ABSTRACTS:

K:	**Keynote Lecture**
I:	**Invited Contribtion**
S:	**Short Communication**
P:	**Poster**

K1: Integrated Systems for Predicting Toxicity and Drug-Drug Interactions – A Use Case for Drug Transporter

M. Pinto, F. Klepsch, G.F. Ecker
Univ. Vienna, Dept Medicinal Chemistry, Pharmacoinformatics Research Group, Vienna, Austria.

Background: Transporters play a pivotal role in the translocation of molecules across biological membranes. Transporters regulate essentially all fundamental biological processes, including metabolism and energy supply, cytoplasmic concentrations of physiologically important ions, signal transduction and defense against potentially toxic agents. Moreover, membrane transporters play a vital role in the pharmacokinetics and pharmacogenomics of drugs. They are linked to clinically important drug-drug interactions (DDI), thus posing a significant challenge to the pharmaceutical industry.

Important examples for these non-target proteins are the product of the MDR1 gene (multidrug resistance protein 1 or P-glycoprotein = P-gp, ABCB1) and other members of the ABC-transporter family, including BCRP (ABCG2), MRP1 (ABCC1), MRP2 (ABCC2), and BSEP (ABCB11). They are mainly responsible for active transport out of cells. In addition, there is the large family of Solute Carriers, which facilitate active transport into cells. At physiological barriers and organs, such as the blood-brain barrier, the intestine, liver, and kidney, they exert a complex interplay modulating uptake and elimination of drugs.

Results and Discussion: Intensive literature search including the Open PHACTS platform and TPsearch allowed to creating data sets suitable for large scale classification of compounds with respect to their transporter interaction profiles. These models were further integrated into a web-based prediction tool for drug-transporter interaction. This will allow to assessing the risk of certain compounds for adverse side effects. Finally, an advanced structure-based approach combining ligand-based information with docking into protein homology models allowed to establishing a classification model for P-glycoprotein inhibitors based on ligand docking.

We gratefully acknowledge financial support provided by the Austrian Science Fund under the framework of the special research programme SFB35 “Transmembrane Transporters in Health and Disease (F3502)”. The research leading to these results has also received support from the Innovative Medicines Initiative Joint Undertaking under grant agreements n° 115002 (eTOX) and n° 115191 (Open PHACTS), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies’ in kind contribution.

K2: Transcriptomics Responses and Functional Endpoints in New Human Cell Based Test Systems for Developmental Neurotoxicity

M. Leist

Doerenkamp-Zbinden Chair for in vitro toxicology and biomedicine, Box M657, University of Konstanz, Germany.

Background: Developmental neuro-toxicity (DNT) and many forms of reproductive toxicity (RT) often manifests themselves in functional deficits that are not necessarily based on cell death, but rather on minor changes relating to cell differentiation or communication. The fields of DNT/RT would greatly benefit from in vitro tests that allow the identification of toxicant-induced changes of the cellular proteostasis, or of its underlying transcriptome network. **Objectives/Methods:** To establish stem cell based test systems, and to use transcriptomics and functional endpoints to assess toxicity and its underlying mechanisms.

Results and Discussion: Pure populations of neuroepithelial precursor cells or of neural crest cells were generated from human embryonic stem cells. The cells were characterized by whole genome transcript profiling and by their migration capacity. Developmental toxicants showed highly specific effects in the different cell populations.

References: Zimmer B et al. (2012). Evaluation of developmental toxicants and signaling pathways in a functional test based on the migration of human neural crest cells. Env Health Perspect, 120, 1116

Balmer NV et al. (2012) Epigenetic changes and disturbed neural development in a human embryonic stem cell-based model relating to the fetal valproate syndrome. Hum Mol Genet 21, 4104

I1: Food-Drug Interaction: Anthocyanins-rich Preparations Affect Targets Relevant in Chemotherapy

D. Marko, C. Kropat, M. Esselen
University of Vienna, Department of Food Chemistry and Toxicology, Vienna, Austria.

Background: Berry anthocyanins have been reported to modify cancer biomarkers in vitro e.g. reducing DNA damage or inhibiting tumor cell growth. Several overlapping mechanisms of action have been reported, including the inhibition of receptor tyrosine kinases, induction of apoptosis and the interference with human topoisomerases. Progress has been made in understanding the mechanism of berry anthocyanins as well as the free aglycons, the so called anthocyanidins, as topoisomerase inhibitors in vitro. Delphinidin, cyanidin (cy) and several anthocyanin-rich extracts have been characterized as catalytic topoisomerase inhibitors in human colon carcinoma cells modulating DNA-damaging properties of the topoisomerase poisons camptothecin and doxorubicin. Camptothecin and its analog irinotecan represent topoisomerase I poisons. Irinotecan is a prodrug that is endogenously bioactivated to its active compound SN-38. We addressed the question whether in rats oral uptake of a complex anthocyanin-rich blackberry extract interacts with the topoisomerase-poisoning and DNA-damaging effect of irinotecan and compared this effect with the lead anthocyanin cy-3-g or the respective main aglycon cy.

Methods: Wistar rats (9/per group) received blackberry extract, cy-3-g, cy or NaCl solution via intragastric gavage. "In vivo Complexes of Enzyme to DNA" bioassay was used to detect irinotecan-induced stabilization of topoisomerase I/DNA complexes and single cell gel electrophoresis to determine DNA strand break induction in the colon of male Wistar rats.

Results and Discussion: The intraperitoneally treatment of Wistar rats with 100 mg/kg bw. irinotecan resulted in a significant increase of covalent topoisomerase I/DNA intermediates in the colon compared to the control group receiving 0.9 % NaCl. When blackberry extract was administered prior to irinotecan treatment the amount of topoisomerase I bound to DNA was significantly decreased in the colon of the rats. Application of cy-3-g or its aglycon cy also resulted in a slight but not statistically relevant decrease in cleavable complex stabilization by irinotecan. A significant increase of DNA damage was shown after a single dose of irinotecan. Furthermore, a significant reduction of irinotecan-induced DNA-strand breaks after a pre-treatment with cy, cy-3-g and blackberry extract was observed. Taken together the question arises whether anthocyanin-rich preparations e.g. taken up as food supplement, might interfere with chemotherapy or whether, due to low systemic bioavailability, the preparations might provide protective potential in the gastrointestinal tract.

I2: ÖGGM and Forensic Toxicology

W. Rabl
Institute of Legal Medicine, Medical University Innsbruck.

Background: In der Tradition der Gerichtlichen Medizin in Österreich haben neben den morphologischen Fachbereichen, mit denen die Bevölkerung die Gerichtsmedizin vor allem verbindet, auch weitere naturwissenschaftliche Disziplinen große Bedeutung erlangt. Neben dem Fachbereich der Serologie, der durch die Molekularbiologie praktisch lückenlos ersetzt wurde, hat vor allem die forensische Toxikologie eine rasante Entwicklung durchgemacht.

Vor wenigen Jahrzehnten lag der quantitative Schwerpunkt zweifellos noch bei der Blutalkoholanalyse. Durch die gesetzlichen Entwicklungen mit Einführung der beweissicheren Atemalkoholanalyse sind die diesbezüglichen Fallzahlen erheblich zurückgegangen.

Der Aufgabenbereich der forensischen Toxikologie hat sich seither auf andere Substanzen verlagert, seien es Suchtmittel, die im Zusammenhang mit Beeinträchtigungen im Straßenverkehr, Kriminaldelikten oder auch klinischen Drogensubstitutionsprogrammen erheblich an Bedeutung gewonnen haben, seien es medikamentöse Substanzen, die im Rahmen von klinischen Studien, therapeutischem Drug-monitoring oder auch ärztlichen Zwischenfällen qualitativ und quantitativ nachgewiesen werden müssen.

Ausblick: In den letzten Jahren hat sich ein Trend zur Spezialisierung der gerichtsmedizinischen Abteilungen abgezeichnet, der auch in der nächsten Zukunft noch anhalten und sich sogar noch verstärken wird. Bedingt durch einen immer mehr zunehmenden personellen und apparativen Aufwand wird es den einzelnen Instituten an den Universitäten nicht mehr möglich sein, eine umfassende forensisch-toxikologische Analytik anbieten zu können.

Die derzeitigen Entwicklungen in Deutschland lassen zudem befürchten, dass der Wettbewerb in naher Zukunft noch deutlich härter werden wird. Dem kann nur durch entsprechendes Qualitäts-management in Verbindung mit einer beständigen wissenschaftlichen Weiter-entwicklung begegnet werden. Eine Akkreditierung der Labors wird in Zukunft die Voraussetzung dafür sein, dass die forensischen Abteilungen weiter bestehen bleiben.

I3: Forensic Toxicology – An Interdisciplinary Field of Research

H. Oberacher, F. Pitterl, B. Beer, B. Schubert
Institute of Legal Medicine and Core Facility Metabolomics, Innsbruck Medical University, Austria.

