyclic antidepressants /confusion/, Opioids /protection from oral opiate dependence/, Phenformin /lactic acidosis/, Perhexilene /hepatotoxicity/); codeine /poor analgesic efficacy/; tramadol /poor analgesic efficacy/.
- ***CYP3A4*** (Anti-leukaemic agents /treatment-related leukaemia/);
- ***plasma butyrylcholinesterase*** (Succinylcholine /prolonged apnoea/);
- ***N-acetyltransferase*** (Sulfonamides /hypersensitivity/, Amonafide /myelotoxicity/, Procainamide, hydralazine, isoniazid /lupus/);
- ***thiopurine methyltransferase*** (6-Mercaptopurine, azathioprine /myelotoxicity/);
- ***dihydropyrimidine dehydrogenase*** (5-Fluorouracil /myelotoxicity/);
- ***UDP glucuronosyl transferase*** **1A1** (Irinotecan /diarrhoea, myelosuppresion/).

Increased risk of toxicity or failure to response can be found in patients with **extensive expression** (**EM**) of **CYP 2D6** as:
- codeine (morphine toxicity);
- encainide (possibly proarrhythmias)
- nortriptyline (poor antidepressant efficacy at normal doses);
- propafenone (poor antiarrhythmic efficacy at normal doses);
- tropisetron and ondansetron (poor antiemetic efficacy at normal doses)

In some cases, the appearance of **selective organ toxicity** by some drugs can be explained by their **metabolic activation** to reactive, toxic metabolite (s):
- paracetamol (liver and kidney toxicity)
- amiodarone (o2, lung and skin toxicity)
- valproic acid (reye-like syndrome)
- isoniazid (polyneuritis)
- furosemid (pancreatitis)
- nitrofurantoin (lung toxicity)
- cimetidine (liver toxicity)
- diclofenac (liver toxicity)
- ranitidine (liver toxicity)

II. Genetic polymorphism of therapeutic target – enzyme or receptors

There are many examples of genetically determined different pharmacodynamic response or ADR due to defective pharmacological target (**enzyme)** as:
- **Glucose-6-phosphate dehydrogenase deficiency** – hemolytic anemia after antimalaric drugs;
- **Thiopurin S-methyltransferase deficiency** – toxic effects after azathioprine in treatment of leukemia and autoimmune diseases;
- **ALOX-5 (5-lipoxygenase)** - asthmatic patients who carry mutations of the core promoter of 5-lipoxygenase (ALOX-5) respond poorly to ALOX-5 inhibitors such as Zileuton;
- **ACE – ACE*DD***-genotype has two times higher maximal velocity and 1.5 higher concentration in the body compared with the wild **ACE*II***-genotype, thus lead to dramatic differences in therapeutic effects of ACE-inhibitors.

There are many ADR or therapeutic failure due to defective pharmacological target (**receptors**) as:
- **Serotonin transporter (5-HTT) gene** is reportedly a determinant of response to fluvoxamine, a selective serotonin re-uptake inhibitor (SSRI);
- **Arg16/Gly16 or Gly16/Gly16 variants of b2-adrenoceptors** have been display a much less favourable immediate bronchodilatory response to salbutamol;

- Patients with homozygote mutation **Gly17Arg of beta-2 adrenoreceptor** exerted increased asthmatic outburst after treatment with "normal" doses of albuterol;
- Mutation of **Apolipoprotein E (**ApoE4**)** gene leads to decreased response to tacrin in treatment of Alzheimer disease;
- **P-glycoprotein (MDR)** mutation – abolished the effectiveness of alkylating chemotherapeutics because of their increased excretion out of cancer cells;
- Patients with **Ryanodine receptors mutation** in skeletal muscles are exposed to malignant hypothermia after anesthesia with halothane;
- **Single point mutation in serotonin receptor** leads to great variability in effectiveness of sumatriptan.

