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## Session 13: Metabolic Activation of Xenobiotics – Therapeutic Target and Drug Monitoring

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### Lectures

#### L13.1

##### Metabolic coordination through metabolite-protein interactions

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How do bacteria know what goes on in their environment and how to they make appropriate decisions? While some bona fide extracellular sensors are known, there are far more environmental conditions and cellular responses than could possibly be dealt with through dedicated sensors. Instead, most microbial responses are based on intracellular changes to environmental changes. One of the first affected networks to just about any extracellular change is metabolism that passively responds to nutritional or chemical/physical challenges. Since fluxes and intracellular metabolite levels respond within seconds, allosteric binding of metabolites to regulatory proteins and enzymes is a highly effective and rapid sensing mechanism. Different from well-established methods to assess physical interaction between proteins and between proteins and nucleic acids, however, methods to assess metabolite-protein interactions are still in their infancy. At present we know on the order of 1500 unique regulatory metabolite-protein interactions [1], which is only the tip of the iceberg [2]. Beyond knowing the interaction topology, I will focus in this talk on the even more challenging and conceptual problem: understanding which of the many regulation mechanisms actually matter for a given adaptation to elicit an appropriate physiological system response. The surprising result for *E. coli* is that only very few regulation events appear to be required for a given transition, typically involving less than a handful of active regulators [3]. Understanding the traffic regulation system of microbes is a foundation for their rational redirection.

##### References:

1. Reznik, Christodoulou, Goldford, Briars, Sauer, Segre, Noor (2017) *Cell Reports* **20**: 2666-2677.
2. Piazza, Kochanowski, Cappelletti, Fuhrer, Noor, Sauer, Picotti (2018) *Cell* **72**: 358-372.
3. Kochanowski, Gerosa, Brunner, Christodoulou, Sauer (2017) *Molecular Systems Biology* **13**: 903.

#### L13.2

##### The metabolic activation of methylated resveratrol analogue 3,4,4',5-tetramethoxystilbene (DMU-212) to 3'-hydroxy-3,4,5,4'- tetramethoxystilbene (DMU-214)

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Currently available chemotherapeutics eliminate both, cancer and normal cells. Hence, the development of pro-drugs, which are activated to the cytotoxic compounds mainly at the site of tumor is still required. It is known that the enzymes of CYP1 family, CYP1A1 and CYP1B1, are overexpressed in many types of cancer cells comparing to their normal counterparts. DMU-212 (3,4,4',5-tetramethoxystilbene) is the methylated resveratrol analogue, which has been shown to exert strong anti-cancer activity. It has been hypothesized that DMU-212 is metabolized by CYP1B1. However, the role of the enzymes of CYP1 family in biotransformation and biological activity of DMU-212 is still elusive. In our previous study we have shown that DMU-212 is converted to DMU-214 (3'-hydroxy-3,4,5,4'-tetramethoxystilbene), which displayed stronger cytotoxic effects. Here, we discussed whether the biological activity of DMU-212 is related to its conversion to DMU-214. Furthermore, we aimed to assess which enzymes of CYP1 family are involved in the biotransformation of DMU-212. In contrary to other authors' suggestions we found that CYP1A1 is the major enzyme of CYP1 family involved in the metabolic activation of DMU-212. Since the distinctly weaker cytotoxic effects of DMU-212 against non-cancerous cells have been associated with the lack of the expression of CYP1A1, we suggest that the biological activity of the parent compound is related to its metabolic activation to DMU-214 and the level of this enzyme.

## Oral presentations

### O13.1

#### Hyperhomocysteinemia caused by highly active antiretroviral therapy (HAART) of HIV infection: focus on mitochondria

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In the last 10 years, hyperhomocysteinemia (hHcy) has been regarded as a marker and a definite risk factor for many other diseases. The main cause of hHcy is a dysfunction of enzymes and cofactors associated with the process of homocysteine biosynthesis. Other causes include excessive methionine intake, certain diseases and side effects of some drugs. The accumulation of Hcy leads to increased cellular oxidative stress, mitochondrial dysfunction and leads to cytoskeletal de-arrangement and cellular remodeling or apoptosis. We speculate that a high level of Hcy can be the sole reason or an aggravating factor in drug induced liver injury (DILI) in HIV-infected patients, depending on the use of antiretroviral drugs. The method for determining homocysteine is based on HPLC. With the development of laboratory signs of toxic liver damage (when taking HAART), a violation of metabolic processes of one-carbon groups is revealed, which is manifested in the development of hyperhomocysteinemia – 8.47 [6.38; 11.43]  $\mu\text{mol/l}$ . This metabolic imbalance is an unfavorable factor in predicting the development of DILI during HAART of HIV-infection. At the same time, a decrease in the level of reduced glutathione is observed, which indicates a violation of the antioxidant balance (possibly due to a malfunction of the respiratory chain and the triggering of the mitochondrial pathway of apoptosis) in patients with laboratory signs of DILI.