Traditionally, forensic toxicology is a discipline getting input from diverse fields of research, including medicine, toxicology, and analytical chemistry. The aim of this presentation is to exemplify the importance of such interdisciplinary research for scientific progress in medico-legal examination. We will demonstrate the usefulness of integrating state-of-the-art analytical techniques (e.g. LC/MS) in drug screening procedures to obtain increased reliability and sensitivity.

Moreover, we will discuss the potential of genetic markers to personalize diagnosis in forensic medicine. Finally, we will show that drug metabolite information generated by *in* vitro and *in vivo* methods can be of medico-legal interest to prevent false accusations and potential negative legal consequences for examined persons.

I4: Forensic and Doping Analysis: Similarities, Dissimilarities, Disseminations

G. Gmeiner

Doping Control Laboratory, Seibersdorf Laboratories, Seibersdorf, Austria.

Background: Forensic as well as doping analysis are two highly specialized fields of analytical chemistry. Both are dealing with the unequivocal detection of active compounds, their markers and/or metabolites using highly sophisticated analytical instruments, mainly dominated by mass spectrometry after chromatographic separation in its various forms.

While doping analysis is a strictly regulated area of chemical analysis, where the analytical result serves in most of the cases as stand-alone proof for an anti-doping rule violation, forensic investigations include many different sources of evidence, where analytical evidence may contribute an important role.

Importantly the simple presence of a prohibited drug in sports drug testing is a satisfying proof for an anti-doping rule violation, whereas the forensic approach focusses on the possible effect of the drug in the analyzed bodily specimen, according to the Paracelsus principle.

Objectives: To highlight the different approaches and solutions for complex tasks in each of the two fields of chemical analysis.

Methods: Relevant regulations, analytical tools, selected cases as well as different approaches are presented, including the legal implications in court.

Results and Discussion: The presentation shows the similarities as well as the dissimilarities, pointing out the potential for dissemination of the experiences on both sides.

I5: The Position of Hair Analysis in Solving Forensic-toxicological Cases: Past, Present, Future

H. Sachs
Forensic Toxicological Center Ltd. (FTC), Munich, Germany.

Hair analysis has been used for the detection of drug intake since the early 1980ies, beginning with the examination of opiates by radio-immunological assays. First confirmatory analyses by gas chromatography/mass spectrometry have been carried out around 1985. With the development of screening methods on illegal drugs, hair analysis became widely accepted in workplace testing in the United States and in driving licence re-granting procedures in Europe. These tests were later expanded to legal drugs and became a recognized procedure in solving criminal cases as well. The instrumental development in liquid chromatography/tandem mass spectrometry (LC-MS/MS) in the past 20 years revolutionized hair analysis not only because of its screening capabilities but also because of its superior sensitivity. That way it became even possible to detect a single uptake of sedative drugs, which opened the field for hair examinations in sexual assault cases related to drug administration. Special LC-MS/MS techniques even allow the detection of drugs in a single hair which helps in determining the date of administration more precisely. It will be demonstrated that especially this procedure could clarify criminal cases. In exhumation cases hair is sometimes the only feasible tissue. For example, the detection of antidepressants after 7 years in a grave was only possible by hair analysis. Particularly due to the progress in mass spectrometry-based analytical methods, hair analysis is nowadays an equivalent if not superior tool to urine analysis in abstinence controls. More than 20,000 hair tests annually are performed in Germany for this purpose and probably more than a million worldwide, mostly in workplace testing.

I6: New Synthetic Psychoactive Drugs – A Challenge in Drug Prevention

A. Luf, A. Führer, R. Schmid
Dept. Laboratory Medicine & 'Check iT!' Project, Med. Univ. Vienna, Austria.

Background: In the past five years the number and structure multiplicity of New Synthetic Psychoactive Drugs that appeared on the gray illicit market has dramatically increased and are more and more consumed by (mainly young) adults in the leisure event scene. This behavior may pose a (high) health risk for individuals in this group.

Objectives & Methods: In a pro-active drug prevention approach the project 'Check iT!', funded by the City of Vienna, is providing since 15 years fast 'on-site' analysis of these compound class coupled with low-threshold credible substance prevention information to achieve a harm minimization.

Results and Discussion: Regular on-site drug testing showed, that of the (many) reported new amphetamine-type drugs only a limited number reach significant interest and prevalence in the event scene. Nearly anything is known of their acute and chronic toxicity. In comparison to the better known amphetamines these drugs display a similar positive and negative spectrum of symptoms. Nevertheless, analysis of information from emergency departments show that out of the large number of synthetic drugs certain members seem to display a significant higher toxicity than the rest.

Project support by 'Drogenhilfe Wien gemn.GmbH is gratefully acknowledged.

S1: Development of Mechanism-based Markers to Predict Non-genotoxic Hepatocarcinogens in Early Drug Development

M. Nejabat, T. Riegler, F. Kellner, R. Schulte-Hermann, W. Huber, B. Grasl-Kraupp

Institute for Cancer Research, Medical University of Vienna, Austria.

Background: Many chemical compounds (drugs, hormones) are non-genotoxic carcinogens (NGC). NGC are not mutagenic or genotoxic, nevertheless produce tumors, and can be identified in long-term animal bioassays only.

Conventional notion assumes NGC-driven hepatocarcinogenesis to be a mere epithelial disease. In the present work we characterized the reactivity of both, mesenchymal liver cells (MC) and hepatocytes (HC), towards two NGC, i.e. phenobarbital, an anti-epileptic drug, and cyproterone acetate (CPA), the gestagenic component of some contraceptives.

Objectives: to develop mechanism-based markers which detect reliably NGC in short-term assays.

Methods: Male Wistar rats were treated with PB, while female rats received CPA. The livers were perfused with collagenase and the cell suspension obtained was used to separate hepatocytes (HC) from mesenchymal cells (MC). The composition of MC was characterized by immune-fluorescence and FACS analyses. mRNA was extracted from HC and MC and analysed by oligoarrays (Affymetrix).

Results and Discussion: MC were found to be composed mainly of endothelial, stellate, and Kupffer cells (liver resident macrophages). CPA treatment induced altered gene expression profiles rather in HC than in MC. The opposite held true for PB with more deregulated genes in MC than in HC. MC, isolated from CPA- or PB-treated rats, showed a pronounced elevation of the messages of many chemo-/cytokines of the CXCL- and CCL-family, interleukins, and TNFα. As a consequence, HC, presumably exposed towards these cytokines, revealed activation of the TNFα- or NFkB-mediated signal transduction pathways.

To conclude, HC appear to be severely affected by NGC-activated MC, which has to be considered for a better mechanistic understanding of NGC.

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S2: Tumor Necrosis Factor (TNF-α) and TNF-α Lectin-like Domain Derived Peptides (TIP) in Alveolar Liquid Clearance

W. Shabbir[1], S. Tzotzos[2], P. Hazemi[1], V. Prymaka[1], M. Hasanovic[1], H. Fischer[2], H. Pietschmann[2], B. Fischer[2], R. Lemmens-Gruber[1].

[1]*Dep. Pharmacol. Toxicol., Univ. Vienna, Austria.*

[2]*APEPTICO GmbH, Vienna, Austria.*

Background: Impaired alveolar liquid clearance (ALC) in acute lung injury and acute respiratory distress syndrome patients is associated with a prolonged stay in the intensive care unit and longer mechanical ventilation. Reactive oxygen species (ROS) disrupt pulmonary endothelial barrier integrity and inhibit epithelial sodium channel (ENaC) activity. However, sodium absorption by ENaC is the main driving force of ALC. On the one hand TNF-α activates ROS production and reduces ENaC expression. On the other hand, TNF-α increases ALC in rats with bacterial pneumonia and increases sodium uptake in human alveolar adenocarcinoma cells that overexpress ENaC.

Objectives: To investigate the molecular mechanism underlying the modulation of ENaC current by TNF-α and TIP peptides.

Methods: Patch-clamp technique in whole cell and cell-attached configuration. ENaC in human alveolar adenocarcinoma cell line (A549); transiently transfected hENaC subunits in CHO and HEK cells; primary dog, pig and rat alveolar type II cells; human nasal epithelial cell line RPMI2650.

Results and Discussion: Similar to TNF-α, TIP peptides activate ENaC in A549, primary alveolar type II cells and transiently transfected αβγ-hENaC by increasing open probability of the channel, without exerting the cytotoxic effects of TNF-α. In contrast, an effect of TIP peptides was barely observed when applied to RPMI2650 cells. Two different populations of ENaC are expressed in cells, proteolytically cleaved, active ENaCs with high open probability, and silent/near silent ENaCs with low open probability. In RPMI2650 cells ENaCs are near silent. To activate these channels trypsin was applied and subsequently TIP peptide modulated the sodium current considerably. The cell assay data are confirmed by various preclinical animal models showing the capability of TIP peptide to accelerate oedema clearance.

Grant support by FFG is gratefully acknowledged.