III. Immune-mediated drug toxicity (ADR type B)

Requirement for development of the immune response to certain drug is appropriate hapten formation in the body through:

1. **Direct haptenization** (penicillin, penicillamine, captopril)

The clinical sings of most common hypersensitivity reaction to drugs are:

- **Anaphylaxis** – type I reaction after betalactame antibiotics, NSAID, sulfonamides;
- **Hemolytic anemia** – penicillin, cephalosporin, methyl-DOPA, nomifensin;
- **Agranulocytosis** – aminopyrin, levamizol, captopril, mianserin, propylthiouracil, peniccilin-G, sulfasalazin, sulphmetoxazol);
- **Skin reacions** – trimetoprim, anticonvulsants, cephalosporin, penicillin).

2. On the other hand, there are many drugs known to cause ***immune-mediated toxicity***, which undergo **bioactivation by different cytochrome p450** isoforms. Most of them are mechanism-based inactivators, which **reactive metabolites covalently bind to the corresponding CYP protein**. By still not fully understand mechanism, organism develops antibodies against this complex. The consequence of this is the appearance of different immune mediated organ toxicities, like:

- **hepatotoxicity** (dihydralazine /CYP1A2/, halothane and ethanol /CYP2E1/, phenytoin /CYP2C9/, tienilic acid /CYP2C9/, sulphamethoxazole and carbamazepine /CYP3A4, CYP2C9/;
- **skin toxicity** (sulphamethoxazole, carbamazepine, phenytoin).

3. Metabolism of drugs by **activated leukocytes** (NADPH oxidase and myeloperoxidase) leads to:

- **skin reactions; agranulocytosis (lupus)** – sulfamethoxazole, dapsone, propylthiouracil, levamisole, ticlopidine, clozapine, 5-aminosalicylic acid, procainamide, mianserin.

Conclusion: The main goal of contemporary personalized medicine is to find the *right* **dose** of the *right* **drug** for the *right* **indication** for the *right* **patient** at the *right* **time**. This approach should decreased drastically in the future the incidences of adverse drug reactions. A broad spectrum of our society could contribute for the success of this goal.

The important role of different participants in drug synthesis and usage to decrease the incidences of ADRs could be described as follows:

Producers – to perform directed synthesis of new compounds;

Scientists – to find new markers for testing gene and phenotype polymorphism and adverse drug reactions;

Teachers - to train knowledgeable clinical pharmacologists and pharmacists;

Centrum for ADR – to do better analysis of the signals for ADR and spreading of the information;

Physicians and pharmacists - to achieve individualization of the pharmacotherapy;

Patients – to have better information and less self-medication.

MEDICAL TREATMENT OF CUSHING'S SYNDROME

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Cushing's syndrome (CS) comprises a large group of signs and symptoms that reflect prolonged and inappropriately high exposure of tissue to glucocorticoids (1). The most common cause is the iatrogenic form. Endogenous CS is a rare disease (the incidence is estimated of 2-3 cases per 1 million inhabitants per year). Excess cortisol production may be caused by either ACTH secretion (from a pituitary or other ectopic tumour) – ACTH-dependant CS or independent adrenal overproduction – ACTH-independent CS. The ACTH-dependant CS is more frequent (80% of cases).
CS is a heterogeneous disorder and requires a multidisciplinary and individualized approach to patient management. As CS is caused by a tumour (pituitary, adrenal or ectopic), producing either ACTH or cortisol, the most common may of treatment is the surgical resection of the tumour, performed by an experienced surgeon, although this is not always possible. Second-line treatments include more radical surgery, radiation therapy (for Cushing's disease), medical therapy, and bilateral adrenalectomy. Because of the significant morbidity of Cushing's syndrome, early diagnosis and prompt therapy are warranted.
The medical therapy of CS may be adrenal-directed and tumour-directed (2).

