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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 is 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%) (Venkatakrishnan 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 transformed by drug metabolizing enzymes to toxic metabolites (metabolic activation, bioactivation). Conversely a toxic drug may be transformed 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 prospective 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 sub cellular 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

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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 lactate dehydrogenase (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 appropriate experimental models of toxicity, provoked by different agents - CCl_4, chlorpromazine, metoprolol, paracetamol, t-BuOOH, which possess different toxic properties.

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

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

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

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 stabilizing effect of the compound.

On a cellular 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 to those of silymarin. In Chang liver cells, diosgenin showed pro-apoptotic effect, however ROS levels remained unchanged.

In vivo, after multiple administrations, diosgenin acted as a CYP 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 the potential risk of tolerance and dependence developing, multiple administrations of psychoactive compounds are 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 was cytotoxic, 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 *et 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 isoforms of cytochrome P450, including CYP3A (Drocourt *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 the compounds (Vitcheva &Mitcheva, 2007).

Multiple co-administrations of nifedipine and amphetamine resulted in changes in some parameters of drug metabolism that differed from those observed after their administration alone. Regarding the metabolic pathways of amphetamine and nifedipine, we suggest a metabolic interaction, involving 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 &Mitcheva, 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 diol-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 co-administration of Paracetamol and naphthalene, resulted in decreased naphthalene toxicity. This effect is probably due to a competition for one isoform of cytochrome P 450.

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

Conclusions The results obtained, confirmed that it is necessary to know of the possible interactions between different compounds that are substrates and/or inhibitors of one particular cytochrome P450 isoform, with a view to prevent adverse drug-drug interactions, which can have serious clinical consequences, as well as to avoid the discontinuation of needed pharmacotherapy.

In vitro based experimental systems 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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