S3: In Silico Predictive Toxicological Approaches in Early Drug Development - From a Regulatory Perspective

C. S. Meissner

AGES-Austrian Agency for Health & Food Safety, Institute for Marketing Authorisation of Medicinal Products & Lifecycle Management, Dept. for Clinical Trials, Preclinical and Statistical Evaluation, Traisengasse 5, 1200 Vienna, Austria.

Background: Computational toxicology data are submitted on a voluntary basis to national competent authorities in early drug development i.e. for approval of clinical trials.

Objectives: This presentation wants to give an overview on the frequency and form of *in silico* data that is submitted for clinical trial applications.

Results and Discussion: Regulatory questions raised for *in silico* approaches are discussed for *in silico* tool DEREK (http://lhasa.harvard.edu/?page=aboutDEREK.htm) used for the assessment of potential genotoxic impurities introduced during manufacturing processes of active pharmaceutical ingredients and other examples.

S4: Analytical and Toxicological Aspects of Recreational Use of Synthetic Cannabinoids

W. Bicker
FTC-Forensic-Toxicological Laboratory Ltd., Vienna, Austria.

Synthetic cannabinoid receptor agonists are evaluated since a few decades for therapeutic purposes, e.g. pain relief. In the past five years this scientific knowledge, especially concerning psychoactive side effects, was misused for introducing a new class of recreational drugs, in Europe called the "Spice" phenomenon. Preparations are made by spraying synthetic cannabinoid solutions on natural herbs. These herbal blends are marketed as "legal" alternative to marijuana. Synthetic cannabinoids mimic the psychoactive effects of delta-9-tetrahydrocannabinol (THC) but almost nothing is known at present about the associated mid- and long-term health risks.

The analytical detection of synthetic cannabinoids in biofluids poses veritable challenges: (i) Common immunochemical urine tests for detecting marijuana consumption do not show any cross-reactivity with synthetic cannabinoids and their metabolites, respectively. A broad spectrum on-site testing is thus not possible with immunochemical tests, albeit some recent technical developments hold promise. (ii) Most synthetic cannabinoids have much higher cannabinoid receptor affinities than THC and thus blood concentrations well below 1 ng/ml can already cause psychoactive effects and acute impairment, respectively. Such low concentration levels makes implementing synthetic cannabinoid screening in routine procedures rather difficult, despite using highly sensitive methods such as liquid chromatography-tandem mass spectrometry. (iii) In urine synthetic cannabinoids are almost exclusively excreted as metabolites and knowledge on biotransformation pathways is just about to evolve. (iv) The compound spectrum in "Spice"-type herbal blends changes rapidly, *i.e*. as soon as certain synthetic cannabinoids are made illegal alternative compounds quickly enter the market. Therefore analytical procedures have to be up-dated regularly and a permanent knowledge exchange between authorities and laboratories is indispensable.

The present contribution attempts to give an overview on the complex situation of current recreational use of synthetic cannabinoids. Problems associated with toxicological risk assessment and analytical detection will be discussed with the aid of own experiences.

P1: Classification of Substrates of ADMET-Relevant ABC-Transporters

M. Pinto, F. Klepsch, G.F. Ecker
Univ. Vienna, Dept Medicinal Chemistry, Pharmacoinformatics Research Group, Vienna, Austria.

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Background: ATP-binding cassette (ABC) transporters comprise a large number of membrane proteins that are actively transporting solutes across the lipid bilayer. This superfamily can be divided into 7 different subfamilies, showing distinct substrate selectivities, ranging from small hydrophobic compounds to amino acids and lipids. Because of that, a number of ABC-transporters were shown to interfere with ADMET (Absorption, Distribution, Metabolism, Elimination, Toxicity) of many drugs. Especially the transporters expressed in epithelial cells of the intestine, the liver, and the blood brain barrier, have been identified to play a critical role in altering the pK properties of compounds. Thus, early identification of substrates of these proteins is of high interest for drug development.

Objectives: Development of in silico prediction models for the identification of ABC-transporter substrates.

Results and Discussion: In silico classification models for putative substrates and non-substrates of the ABC-proteins ABCB1, ABCB4, ABCB11, ABCC2 and ABCG2 have been developed. Due to dataset imbalances, cost-sensitive algorithms have been applied, sampling different combinations of FP and FN costs. As the contribution of the physicochemical properties seemed to be similar for all subtypes, no clear substrate profiles deduced from the results of this study. However the performance of the classification models showed to be quite good, with accuracy values ranging from 0.74 (ABCC2) to 0.88 (ABCB11).

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P2: Crucial Features for the Brest Cancer Resistance Protein (BCRP/ABCG2) Inhibition

F. Montanari, G.F. Ecker
University of Vienna, Department of Medicinal Chemistry, Pharmaco-informatics Research Group

Background: BCRP is an efflux transporter from the ABC transporter family. It is expressed in the apical membrane of hepatocytes, as well as overexpressed in some cancers. As an efflux transporter, it can be responsible for resistance to drug treatments, or, when inhibited, for liver injuries. It is of great interest to be able to predict the probability of a small molecule to be an ABCG2 inhibitor.

Objectives: Collect and mind a sufficiently large dataset of BCRP inhibitors and non-inhibitors to gain a better understanding of the mechanisms of inhibition.

Methods: Data was retrieved from published studies and carefully curated. Descriptors were computed using several software. Models were built using Weka and RDKit and validated with a 10-fold cross-validation.

Results and Discussion: A dataset of 916 compounds extracted from 41 published SAR studies was generated. Analysis of the data demonstrate that inhibitors tend to have more than 5 rings, a high number of hydrophobic atoms, more than 20 aromatic bonds, and a high value of LogP(w/o), many SP2 and SP3 carbon atoms, a positive polar surface area, and less H bond donors and acceptors than the inactive compounds. These important features might provide ideas about the interactions between the inhibitor and the transporter. One could speculate that the high hydrophobicity in combination with the high aromaticity of the inhibitors would result essentially in hydrophobic and pi-pi interactions with the transporter.

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P3: An Assay-tailored Threshold Ameliorates Accuracy of Classification Models for P-Glycoprotein Inhibitors

B. Zdrazil[1], M. Pinto[1], J. Mestres[2], G.F. Ecker[1]

[1]*University of Vienna, Department of Medicinal Chemistry, Pharmacoinformatics Research Group, 1090 Vienna, Austria.*

[2]*Chemogenomics Laboratory, Research Programme on Biomedical Informatics, Universitat Pompeu Fabra, 08003 Barcelona, Spain.*

Background: P-glycoprotein (Multidrug resistance protein 1, ABCB1) inhibitors are promising agents for the use in cancer chemotherapy in order to overcome multidrug resistance. In addition, they are involved in bioavailability and safety of drugs. Thus, reliable *in silico* procedures to predict P-glycoprotein inhibiton are of great interest.

Objectives: Examining whether a classification dataset taking the individual values of a standard compound (e.g. verapamil) measured in every assay as separation criterion, is performing better than by taking a generalized threshold.

Methods: Compound bioactivity data for P-glycoprotein inhibitors was retrieved from ChEMBL_15 (410 compounds). 2D descriptors were calculated using the MOE software. Attribute selection and model building was done in WEKA 3.7.8 by using different classifier algorithms. Validation was done by 10-fold cross validation as well as by prediction of an external test set, consisting of *in-house* tested compounds.

Results and Discussion: Models built under the pre-condition of taking an assay-tailored verapamil threshold in order to separate inhibitors from non-inhibitors outperform the models with a generalized threshold when it comes to the prediction of an external test set. Best models (e.g. based on support vector machines) correctly classify positives (inhibitors) up to a rate of 90%.

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreements n° 115002 (eTOX) and 115191 (Open PHACTS), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.

P4: Impact of Oxidative Metabolism on the Toxicological Potential of Genistein in Human Colon Cancer Cells

K. Stornig, A. Schroeter, D. Marko
Department of Food Chemistry and Toxicology, University of Vienna, Austria.

Background: Genistein is one the major isoflavones found in soy and soy-based products. It has been associated with a broad spectrum of biological effects. However, beside potential beneficial effects also undesired genotoxic properties have been reported [1]. After consumption genistein might undergo phase I oxidative metabolism resulting in the formation of hydroxylated metabolites [2].

Objectives: In the present study the question was addressed whether oxidative metabolism of genistein will lead to a change in the toxicological potential focusing on cytotoxic and genotoxic effects in human colon cancer cells. **Methods**: Cytotoxic effects of genistein metabolites on the human colon cancer cell line HT29 was investigated in the LDH Assay and WST-1 Assay. The impact of the metabolites on DNA-integrity was determined by single cell gel electrophoresis (comet assay).

Results and Discussion: After 1 h of incubation of HT29 cells neither genistein nor the metabolites 6-hydroxygenistein (6-HO-GEN) and 3´-hydroxygenistein (3´-HO-GEN) mediated cytotoxic effects up to 500 µM. However, after 24 h a significant decrease in mitochondrial activity of the cells (WST-1 assay) was observed for genistein (≥ 200 µM) and 3'-OH-GEN (≥ 200µM) whereas 6-OH-GEN did not show significant toxic effects up to 500 µM. In the comet assay 3´-HO-GEN induced a significant increase in DNA damage, exceeding significantly the effects of 6-HO-GEN and the parent isoflavone genistein. **In summary** oxidative metabolism of the isoflavone genistein appears to modulate its toxicological potential.