Adrenal-directed therapy: steroidogenesis inhibitors
Adrenal-directed therapy (steroidogenesis inhibitors) may be highly effective but does not treat the underlying tumour or restore normal HPA secretory dynamics. Most experience with steroidogenesis inhibitors has been acquired with **ketoconazole** and **metyrapone**, which appear to be more effective and better tolerated than aminoglutethimide (3-12).
Ketoconazole was initially used as antifungal drug. Besides this action, it was proven to inhibit the steroïdogenesis at several levels: 20, 22 desmolase (CYP11A1), 11-β-hydroxylase (CYP11B1), and 17, 20 lyase (CYP17). The usual doses are 200 mg twice daily to 400 mg three times daily (total daily dose of 1200 mg)(2). Mild elevations in liver enzymes (up to 3-fold normal), which are transient, are not a contraindication to medical therapy with ketoconazole, but liver function should be monitored carefully because of the rare complication of liver failure. The possibility of the development of hypogonadism in men during ketoconazole therapy may favour the initial use of metyrapone in this population. Conversely, the association of hirsutism with metyrapone treatment in women may make ketoconazole a better choice in this population. Interestingly, in contrast to subjects with an intact HPA axis, patients with pituitary-dependent Cushing's disease show no compensatory rise, or decrease, in ACTH levels upon prolonged administration of ketoconazole. According to human and animal studies, however, this phenomenon does not seem to involve a direct effect on ACTH secretion but rather an adjustment in the sensitivity of the HPA axis (4, 5, 9, 10). Moreover, the ACTH response to CRH in patients with Cushing's disease was enhanced or unchanged during ketoconazole treatment compared with the pretreatment response (4). Taken together, these findings argue against an additional site of inhibition at the pituitary level, although it was suggested by *in vitro* studies of pituitary corticotrophs (13).
Metyrapone acts by blocking the action of CYP11B1. Metyrapone treatment leads to marked inhibition of aldosterone biosynthesis and accumulation of aldosterone precursors with weak mineralocorticoid activity. Electrolyte balance and blood pressure levels vary individually with the degree of aldosterone inhibition and 11-deoxycorticosterone stimulation. Adverse effects due to increased 11-deoxycorticosterone levels (hypokalemia, oedema, and hypertension) are infrequent (12). At present, metyrapone is not commercially available in the United States, but it can be provided for compassionate use by contacting the manufacturer (Novartis) directly. The usual doses of Metyrapone are 250 to 1500 mg four times daily (total daily dose of 6000 mg) (2).
Mitotane (o'p'-DDD) may prove highly effective in the longterm suppression of hypercortisolism in the majority of patients with CS because of its specific adrenolytic action. Its mechanism of

action also prevents the risk of escape phenomenon in response to the ACTH rise that occurs in Cushing's disease when plasma cortisol is decreased (15). However, its onset of action is slow (weeks or months), and the adverse effects associated with mitotane therapy (mainly digestive and neurological) require careful monitoring of drug levels, and it is routinely used in only a few centres.
Aminoglutethimide is an anticonvulsant drug that inhibits the CYP11A1 and the 11-β-hydroxylase CYP11B1. Because of its serious side effects, it is no longer available worldwide.
In situations where rapid control of cortisol levels is required and oral therapy is problematic, i.v. **etomidate** therapy may be considered (14) (bolus of 0.03 mg/kg i.v. followed by infusion of 0.1 mg/kg/h. the maximal dosage is 0.3 mg/kg/h (2). Etomidate acts by inhibiting the CYP11B1.
Treatment with the glucocorticoid receptor-antagonist **mifepristone (RU486)** has been reported in fewer than 20 patients with ectopic ACTH secretion, and its use for this indication is currently investigational (16). There is no significant experience reported yet with this agent in patients with Cushing's disease, and assessment of its efficacy in the absence of a biochemical marker is challenging.

Follow-up evaluations should include the examination of clinical features and 24-h urinary free cortisol (UFC) levels, aiming for normalization of both. A few centers use a cortisol day curve with five measurements of serum cortisol over 12 h, with a goal of maintaining the mean level within normal limits. Blood samples are taken at 0900, 1200, 1500, 1800, and 2100 h, and the mean cortisol levels are calculated. Several assessments may be advisable, because control may be variable with cyclical disease.
The choice of UFC assay should be considered carefully, with tandem mass spectrometry considered most specific, and it is important to note that normal ranges vary greatly depending on the assay method. Although salivary cortisol measurements may be an important endpoint in establishing efficacy and restoration of normal cortisol levels, validation data in patients treated for Cushing's disease are needed. Whichever technique is used, the aim is to restore a 24-h production rate of cortisol within the normal range, although circadian rhythmicity may not necessarily be restored. However, the clinical impact of these abnormal rhythms remains unclear.