### O13.2

#### Metabolic stability as a challenge in the early stage of drug discovery process – *in vitro* determination of metabolic pathways for new promising 5-HT<sub>7</sub>R ligands

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Although the 5-HT<sub>7</sub> serotonin receptor (5-HT<sub>7</sub>R) was recognized as a promising therapeutic target, no drug acting selectively *via* 5-HT<sub>7</sub>R has got pharmaceutical market, to date. Metabolic instability is one of the main reasons of drug-candidates failures. Thus, studies on the metabolic pathways and metabolic stability for new 5-HT<sub>7</sub>R ligands were performed using human (HLMs) and mice microsomes (MLMs). To determine the routes of metabolic biotransformation, the 5-HT<sub>7</sub>R ligands were incubated with proper microsomes for 2h and the reaction mixtures were analyzed by UPLC-MS with ion fragmentation. Moreover, the metabolic stability of 5-HT<sub>7</sub>R ligands were determined in pharmacokinetic *in vitro* studies, where the reactions were terminated in the five time points in order to calculate the half-life period ( $t_{1/2}$ ) and hepatic metabolic clearance  $CL_{int}$ . In human *in vitro* model, metabolic biotransformations included hydroxylations, dehydrogenations and dealkylations. Additional compounds' decomposition and oxidations were found in the mice metabolic pathways. The most probably structures of metabolites were proposed, supported by *MetaSite* 6.0.1 *in silico* simulation. The 5-HT<sub>7</sub>R ligands tested at HLMs showed good metabolic stability with  $CL_{int} \leq 16.56$  ml/min/kg. Compounds MF8 and KKB16, selected as the most stable leads, showed an excellent metabolic stability in human ( $CL_{int} = 8.2$  ml/min/kg for MF-8 and not calculable for KKB16) and good, comparable stability in mice ( $CL_{int} = 17.2$  ml/min/kg).

## Posters

### P13.1

#### Metabolism of antitumor unsymmetrical bis-acridines in liver microsomes and cytosol: Identification of the metabolites and metabolic pathways of the compounds

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Unsymmetrical bis-acridines (UAs) are a novel potent class of antitumor agents with marked antitumor efficacy *in vitro* and *in vivo*. During drug development process particular attention is paid to drug metabolite formation and identification of metabolic pathways. Thus, in this work we have reported the results of preliminary *in vitro* metabolism studies of the selected UAs in liver microsomes and cytosol. UAs show different physicochemical properties (*i.e.*, solubility, acid-base and spectroscopic properties) compared to their monomeric substrates (imidazoacridinone and 1-nitroacridine derivatives), hence new reaction and liquid chromatographic conditions have been developed. Metabolism of UAs in rat liver microsomes revealed the formation of several different metabolites. Further, the use of phenobarbital-, 3-methylcholanthrone- or dexamethasone-activated microsomes indicated that mainly CYP3A isoenzymes are involved in UA metabolism. When incubated with rat liver cytosol, UAs were transformed into one intense metabolite. It was more rapidly generated with the addition of endogenous glutathione but its formation did not require the participation of NADPH cofactor. The chemical structures of the observed metabolites were identified using mass spectrometry. Our results indicated that the metabolic pathways of the studied compounds are extremely complicated. This issue will require further extensive studies both in cell-free systems and tumor cells.

### P13.2

#### The effect of phytochemicals and their combination on the expression and activity of Nrf2 in human pancreatic cancer cells

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In this study, the modulation of Nrf2 expression and activity by phenethyl isothiocyanate (PET), indole-3-carbinol (I3C), resveratrol (RES), xanthohumol (X) and their combinatorial treatment was evaluated in human pancreatic cancer cell line PANC-1. The expression of *Nrf2* gene and *GSTP*, *NQO1*, *SOD* genes controlled by this transcription factor was assessed by RT-PCR and Western blot. The highest transcript and protein levels were observed as result of treatment with X and PET and their combination. The increased expression of Nrf2 accompanied its enhanced activation, which was shown by translocation from cytosol to nucleus and increased binding to DNA –ARE sequence. As result of Nrf2 activation the induction of GSTP1 and SOD was observed after treatment with all phytochemicals. In general no significant differences were found in the effect of single phytochemical treatment and their combinations.

The activation of Nrf2-ARE pathway in pancreatic cancer cells, particularly by X and PET may contribute to development cancer resistance to chemo- or radiotherapy. On the other hand these compounds showed the highest cytotoxicity suggesting that using other mechanisms may exert anti-cancer effect.

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## P13.3

### Generation of hydrogen peroxide by dietary antioxidants

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Studies of 41 antioxidants of various structure revealed that 17 of them, mainly polyphenols, generated hydrogen peroxide when added to Dulbecco's Modified Minimal Eagle's Medium (DMEM) and other media used for culturing mammalian and yeast cells. The most active compounds were: propyl gallate, epigallocatechin gallate and quercetin, generating in DMEM  $91.1 \pm 4.3 \mu\text{M}$ ,  $52.3 \pm 4.3 \mu\text{M}$  and  $51.4 \pm 5.1 \mu\text{M}$ , respectively, upon incubation their 1 mM solutions in the medium at 37°C for 3 h. The amount of H<sub>2</sub>O<sub>2</sub> generated depended on the the medium, was not augmented by light and was higher in PBS than in the cell culture media. Chelex treatment and iron chelators decreased H<sub>2</sub>O<sub>2</sub> generation suggesting that transition metal (mainly Fe) ions catalyze antioxidant autoxidation and concomitant hydrogen peroxide production. The autoxidation of polyphenols proceeds most probably via two one-electron steps since formation of semiquinone radicals was demonstrated by ESR spectroscopy and superoxide was evidenced by superoxide dismutase-inhibitable NBT reduction and dihydroethidine oxidation. Green tea also generated H<sub>2</sub>O<sub>2</sub>; tea prepared on tap water generated significantly more hydrogen peroxide than tea prepared on deionised water. Ascorbic acid (except at low concentrations in tap-prepared tea) decreased H<sub>2</sub>O<sub>2</sub> production. Lemon added to the tea significantly reduced generation of hydrogen peroxide.