References [1] Mortensen, A., Kulling, S. E., Schwartz, H., Rowland, I., et al., Molecular Nutrition & Food Research 2009[2] Kulling, S. E., Honig, D. M., Metzler, M., J Agric Food Chem 2001

The study was performed as part of the Doctoral College BioProMoTION.

P5: Alternariol and Altertoxin II Act as Possible Inducers of the AhR Pathway

C. Tiessen, C. Schwarz, N. Kahle, G. Pahlke, D. Marko

University of Vienna, Department of Food Chemistry and Toxicology, Währinger Straße 38, 1090 Vienna, Austria.

Background: Several mycotoxins formed by Alternaria alternata are widespread contaminants and relevant to the spoilage of food and feed (Li and Yoshizawa 2000). They are known to possess genotoxic properties thus representing a potential risk for human health. Especially alternariol (AOH) and altertoxin-II (ATX-II) have been recently reported to induce DNA strand breaks in mammalian cells (Fehr et al. 2009; Schwarz et al. 2012). However, the different modes of action for both toxins are not completely elucidated yet.

Objectives: In the present study we addressed the question whether the toxins activate the AhR pathway thus modulating the transcription of phase I enzymes.

Methods: CYP1A1 transcripts in human esophageal cells (KYSE510) were analysed by q-PCR. Furthermore CYP activity was measured with 7'-ethoxyresorufin-O-deethylase (EROD) assay.

Results and Discussion: The onset of cytotoxicity was observed after 24 h of incubation with 50 µM AOH, respectively. For ATX-II, a decrease of 30 % in cell viability was already detected at a concentration of 0.5 µM, which resulted in a different concentration range for AOH and ATX-II. Q-PCR revealed the highest induction of the transcription level of CYP1A1 at 10 µM AOH (10-fold), followed by 0.1 µM ATX-II (4-fold). ATX-II enhanced CYP activity concentration-dependently up to 130 %, whereas AOH induced an increase above 150 %. To conclude, incubation of KYSE510 cells with AOH or ATX-II leads to an increase in the transcription of CYP enzymes followed by enhanced CYP activity detected in the EROD-assay. Therefore it is conceivable that Alternaria toxins act as possible inducers of the AhR-Pathway.

Considering that AOH represents a potential substrate for CYP1A1, functionalization by enhanced CYP-activity might result in the formation of quinone structures thus potentially generating oxidative stress. Owing to the aromatic ring structure of ATX-II, it might also represent a potential substrate for CYP enzymes.

P6: The Mesenchymal – Parenchymal Interactions are Critical for Tumor Promotion by Phenobarbital

T. Riegler, M. Nejabat, F. Kellner, R. Schulte-Hermann, W. Huber and B. Grasl-Kraupp

Institute for Cancer Research, Medical University of Vienna, Austria

Background: Non-genotoxic carcinogens (NGC) do not interact with DNA or induce mutations, but induce tumor formation by promotion of "spontaneously" occurring mutated/preneoplastic cells. A large number of chemical compounds, including drugs, fall into the category of NGC. Mechanisms underlying the tumor promoting activity of NGC are incompletely understood. Better knowledge of these mechanisms would improve the early identification of NGC in drug development.

Cancer formation is not a mere epithelial disease. In the present work we address the question whether the NGC phenobarbital (PB), an antiepileptic drug, and the gestagen cyproterone acetate (CPA), a component of contraceptives, interfere with epithelial-mesenchymal interactions during hepatocarcinogenesis.

Objectives: to understand the mechanisms underlying the tumor promoting effect of PB and CPA.

Methods: Male Wistar rats received PB or tap water for 7 days, female rats were treated with a single oral dose of CPA or corn oil. Livers were perfused with collagenase and the cell suspension obtained was used to separate hepatocytes (HC) and mesenchymal cells (MC) for culture. mRNA was isolated from HC and subjected to qRT-PCR. In parallel experiments male rats received a single dose of N-nitrosomorpholine to induce the formation of preneoplastic HC. Livers were perfused, unaltered and preneoplastic HC were isolated and cultured. The secretome of cultured MC, obtained from PB- or CPA-pretreated animals, was transferred to unaltered and preneoplastic HC. DNA synthesis of the cells was determined by ^{3}H-incorporation and autoradiography.

Results and Discussion: We found that the secretome of MC, which had been isolated from PB-treated rats, induced a pro-inflammatory reaction in HC. Furthermore, an increase in the DNA replication was found, which was more pronounced in preneoplastic HC than in unaltered HC. However, the secretome obtained from CPA-pretreated MC failed to elicit a pro-inflammatory reaction and DNA synthesis induction in HC. These data indicate that (i) PB activates the hepatic mesenchyme to release pro-inflammatory cytokines and growth factors acting on unaltered and preneoplastic HC, which may contribute to tumor promotion, and (ii) the mechanisms underlying the carcinogenic action of CPA differ from that of PB. Taken together, epithelial-mesenchymal inter-actions appear to be critical for the action of certain NGC.

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P7: Adaptive Responses of Preneoplastic Hepatoctyes: Essential for Tumor Promotion by Non-genotoxic Hepato-Carcinogens?

F. Kellner, T. Riegler, M. Nejabat, R. Schulte-Hermann, W. Huber and B. Grasl-Kraupp

Institute of Cancer Research, Medical University of Vienna, Austria.

Background: Carcinogenesis is a multistep process characterized by genotoxic initiation of the cells, followed by promotion and progression. Non-genotoxic carcinogens like phenobarbital (PB) are drivers of promotion. Initiated rat hepatocytes are faced with selection pressure during tumor promotion. Our group observed that treatment with PB activates the mesenchyme in the liver, which may cause release of factors inducing stress and selecting premalignant hepatocytes. We hypothesize that these premalignant hepatocytes are better adapted and less impaired by the released factors than the surrounding unaltered hepatocytes which selects for their outgrowth.

Objectives: to investigate the possible adaption of premalignant hepatocytes to stress factors, released by the PB-activated hepatic mesenchyme.

Methods: Initiation-promotion protocol: Male wistar rats were treated with 250 mg N-nitrosomorpholine / kg body weight at the age of 3 weeks. After 3 weeks of recovery time rats received a daily dose of 50 mg PB / kg per day admixed to the diet. Animals were sacrificed after 15-18 months of treatment and liver and tumor samples were obtained. An aliquot of each tumor was used for histological diagnosis. Total RNA was extracted from control tissue, dysplastic nodules and hepatocellular adenoma. The extracted RNA was processed and labelled for application to the Affymetrix Rat Genome 230 2.0 Array.

Results and Discussion: Analysis of the transcriptome profile revealed 950 deregulated genes in PB-induced adenomas, 250 in nodules. Signal transduction pathways, which are usually altered in carcinogenesis, like Wnt-, Tgf-β- or Ras-pathway, were hardly affected in the hepatocellular adenoma. However, various metabolic pathways were altered, i.e., adenoma showed upregulation of enzymes responsible for carbohydrate metabolism and fatty acid and cholesterol synthesis. Upregulation of both oxidative and nonoxidative pentose-phosphate pathway suggests provision of riboses for nucleotide synthesis and NADPH for the production of lipids. An altered cytokine pattern together with upregulated enzymes producing lactate may cause immune surveillance.

Generally, profound alterations in the transcriptome profile were observed, indicating outgrowth of adapted premalignant hepatocytes in a stressful environment.

Grant support by the European Union Innovative Medicines Initiative (IMI JU) under Grant Agreement no 11 5001 is gratefully acknowledged.

P8: On the Mechanism of hERG Channel Block by Propafenone Analogs

P. Saxena, T. Erker, F. Bauer, A. Weinzinger, T. Linder, S. Hering, E. Timin

Department of Pharmacology and Toxicology, University of Vienna.

Department of Medicinal Chemistry, University of Vienna.

Background: hERG (*human ether-a-go-go related gene*) channel inhibition can delay the repolarization of the cardiac action potential, induce the prolongation of the QT interval (long QT-syndrome) and increases the risk of life-threatening *torsade de pointes* (TdP) arrhythmia. Cardio toxic effects due to hERG channel block are cause by a surprisingly diverse group of drugs. Understanding the mechanisms of channel inhibition is therefore important for the development of safe pharmaceuticals.

hERG (Kv11.1) channel inhibitors can be trapped in the channels at rest. The structural peculiarities of hERG blockers that enable trapping or alternatively resting state dissociation are currently unknown. Propafenone (small molecule, MW 341 g/mol) is efficiently trapped in the closed hERG channel pore [(1)].

Objective: To investigate whether the size of the blocking molecule plays a role in trapping we synthesized bulky attached by piperazine linkers, with molecular weights propafenone derivatives containing benzoyl and trimethylphenyl side chains, of 500 (Fba212) and 650 g/mol (Fba213) respectively.