Adrenal-directed medical therapy is effective in the majority of patients in a dose-dependent manner. Its indications might include the preoperative preparation of patients to correct severe complications of the disease quickly. In this context, the possibility of avoiding hypoadrenalism immediately after surgery by normalization of cortisol production for a sufficient length of time preoperatively, pertains to clinical observation rather than randomized clinical trials and should be better explored. Drug control of hypercortisolism is also suitable for patients, awaiting a response to radiation therapy and whenever a palliative treatment is needed. In general, definitive therapy, either surgery or radiotherapy should be considered for all patients, and longterm medical therapy alone is rarely indicated.

Tumour-directed medical therapy
Pituitary-directed therapy targets the underlying cause of the disease, and therefore, several investigational agents are under evaluation. Despite initial promise, subsequent studies do not support a routine clinical role for the use of **peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists**, such as rosiglitazone and pioglitazone (17). Although **retinoic acid** is effective at reducing ACTH in animal models and in dogs with Cushing's disease, the effective dose used is high and human clinical trial results are not currently available. Current medical therapies targeted to the corticotroph tumour itself have not been uniformly successful. However, a medical therapy that acts directly on the pituitary tumour to normalize ACTH secretion and inhibit tumour growth would represent a major non-surgical advance in the treatment of this disease. Molecular studies provide a rationale for the use of **somatostatin receptor ligands** for the treatment of corticotroph adenomas, because these tumours express somatostatin receptor subtypes sst1, sst2, and sst5, although expression of sst5 predominates (18). The commercially available somatostatin analogs octreotide and lanreotide are predominantly sst2-selective ligands and are mostly ineffective in

treating Cushing's disease. Somatostatin analogs with a broader somatostatin receptor-subtype affinity might be more effective. **Pasireotide** (SOM230; Novartis, Basel, Switzerland), which has high affinity for sst1–3 and especially sst5, shows promise as a tumour-directed medical therapy in patients with Cushing's disease (18). Longer-term trials are needed to determine the safety and efficacy of pasireotide. The dopamine D2 receptor is expressed in more than 75% of corticotroph pituitary adenomas (19). In long-term studies with **bromocriptine**, disease remission was confirmed in only a small minority of patients. A small, short-term study suggests that **cabergoline** at dosages of 2–3.5 mg/wk may be effective in treating a subset of patients with Cushing's disease (19). However, more data are required not only for efficacy but also to address the long-term safety of cabergoline in these patients. The use of combination pituitary-directed drug therapy (*e.g.* a dopamine D2 receptor agonist plus a sst receptor ligand) is an exciting concept that has not been evaluated to date. Previous studies have shown that serotonin antagonists and γ-aminobutyric acid (GABA) agonists are generally ineffective and are not routinely recommended.

Treatment strategies

The treatment of choice for Cushing's disease (the most frequent cause of CS) is the selective trassphenoïdal surgery. Medical treatment can be considered before the operation in cases of severe hypercortisolism in order to diminish the morbidity and the mortality, linked to the intervention. The most frequently used drug are Ketoconazole and o'p'-DDD. When surgery is not successful or is contraindicated, o'p'-DDD is prescribed. Because of its slow start of action, Ketoconazole should be administered in the beginning of treatment.

In cases of adrenal adenoma, responsible for CS (ACTH-independent CS), surgery is preferable. Medical treatment (Ketoconazole) can be prescribed in the preoperative period in order to diminish the hypercortisolism and to prepare better the patient for the operation. o'p'-DDD treatment is not advisable in order not to alter the contralateral adrenal gland.

After surgical treatment of adrenal carcinoma, responsible for CS, o'p'-DDD is most often prescribed because of its adrenolytic action with or without Ketoconazole.