Methods: hERG channels were expressed in Xenopus laevis oocyte and potassium current inhibition was studied using the two-microelectrode voltage clamp technique.

Result and discussion: It was found: first, both compounds are potent hERG blockers with IC50 3.7µM (Fba212) and 52µM (Fba213). Secondly, channel block by Fba212 and 213 was prevented by mutations Y652A and F656A as previously shown for propafenone. Third, both the propafenone derivatives were trapped at rest. To obtain insights into the molecular mechanism of channel block docking experiments with Fba212 and Fba213 in closed and open conformation were performed. Both compounds interact with the propafenone binding site (Y652A and F656A). Fba213 was found to exceed the size of the closed channel cavity of our hERG homology model. We conclude that drug trapping in hERG channels does not necessarily require full closure of the activation gate.

References: 1. Witchel HJ, Dempsey CE, Sessions RB, Perry M, Milnes JT, Hancox JC, Mitcheson JS.
Mol Pharmacol. 2004 Nov; 66(5):1201-12.

P9: Predictive Toxicology in Applied Risk Assessment Regarding Material Qualification

B.J. Majer, R. Stidl, B. Dietrich, E.M. Muchitsch
Preclinical Pharmacology & Toxicology
Baxter Innovations GmbH.

The prediction of toxic effects and adverse events is a central element in drug safety assessment. Usually a tolerable exposure is extrapolated from available *in vivo* data by use of appropriate uncertainty factors. In many cases, especially in the evaluation of extractables & leachables in the context of material qualification, experimental toxicity data are unavailable. In these cases *in silico* tools for predictive toxicology represent an alternative to animal testing. The first step of an *in silico* assessment is read-across analysis by means of chemical similarity. If enough experimental data from similar compounds are available, a tolerable exposure can be extrapolated based on expert judgement. Otherwise (quantitative) structure-activity relationship [(Q)SAR] tools may be applied to support an appropriate safety limit. Yes/No decisions (e.g. on genotoxicity) can often be established by open source software, whereas quantitative analysis is mainly limited to commercial tools. In some challenging cases the substances of concern (e.g. impurities) cannot be structurally identified by chemical analysis. Here the threshold of toxicological concern (TTC) concept is applied to establish an appropriate threshold level. In this regard the Cramer decision tree can be a useful tool to derive conservative safety levels.

P10: Piperine Derivatives as New GABA(A) Receptor Ligands

A. Schöffmann[1], L. Wimmer[2], T. Schwarz[3], T. Erker[3], M. D. Mihovilovic[2], S. Hering[1].

[1]Inst. of Pharmacology and Toxicology, University of Vienna, Austria; [2]Inst. of Applied Synthesis, Vienna University of Technology, Vienna; [3]Inst. of Pharmaceutical Chemistry, Univ. of Vienna.

Background: GABA(A) receptors are the major inhibitory receptors in the central nervous system. Among eleven subtypes with distinct pharmacological properties, the α1β2γ2S subtype is the most abundant subunit combination in the human CNS. Clinically applied pharmaceuticals acting via the GABAA receptor, such as benzodiazepines and barbiturates, display serious side effects, which in contrast are rarely observed with natural products. In Asian and African folk medicines, black pepper (*Piper nigrum*, Piperaceae) is known as sleep inducing and sedative remedy, and was proposed to hold antiepileptic potential. Piperine could be demonstrated to be a positive GABA(A) receptor allosteric modulator(1) offering a new scaffold for antiepileptic drug development.

Objectives: To examine a series of 78 piperine derivatives for their effect on $GABA_A$ receptors in an in-vitro functional assay.

Methods: Modulation of GABA-induced chloride currents through $GABA_A$ $\alpha_1\beta_2\gamma_{2S}$ receptors is determined using two-micorelectrode voltage-clamp by means of a fast perfusion system(2).

Results and Discussion: A screening of 78 derivatives at 100 μM yielded 14 compounds potentiation the $GABA_A$ α1β2γ2S receptor more efficiently than piperine. Concentration-response curves evaluated efficacy and potency for these compounds, revealing distinctive structure-activity patterns.

Efficacy of piperine derivatives could be significantly increased by substituting the piperidine moiety with *N*,*N*-dipropyl, *N*,*N*-diisoprpyl, *N*,*N*-dibutyl, p-methylpiperidine, or *N*,*N*-ditrifluorethyl groups. Potency of piperine derivatives could be enhanced through modifying the piperidine moiety to *N*,*N*-dibutyl, *N*,*N*-diisobutyl, or *N*,*N*-trifluoroethyl groups. Rigidification of the linker moiety did not influence the effect on $GABA_A$ receptor as previously expected, but provided reasonable efficacy and potency. The combination of these approaches seems promising in the development of a new scaffold for antiepileptic drugs derived from natural products.

References: 1. Zaugg J, Baburin I, Strommer B, Kim HJ, Hering S, Hamburger M. J Nat Prod. 2010 Feb 26;73(2):185-91. doi: 10.1021/np900656g.

2. Baburin I, Beyl S, Hering S. Pflugers Arch. 2006 Oct;453(1):117-23. Epub 2006 Sep 5.

P11: Effects of Oxime K048 on Acetylcholinesterase Activity and Oxidative Response in Tabun Exposed Rats

S. Žunec, A. Lucić Vrdoljak, N. Kopjar
Institute for Medical Research and Occupational Health, Zagreb, Croatia.

Background: The widespread use of organophosphorous (OP) compounds as pesticides and the misuse of nerve agents emphasize the need for effective antidotal preparedness. The toxic symptomatology of OP is caused primarily by irreversible inhibition of the serine-protease acetylcholinesterase (AChE), but the data on OP overall mechanisms of toxicity are still incomplete. Anticholinergics such as atropine and AChE reactivators - oximes are used as first aid antidotes for OP intoxications. However, reactivation by oximes is not possible in all cases of OP poisoning, especially in the case of nerve agent tabun.

Objectives: To evaluate the efficiency of oxime K048 as therapy against tabun poisoning and to study whether the oxidative stress is involved in the mechanism of OP toxic effects.

Methods: Male rats were injected subcutaneously with sublethal dose of tabun, and treated intraperitoneally 1 min later with K048 plus atropine. Plasma and tissue samples were analyzed for AChE activity and levels of oxidative stress and DNA damage (lipid peroxidation, superoxide dismutase and the alkaline comet assay).

Results and Discussion: Tabun exposure resulted with a high degree of AChE inhibition (~90% in the plasma and 75% in the brain). Therapy combined of K048 and atropine efficiently counteracted tabun poisoning by restoring ~50% of AChE activity in plasma up to 1 h. This result indicates good reactivation potential of oxime K048. Beside anticholinesterase activity, tabun showed stressogenic effects by increasing LPO, SOD activity and primary DNA damage in the brain during 24 h exposure. We can conclude that tabun poisoning caused an excessive formation of free radicals which could associate oxidative stress with the nerve agent's neurotoxicity.

P12: FGFR3 May be a Therapeutic Target for Hepatocellular Carcinoma

J. Paur, D. Huber, C. Maier, W. Schrottmaier, B. Marian, W. Berger, M. Grusch, K. Holzmann, B. Grasl-Kraupp
Institute for Cancer Research, Medical University of Vienna, Austria.

Background: Hepatocellular carcinoma (HCC) is often caused by toxic effects of chemicals like ethanol, nitrosamines and aflatoxin B1. While the etiological factors are often known, the pathogenetic mechanisms involved in tumor formation and progression are incompletely understood.

The prognosis of this disease is poor and therapeutic options are limited and require urgently the identification of new targets. Upregulation of fibroblast growth factor receptor 3 (FGFR3) was found in a subset of HCC. Therefore, we investigated the impact of FGFR3 in the malignant behaviour of HCC and examined the effects of FGFR3 knockdown on clone formation at low-density and anchorage-independent growth.

Objectives: To examine the role of FGFR3 for the malignant phenotype of HCC cells.

Methods: FGFR3 expression of human hepatoma/ hepatocarcinoma cell lines was determined by Western blotting. In vitro assays were performed with human HCC model cell lines transiently over-expressing adenoviral KD3, a kinase-dead form of FGFR3. Alternatively, FGFR3 was knocked down in the cells by siRNA. Survival and clonogenic growth at low density was investigated. Anchorage-independent growth, which presents a meaningful tool to estimate the cells´ capability to form tumors in-vivo, was determined by the soft agar assay.

Results and Discussion: In all HCC cell lines used, knockdown of FGFR3 through siRNA as well as KD3 reduced the growth and clone formation at low-density. Anchorage-independent growth was also impaired after knockdown of FGFR3.

Based on our results we postulate that FGFR3 plays an important role in the progression of HCC. Accordingly, impaired FGFR3-mediated signalling may be an interesting approach in tumor therapy. Further mechanistic studies are required to improve our understanding on the significance of FGFR3 as therapeutic target in HCC patients.

Grant support by FWF is gratefully acknowledged.

P13: Impairment of the Interaction between Hepatocarcinoma and Endothelial Cells by Knock-down of FGFR3 – a Promising Anti-metastatic Therapy?

D. Huber[1], J. Paur[1], B. Marian[1], K. Holzmann[1], W. Berger[1], G. Krupitza[2], M. Grusch[1], B. Grasl-Kraupp[1]

1 Institute for Cancer Research, Medical University Vienna; 2 Clinical Institute of Pathology, Medical University Vienna.

Background: Hepatocellular carcinoma (HCC) is one of the most common cancer entities worldwide with a steadily increasing incidence in many countries. Therapeutic options are poor and the mortality rate is extremely high due to early hematogeneous or lymphatic dissemination. Impairment of this pro-metastatic process would be a valuable therapeutic tool.

Upregulation of fibroblast growth factor receptor 3 (FGFR3) was found in a subset of HCC. Therefore, we investigated the impact of FGFR3 on the interaction of hepatocarcinoma and lymphatic/blood endothelial cells by knocking down FGFR3.

Objectives: To understand the impact of FGFR3 on cell-cell interaction.

Methods: In human hepatocarcinoma cell lines the expression of FGFR3 was analyzed by qRT-PCR and Western blotting after knock-down by siRNA or by an adenoviral construct (KD3), which harbours a mutation in the kinase domain of FGFR3. The interaction between human HCC and blood or lymphatic endothelial cells was examined by the gap assay.

Spheroid formation of HCC cell lines was induced by methylcellulose. The spheroids formed were placed on a lymphatic/blood endothelial cell monolayer (stained with cytotracker) in order to enable cell-cell interactions and gap formation in the monolayer.

Results and Discussion:

Knock-down of FGFR3 through siRNA significantly reduced the gap size in two types of endothelial cells by HCC-1.2 cells. The adenoviral (KD3) knock-down did not affect the interaction of HCC-1.2 cells with the endothelial monolayers but reduced significantly the gap forming capacity of HCC-3 and Hep3B cells.

To conclude, knock-down of FGFR3 by two different approaches impaired the interaction of hepatocarcinoma cells with two types of endothelial cell monolayers. Based on these results we postulate that interference with FGFR3-mediated signalling may decrease the probability of the extravasation or intravasation of aggressive hepatocarcinoma cells through lymphatic and blood vessels. The application of FGFR3-targeting appraoches as an anti-metastasis therapy deserves further investigations.

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P14: Impact of Glutathione Peroxidase 4 Overexpression on Human Hepatocellular Carcinoma Cells.

N. Rohr-Udilova[1], D. Stoiber[2], R. Brigelius-Flohe[3], K. Stolze[4], R. Eferl[5], M. Peck-Radosavljevic[1]

[1]Department of Gastroenterology and Hepatology, CIM III, Med. Univ. Vienna, Austria; [2]LBI for Cancer Research, Vienna, Austria, [3]German Institute for Nutrition, Potsdam, Germany; [4]Veterinary University of Vienna, Vienna, Austria, [5]Institute for Cancer Research, CIM I, Med. Univ. Vienna.

Background: Glutathione peroxidase 4 (GPx4) is a selenium containing antioxidative enzyme which efficiently reduces lipid hydroperoxides. Inhibition of GPx4 by siRNA has been shown to increase VEGF and IL-8 formation of hepatocellular carcinoma (HCC) cells (Rohr-Udilova, Hepatology, 2012). **Objectives:** We intend to investigate how GPx4 regulates these factors since elevated levels of both, VEGF and IL-8, are associated with bad prognosis of HCC. **Methods**: For that purpose, expression plasmids with the porcine GPx4 gene under control of the CMV promoter were transfected into cultured human HCC-3 hepatocarcinoma cells. The GPx4 transfection efficiency was evaluated by real time RT-PCR, western blotting and activity measurements. Intrinsic and induced oxidative stress, cell cycle progression and expression of IL-8 and VEGF genes were investigated. The effect of GPx4 on tumour growth in vivo was assessed using subcutaneous transplantation of HCC cells into recipient mice.

Results and Discussion: GPx4 overexpression increased the survival of HCC cells treated with ROS-inducing agents such as hydrogen peroxide or linoleic acid peroxide (LOOH). Moreover, the DHFC fluorescence was reduced in untreated and LOOH-treated GPx4 overexpressing HCC cells. However, overexpression of GPx4 impaired cell cycle progression. The increase of HCC cells in G2/M phase as observed after LOOH treatment was prevented by GPx4 overexpression. The induction of IL-8 but not VEGF by LOOH was also prevented in GPx4 overexpressing cells. In a pilot in vivo experiment, less and smaller tumours were formed by transplanted GPx4 overexpressing HCC cells. Mouse VEGF and the IL-8 analogue CXCL1 were expressed at lower levels in tumours derived from GPx4 overexpressing cells.

Thus, GPx4 overexpression decreases the malignant potential of HCC cells.

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P15: The Organic Anion Transporting Polypeptide OATP4A1 May Contribute to Immunactivation in Colorectal Cancer and Inflammatory Colon Disease.

S. Zotter[5], A. Larijani[5], V. Buxhofer-Ausch[1,3], C. Ausch[2,3], H. Bauer[3], M. Mollik[4], E. Bajna[5], C. Sebesta[1], R. Zeillinger[3], A. Reiner-Concin[4], T. Thalhammer[5]

[1]2nd Department of Medicine, Donauspital, Vienna, Austria; [2]Department of Surgery, Donauspital, Vienna, Austria; [3]Ludwig Boltzmann Society, Cluster for Translational Oncology, Vienna, Austria; [4]Department of Pathology, Donauspital, Vienna, Austria; [5]Department of Pathophysiology, Medical University of Vienna, Vienna, Austria.

Background: Organic anion transporters (OATPs) including OATP4A1 may influence tumor progression by mediating uptake of hormones, cyclic nucleotides, second messenger proteins and drugs.

Methods: We analysed OATP4A1 in paraffin-embedded specimens from colorectal cancer and non-malignant diverticulitis by immunohistochemistry using an automatic quantitative microscopic image analysis system (TissuesFaxs, Histoquest program). To identify OATP4A1 expressing cells, double-immunofluorescence staining was done with antibodies against markers for epithelial, stroma and immune cells.

Results: OATP4A1 was abundantly present in the membrane and cytosol of colon cancer cells. The immunoreactive score (IRS), calculated from the staining intensity and the number of OATP4A1-positive cells, was significantly higher in the cancerous than in

non-cancerous areas of the tumors (1528±326 vs.376±218, n=50). Highest OATP4A1 levels were observed in immune cells in the tumors, while OATP4A1 in tumor stroma was low (2839±381 vs.298±56). Importantly, immune cells in tumors with early relapse had significant less OATP4A1 compared to relapse-free tumors (IRS:2784±298 vs IRS:6012±672, $p>0.05$). High values for OATP4A1 were also seen in diverticulitis samples for immune cells, but not for mucosa cells. OATP4A1 expressing immune cell subtypes in cancer and diverticulitis sections included CD45+ leucocytes, CD3+ T- and CD20+ B-lymphocytes.

Conclusion: The higher expression of OATP4A1 in immune cells in samples from diverticulitis and colon cancer without early relapse suggests a role of the transporter in the activation of the immune system. It may give a potential target for immunostimulatory drugs.

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P16: The Estrogen Sulfatase Pathway: A Potential Target in Ovarian Cancer Treatment

L. Ndue[1], P. Allinger[1], S.Aust[2], M. Svoboda[1], L. Klameth[1], L. Secky[1], D. Cacsire Castilo-Tong[2], R. Zeillinger[2], T. Van Gorp[4], I. Vergote[5], I. Braicu[5], J. Sehouli[6], S. Mahner[7], M. Fogel[8], T. Thalhammer[1]

[1]Department of Pathophysiology and Allergy Research, [2]Molecular Oncology Group, Clinic for Gynecology and Obstectics, Medical University of Vienna, Austria,, [3]Department of Gynecology, European Competence Center for Ovarian Cancer; Campus Virchow Klinikum, Charité - Universitätsmedizin Berlin, Berlin, Germany, [4]Division of Gynaecological Oncology, Department of Obstetrics and Gynaecology, Universitaire, [5]Ziekenhuizen Leuven, Katholieke Universiteit Leuven, Leuven, Belgium; [6]Department of Gynecology and Gynecologic Oncology, [7]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [8]Department of Pathology, Kaplan Medical Centre, Rehovot, Israel.

Background: Expression of estrogen receptors (ERs) alpha/beta and local formation of active estrogens from inactive estrone-3-sulfate (E1S) by estrone sulfatase may be implicated in the progression of epithelial ovarian cancer (EOC), particularly if associated with inflammation.