References:

1. **Nieman L.K., B.M.K. Biller, J. Findling et al.** The Diagnosis of Cushing's Syndrome: An Endocrine society Clinical Practice Guideline. JCEM, 2008, 93(5):1526-1540.
2. **Biller B.M.K., A.B. Grossman, P.M. Stewart et al.** Treatment of ACTH-Dependant Cushing's Syndrome: A Consensus Statement. JCEM, 2008, 93:2454-2462.
3. **Beardwell CG, Adamson AR, Shalet SM** 1981 Prolonged remission in florid Cushing's syndrome following metyrapone treatment. Clin Endocrinol (Oxf) 14:485–492
4. **Boscaro M, Sonino N, Rampazzo A, Mantero F** 1987 Response of pituitaryadrenal axis to corticotrophin releasing hormone in patients with Cushing's disease before and after ketoconazole treatment. Clin Endocrinol (Oxf) 27: 461–467
5. **Burrin JM, Yeo TH, Ashby MJ, Bloom SR** 1986 Effect of ketoconazole on adrenocorticotrophic hormone secretion in vitro and in vivo. J Endocrinol 108:37–41
6. **Gartner R, Albrecht M, Muller OA** 1986 Effect of etomidate on hypercortisolism due to ectopic ACTH production. Lancet 1:275
7. **Schteingart DE, Tsao HS, Taylor CI, McKenzie A, Victoria R, Therrien BA** 1980 Sustained remission of Cushing's disease with mitotane and pituitary irradiation. Ann Intern Med 92:613–619
8. **Sonino N, Boscaro M, Ambroso G, Merola G, Mantero F** 1986 Prolonged treatment of Cushing's disease with metyrapone and aminoglutethimide. IRCS Med Sci 14:485–486
9. **Sonino N, Boscaro M, Paoletta A, Mantero F, Ziliotto D** 1991 Ketoconazole: treatment in Cushing's syndrome: experience in 34 patients. Clin Endocrinol (Oxf) 35:347–352
10. **Sonino N, Boscaro M, Fallo F** 2005 Pharmacologic management of Cushing syndrome: new targets for therapy. Treat Endocrinol 4:87–94
11. **SoninoN**1987 The use of ketoconazole as an inhibitor of steroid production. N Engl J Med 317:812–818
12. **Sonino N, Boscaro M** 1999 Medical therapy for Cushing's disease. Endocrinol Metab Clin North Am 28:211–222

13. **Jimenez Reina L, Leal-Cerro A, Garcia J, Garcia-Luna PP, Astorga R, Bernal G** 1989 In vitro effects of ketoconazole on corticotrope cell morphology and ACTH secretion of two pituitary adenomas removed from patients with Nelson's syndrome. Acta Endocrinol (Copenh) 121:185–190
14. **Greening JE, Brain CE, Perry LA, Mushtaq I, Sales MJ, Grossman AB, Savage MO** 2005 Efficient short-term control of hypercortisolaemia by low-dose etomidate in severe paediatric Cushing's disease. Horm Res 64:140–143
15. **Luton JP, Mahoudeau JA, Bouchard P, Thieblot P, Hautecouverture M, Simon D, Laudat MH, Touitou Y, Bricaire H** 1979 Treatment of Cushing's disease by O,p'DDD: survey of 62 cases. N Engl J Med 300:459–464
16. **Johanssen S, Allolio B** 2007 Mifepristone (RU 486) in Cushing's syndrome. Eur J Endocrinol 157:561–569
17. **Pecori GF, Scaroni C, Arvat E, Martin M, Giordano R, Albiger N, Leao AA, Picu A, Mantero F, Cavagnini F** 2006 Effect of protracted treatment with rosiglitazone, a PPAR_ agonist, in patients with Cushing's disease. Clin Endocrinol (Oxf) 64:219–224
18. **Hofland LJ, Van Der Hoek J, Feelders R, van Aken MO, van Koetsveld PM, WaaijersM, Sprij-Mooij D, Bruns C, Weckbecker G, de HerderWW, Beckers A, Lamberts SWJ** 2005 The multi-ligand somatostatin analogue SOM230 inhibits ACTH secretion by cultured human corticotroph adenomas via somatostatin receptor type 5. Eur J Endocrinol 152:645–654
19. **Pivonello R, Ferone D, de Herder WW, Kros JM, De Caro ML, Arvigo M, Annunziato L, Lombardi G, Colao A, Hofland LJ, Lamberts SW** 2004 Dopamine receptor expression and function in corticotroph pituitary tumours. J Clin Endocrinol Metab 89:2452–2462