Methods: We investigated expression of ERalpha/beta, estrone sulfatase (STS), generating active E1 from inactive E1-Sulfate, as well as COX-2, the main enzyme for the production of the proinflammatory prostaglandin E2 in samples from 172 EOC by quantitative RT-PCR and by automated quantitative microscopic image analysis on a subset of paraffin-embedded EOC tissue sections. Correlation of these parameters with clinicopathological features (tumor-free resection, ascites, p53, HER-2) and their prognostic impact were statistically analyzed.

Results: STS, COX-2, ERalpha and ERbeta mRNA were highly expressed in all EOR samples. However, ERalpha, but not ERbeta, expression is a favourable prognostic factor for overall survival in univariate and multivariate analyses (HR = 0.87; 95% CI 0.78-0.96). ERalpha gene expression values obtained from RT-qPCR correlated with the ERalpha immunoreactivity in tumor cells in EOC tissues (p=0.007). Immunoreactive STS, COX-2, ERalpha and ERbeta were detectable in the tumor cells in EOR samples, but STS was mainly located in the tumor stroma in some patients. Remarkably, STS mRNA expression is significantly higher in EOCs from premenopausal women (p=0.007), but is not related to overall and progression-free survival.

Discussion: In patients with serous EOC, high levels of ERalpha, ERbeta, STS and COX-2 indicate the importance of estrogen signalling in these tumors. Therefore, the estrogen-activating system in EOCs might be target for therapeutic intervention.

P17: Conversion of 5-Hydroxymethyl-5-methyl-pyrroline *N*-oxide to the Two Novel Spin Traps 5-Acetoxymethyl-5-methylpyrroline *N*-oxide and 5-Hexanoyloxymethyl-5-methylpyrroline *N*-oxide

K. Stolze[1], P. Jodl[1], N. Rohr-Udilova[2], and T. Rosenau[3].

[1]Institute of Pharmacology and Toxicology, Dept. Biomedical Sciences, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria. [2]Div. Gastroenterology and Hepatology, Clinic of Internal Medicine III, Medical University of Vienna, A-1090 Vienna, Austria. [3]Dept. of Chemistry, Division of Chemistry of Renewables, University of Natural Resources and Life Sciences (BOKU), Muthgasse 18, A-1190 Vienna, Austria.

Background: The free radical trapping properties of two novel compounds synthesized from the spin trap 5-hydroxymethyl-5-methyl-pyrroline N-oxide (HMMPO) are reported. HMMPO and the novel compounds are derivatives of the well-known spin trap 5,5-dimethyl-pyrroline N-oxide (DMPO). They were synthesized as model compounds for the development of more site-specific spin traps such as compounds capable of detecting radicals within mitochondria or other organelles. Objectives: The aim of the present study was to investigate whether a spin trap carrying a reactive group (e.g. a hydroxy group) can be modified according to the desired properties using a variety of different reactive substrates.

Methods: MS, IR, NMR and ESR spectroscopy.

Results and Discussion: A first attempt was made with the respective alkanoyl chlorides in the presence of weak bases capable of binding the evolving hydrogen chloride (e.g. triethyl amine or pyridine). This approach unfortunately caused formation of many dark-coloured degradation products. The use of the respective carbonic acid anhydrides was successful in the case of acetic anhydride and hexanoic acid anhydride, while other anhydrides gave low yields or products that were difficult to isolate and purify. Our approach to use either a symmetric or asymmetric anhydride derived from a triphenylphosphonium (TPP)-containing carbonic acid was, however, not yet successful. The latter TPP-moiety is expected to render the respective spin traps more specific to mitochondria, as has previously been demonstrated by other groups for similarly modified spin traps, e.g. “Mito-DEPMPO”. The spin-trapping activity of these compounds towards different carbon-centered radicals derived from methanol, ethanol, and formic acid, generated in the presence of a Fenton-type system, was also tested.

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P18: Oxidative Stress and DNA Interactions Are not Involved in Enniatin- and Beauvericin-induced Cytotoxicity

R. Dornetshuber-Fleiss[1,2], P.Heffeter[1], R. Lemmens-Gruber[2], L. Elbling[1], D. Marko[3], M. Micksche[1], W. Berger[1]

[1]*Department of Medicine I, Institute of Cancer Research, Medical University of Vienna*

[2]*Department of Pharmacology and Toxicology, University of Vienna*

[3]*Institute of Applied Biosciences, Section of Food Toxicology, University of Karlsruhe*

Background: Beauvericin (BEA) and enniatins (ENN) are cyclodepsipeptidic secondary metabolites of several Fusarium strains which currently came into focus of interest as anticancer drugs. Prior to moving the drug into clinical testing it is crucial to test the possible side effects of drug candidates. For instance deleterious effects of oxidative stress are implicated in a variety of drug-induced toxicities like in the drug-related side effects of doxorubicin-induced cardiac damage, azidothymidine-induced myopathy, and cisplatin-induced ototoxicity.

Objectives: This study aims to clarify whether ROS and DNA interactions are involved in ENN- and BEA-mediated cell death.

Methods: Pro-oxidative properties: cell permeant dye DCFH-DA. Induction of DNA strand breaks by oxidative stress: alkaline comet assay. Topoisomerase I activity: relaxation of supercoiled pGEM1 plasmid DNA by nucleic extract from MCF-7 cells. Topoisomerase II: decatination assay. Impact of repair mechanisms on ENN and BEA-mediated cytotoxicity: cell viability assays.

Results and Discussion Data indicated that oxidative stress does not contribute to ENN- and BEA-induced cytotoxicity. In contrast, both fusariotoxins were shown to exert moderate antioxidative activities. Moreover, only at high concentrations both mycotoxins were found to intercalate substantially into dsDNA and to inhibit the catalytic activity of topoisomerase I and II. The potent cytotoxic activity of ENN and BEA was shown to be widely independent of cellular mismatch- and nucleotide excision repair pathways. Also the ataxia-telangiectasia mutated (ATM) protein kinase, a well-known DNA damage sensor, did not affect BEAs cytotoxic potential while in ENN-induced cytotoxicity ATM had a detectable but not a major modulating influence. Together, our data suggest that ROS and DNA damage are not key factors in ENN- and BEA-mediated cytotoxicity.

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References: Dornetshuber et al. (2009). Oxidative stress and DNA interactions are not involved in Enniatin- and Beauvericin-mediated apoptosis induction. Mol Nutr Food Res 53(9):1112-22

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P19: Acetaminophen Affects Intestinal Cell Membrane Properties

M. R. Lornejad-Schäfer, C. Schäfer, K. R. Schröder
BioMed-zet Life Science GmbH, Industriezeile 36/I, 4020 Linz, Austria.

Background: Drugs can reduce the net intestinal absorption followed by malnutrition and/or drug resistance.

Objectives: The aim of this study was to find out if and how Acetaminophen (APAP) known as paracetamol, a widely used pain reliever and fever reducer, affects intestinal cell membrane properties in a Caco-2 barrier model?

Methods: To construct a Caco-2 barrier model, the Caco-2 cells were seeded onto inserts (Millipore, 0.4µM pore size) for 21 days. After differentiation, the cells were treated apically with 10 mM APAP for 24h. The cell transepithelial electrical resistance (TER) and capacitance (Ccl) were determined by two different impedance measuring systems. The cell surface was investigated using Scanning electron microscopy (SEM). The membrane permeability was tested with different-sized (FITC)-dextran molecules. The tight junction proteins were investigated using western blot analysis.

Results and Discussion: APAP changed the cell membrane properties in the Caco-2 barrier model through different mechanisms: reduction of the number of microvilli and enhancement of MDR1 activity which went along with a significant decrease in the cell capacitance. Furthermore, APAP increased the membrane integrity (TER value) and modulated the tight junction proteins followed by a decreased permeability of small molecules.

Conclusion: Acetaminophen changed the intestinal cell membrane properties which may reduce the net intestinal absorption of administered foods and drugs.

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P20: Influence of engineered silica nanoparticles on the proliferation and signalling pathways in gastrointestinal cells

H. Gehrke, E-M. Fritz, D. Marko.
University of Vienna, Institute of Food *Chemistry and Toxicology, Währinger Straße 38, 1090 Vienna, Austria*

Background: The use of nanostructured silica particles (SiO_2-NPs) has been extended from biomedical and biotechnical fields to applications in cosmetics, varnishes and food. Thus the environmental and health impact of nanoscaled SiO_2 is of great interest. While previous studies mainly focused on the toxicity of SiO_2-NP in the respiration tract, this study contributes to the rarely investigated effects in cells of gastrointestinal origin. Therefore the present study on the toxicological relevance of SiO_2-NPs focused, with respect to the different particle sizes of SiO_2-NPs (12, 40, 200 nm), on proliferative effects as well as on the influence on cellular signalling pathways, including the MAPK/ERK1/2 as well as the Nrf2/ARE pathway [1].

Material and Methods: The investigated particles were all commercially available and were used in a concentration range from 15.6 µg/cm² to 156.3 µg/cm² (50 µg/ml – 500 µg/ml). The study was performed in different human carcinoma cell lines, all originating from the gastrointestinal tract (KYSE510 - oesophagus, GXF251 – stomach, HT29 - colon). Influence on the cell growth of the different cell lines was determined with the SRB-assay, both for different particles sizes and incubation times (24, 48 and 72 h). Furthermore, interference with the MAPK/ERK1/2 pathway was investigated by Western blot analysis, while the effects on Nrf2/ARE-driven genes were analysed by qPCR.

Results and Discussion: The results of this study indicate that the investigated SiO_2-NPs may stimulate the proliferation/cell growth of human carcinoma cells, depending on the incubation time as well as on the particle size. Furthermore, the results showed that the effects discern within the different cell lines. While for the HT29- as well as for the KYSE510-cells the most pronounced effects was observed for the smallest SiO_2-NPs (12 nm), for the GXF251-cells the cell growth was most affected by 40 nm SiO_2-NPs. In contrast, 200 nm SiO_2-NP showed no influence on the cell growth at all. Further investigations in HT29-cells revealed that these effects were mediated by interference with the MAPK/ERK1/2, leading to an increased phosphorylation of ERK1/2. Additionally, interactions with the Nrf2/ARE signalling pathway were observed, by an increased transcription of the Nrf2/ARE-driven gene γ-GCL. However, this effect could be suppressed by co-incubation with a specific MEK-inhibitor (PD98059), indicating a cross-like between these two pathways.

Conclusion: In summary, the results of the study indicate that these SiO2-NPs elicited cell growth may be mediated by interference with the MAPK/ERK1/2 and/or the Nrf2/ARE signalling pathways, both deeply involved in the regulation of cellular processes like the antioxidative defence system as well as the cell cycle progression.

P21: Establishment of a treatment scenario to detect fixed point mutations in human airway epithelia

K. Sommer[1], E. Bradt[1], S. Constant[2], D. Breheny[3], K.R. Schröder[1]

[1]*BioMed zet Life Science GmbH, Industriezeile 36/I, A-4020 Linz, Austria*

[2]*Epithelix Sàrl, 14, Chemin des Aulx, CH-1228 Plan les Ouates, Geneva, Switzerland*

[3]*British American Tobacco, GR&D Centre, Regents Park Road, Southampton, SO15 8TL, United Kingdom*

Background: MucilAir™ is an *in vitro* test-system of primary human respiratory epithelium with a shelf life of about one year. It reflects the natural human bronchial epithelium containing basal cells, epithelial cells with cilia in motion and goblet cells producing surfactant. The long shelf life suggests the model to be used for *in vitro* assays to study long term effects.

Objectives: This system is analyzed for its potential to detect all phases of carcinogenesis, starting with initial events causing DNA lesions, promoting substance effects and progressive outcome at the end of this process.

Methods: Tumour initiators like MNNG and MNU, producing point mutations as a consequence of O^6-alkylguanine adducts and TPA as tumour promoter are used to characterize the test system.

Results and Discussion: For manifestation of the induced lesions, two rounds of replication are necessary. Hence, differentiated cells of the quiescent epithelia had to be forced into mitosis and the right time point for the treatment had to be found. Regenerative growth led to an increase of the mitosis rate 7 days after wounding, when also the treatment was started. The DNA damaging action of MNNG and MNU could be proven with indirect parameters like the phosphorylation of histones upon DNA double strand breaks using Western Blot and Immuno Fluorescence analysis.

The next step is the direct prove of fixed point mutations by "Next Generation Sequencing (NGS)" using specific cancer panels. The aim is to treat the epithelia as harsh as possible to increase the possibility to gain fixed point mutations without inducing DNA breaks. A respective treatment scenario is established.

P22: Rapid Detection of *Amanita muscaria DNA* Traces in Different Matrices

C. Gausterer[1], M. Penker[1], C. Stein[1], T. Stimpfl[2]

[1]*Forensisches DNA Zentrallabor, Med. Univ. Vienna, Austria.*

[2]*Clin. Inst. of Laboratory Medicine, Med. Univ. Vienna, Austria.*

Background: Accidental poisoning with certain mushroom species from the genus Amanita poses a challenge to clinical toxicology worldwide, because of the relatively high mortality rate, the long latency period and the narrow time window for effective medical interventions. Current standard testing is also limited concerning the types of samples that can be used for the detection of the amatoxins.

Objectives: To examine whether a direct PCR approach is feasible for the detection of fungal traces in sample types that may be available in clinical cases of suspected mushroom poisoning.

Methods: Fly agaric (*Amanita muscaria*) and cultivated champignon (*Agaricus bisporus*) specimens served as fungal materials for experimental testing. *A. muscaria* was chosen not only because it belongs to the genus Amanita, but it also does not contain amatoxins. Novel PCR primers were designed and evaluated for the specific detection of *A. muscaria* in samples containing processed (raw, fried, artificially digested) mixed mushrooms and fecal samples (feces mixed with mushrooms, feces from an ingested mushroom meal), respectively.

Results and Discussion: The novel PCR primers allowed application of a rapid two-step cycling protocol. Assay sensitivity was sufficient for single cell analysis. Specific amplification of target DNA was not compromised by a vast excess of non-target DNA (i.e. from champignon, human and human feces, respectively). Target amplification by direct PCR was successful with raw, fried and "artificially digested" mushrooms. Furthermore, it was possible to detect the presence of *A. muscaria* remains in extracts from a stool sample that had been harvested 44 hours after ingestion of a mushroom meal.

P23: Cobalt and Tungsten Carbide: Planning of an Occupational Cohort Study

H. Moshammer
Inst. Env. Health, ZPH, Med. Univ. Vienna, Austria.

Lung cancer due to Cobalt is well established. Is the risk enhanced by the combined impact of cobalt and tungsten carbide in the hard-metal industry? American researchers are coordinating an international historical cohort study of workers in the hard-metal industry. From Austria we will provide data from a large industrial plant.

As a first result of the cooperation between the plant and out institute a cross-sectional study was designed based on a questionnaire directed to all present workers and to past workers with still valid addresses. This questionnaire served two purposes: (1) to announce the aim of the cohort study and (2) to obtain more detailed data on smoking history and general health history than is available in the company records.

In spite of repeated advertising of the questionnaire by the plant management only approximately 10% of all addressees (233 persons in total) responded. Active workers were overrepresented while only 78 (mostly only recently) retired workers completed the questionnaire. Also, current white collar workers were overrepresented (58 persons).

Although respiratory disease or hypertension were each reported by about 10% of respondents the subjective health status was generally good. Better health was reported by office workers while working in departments with the highest dust exposure was not associated with poorer health. Increasing age did not consistently lead to higher symptom rates while smokers reported poorer health not only for respiratory but also for rheumatic and psychiatric symptoms.

We will also report on the progress of our ongoing work on the international cohort study. Data confidentiality issues increasingly pose a problem with epidemiological studies.

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P24: Heavy Metals in Patients with Coronary Artery Disease (CAD): Sex Matters?

M. Sponder, B. Köhler-Vallant, M. Uhl, M. Mittlböck, J. Strametz-Juranek
Department of Cardiology, Med. Univ. Vienna
Institute of Clinical Biometrics, Med. Uni Vienna
Environment Agency Austria, Vienna.

Background: Heavy metals like cadmium (Cd), lead (Pb) and mercury (Hg) are suspected to be associated with cardiovascular disease (CVD), generating a "catchy" basis for the formation of atherosclerotic plaque, interacting with the renin-angiotensin-system increasing angiotensin-converting enzyme and ROS-production leading to endothelial dysfunction. Zinc (Zn), an essential trace element, seems to support NO-derived protection of endothelial cells. **Objectives:** This study aimed to investigate whole blood and urine levels of environmental heavy metal in patients with CAD. **Results and Discussion:** A total of 202 non/ex-smoking CAD-inpatients at the Dept. of Cardiology were studied. Cd was determined in urine after microwave digestion by ICPMS; Pb, Hg and Zn were analyzed after microwave digestion in whole blood by ICPMS resp. AFS. The Human-Biomonitoring-I-values (HBM-I) which indicate no elevated health risks were taken from the German Environment Agency. The detection/quantification/ HBM-I limits were 0,4/2/? µg/l (Pb), 0,067/0,13/5 µg/l (Hg), 14/50/? µg/l (Zn) and 0,12/0,40/1 µg/l (Cd). Males (age 60,35±11,32 years, BMI 27,91 kg/m^2) showed higher median levels for Cd (1,05 vs. 0,89 µg/l), Pb (21 vs. 18 µg/l), Hg (0,60 vs. 0,45 µg/l) as well as Zn (6500 vs. 6000 µg/l) compared to females (age 67,11±8,48 years, BMI 26,74±5,19 kg/m^2) but without statistical significance (p-values 0,063-0,271). Moreover levels of heavy metals failed to correlate with number or stenosis grade of concerned coronary arteries. Although men had higher levels of Cd, Pb and Hg compared to women the studied heavy metals do not seem at present time to play an important role in progression of CVD. Further studies are warranted to investigate if heavy metals might be involved in the development of CVD risk factors.