flurbiprofen together with 20 mg dextromethorphan in water. On day 15, flurbiprofen was administered together with 20 mg omeprazole. Urine was collected for 2 h after drug administration, concentrations of flurbiprofen (FLB) and its CYP2C9-dependent metabolite 4'-OH-flurbiprofen (OHF) were determined, and the respective metabolic ratio (MR_{FLB} = [OHF]/[FLB]) was calculated.
Results There was no statistically significant effect of dextromethorphan on the urinary MR_{FLB}. Co-administration of flurbiprofen with omeprazole, on the other hand, resulted in an increased MR_{FLB} in 22 volunteers. While the concentration of unchanged flurbiprofen did not change significantly, 4'-OH-flurbiprofen was excreted at higher levels (p-value = 0.0041). The 90 % CI of the ratio of the geometric means for MR_{FLB} (with/without omeprazole) was between 115 and 127 %.
Conclusions Concomitant phenotyping for CYP2D6 with dextromethorphan did not affect phenotyping in urine for CYP2C9 with flurbiprofen. Under the given experimental conditions, both probe drugs can therefore be used in a combined phenotyping procedure. Co-administration of omeprazole, on the other hand, had a marked effect on the 2 h-urinary concentrations of 4'-OH-flurbiprofen. As defined by the EMEA, bioequivalence can be assumed if the 90% CI for the ratio of the analysed parameter (test vs. reference) is within an acceptance interval of 80 to 125 %. Judged on this basis, the two sets of MR_{FLB} values (with/without omeprazole) cannot be considered bioequivalent.

248

Citrinin is metabolized to dihydrocitrinone in hepatocytes

Föllmann W., Blaszkewicz M., Hengstler J. G., Degen G. H.
Leibniz-Institut für Arbeitsforschung an der TU Dortmund, Germany

The mycotoxin citrinin (CIT) is known to exert nephrotoxicity in several animal species, and has genotoxic properties. A risk assessment for CIT is hampered by gaps in the toxicological database, scarce knowledge regarding its metabolism, and insufficient data on human exposure [1]. A first biomonitoring study revealed frequent occurrence of CIT and dihydrocitrinone (HO-CIT) in urines from German adults [2], and the metabolite HO-CIT has been characterized recently as detoxication product of CIT [3].
To gain further insight into the metabolism of this mycotoxin, we have now investigated conversion of CIT *in vitro*: Primary cultures of freshly isolated mouse hepatocytes were incubated with CIT (at 10 and 100 µM) for up to 24 hours, and culture medium aliquots were analyzed by HPLC and LC-MS/MS [4]. The analysis clearly showed a decline of parent compound over time and a concomitant appearance of HO-CIT metabolite: It accounts for about 4% of the dose after 3 hours, and about 22 to 31 % of the dose after 24 hours. In principle, these data confirm our assumption that CIT is mainly metabolized in the liver. Further studies aimed to identify the enzymes (oxidoreductases) responsible for CIT conversion to HO-CIT are needed, and a comparison between rodent and human hepatocytes is of interest, since the extent of CIT detoxication could differ between species.
Acknowledgement: This project was supported by the Brigitte and Wolfram Gedek-Stiftung

[1] EFSA Panel on Contaminants in the Food Chain, 2012. Scientific Opinion on the risks for public and animal health related to the presence of citrinin in food and feed. EFSA Journal 10(3), 2605; DOI 10.2903/j.efsa.2012.2605
[2] Ali N, Blaszkewicz M, Degen GH. Occurrence of the mycotoxin citrinin and its metabolite dihydrocitrinone in urines of German adults. Arch Toxicol, online 2014 Sep 17; DOI 10.1007/s00204-014-1363-y
[3] Föllmann WF, Behm C, Degen GH, 2014. In vitro toxicity of citrinin and its metabolite dihydrocitrinone. Arch Toxicol 88:1097-1107
[4] Blaszkewicz M, Muñoz K, Degen GH, 2013. Methods for analysis of citrinin in human blood and urine. Arch Toxicol 87:1087-1094

249

Testosterone and 2,4-toluenediamine metabolism by human skin and reconstructed tissues

Grohmann L., Klipper W., Schäfer-Korting M., Weindl G.
Freie Universität Berlin, Institute of Pharmacy (Pharmacology and Toxicology), 14195, Germany

Reconstructed human skin (RHS) gains increasing interest in preclinical drug development, but as with human skin, knowledge about biotransformation capacity is rather poor although this can be highly relevant for genotoxicity and sensitization testing. We compared the metabolism of the standard compound testosterone and the industrial chemical 2,4-toluenediamine (2,4-TDA) in excised human skin, RHS (Phenion FT, Epiderm-FT) and undifferentiated keratinocytes and fibroblasts. The extent of testosterone biotransformation by RHS and RHE at 24 h outperforms the biotransformation in human skin because of the higher absorption rate and thus enforced testosterone access to the various enzymes of androgen biotransformation. Except for the significantly lower dihydrotesterone amount and higher estradiol formation in EpiDerm-FT metabolite spectrum (other major metabolites: 6β-hydroxytestosterone, androstanedione, androsterone, androstenedione) and enzyme expression in human skin and the constructs (in particular Phenion FT) are very similar. Aromatase expression is most prominent in the RHS EpiDerm-FT. 2,4-TDA readily permeates through human skin and RHS and skin absorption is complete after 24h. The mono-N-acetylated derivative N-(3-Amino-4-methylphenyl)acetamide was the only metabolite found in all test matrices and the formation ranked as: RHS > human skin ~ keratinocytes > fibroblasts. Phase I metabolites were not detected. In summary, RHS exceeded biotransformation in human skin, yet, the metabolite profile was close. Thus, our findings indicate that reconstructed tissues appear to be an adequate test matrix for the investigation of cutaneous metabolism of xenobiotics and thus can be used for non-clinical drug development as well as the investigation of biotransformation-related toxicological endpoints.

250

Degradation of benzo[*a*]pyrene on the human skin leads to the production of cytotoxic/genotoxic metabolites

Sowada J., Lemoine L., Luch A., Tralau T.
Federal Institute for risk assessment, safety of products ans chemicals, Berlin, Germany

The skin is not only our largest organ but also the first one getting in contact with the surrounding environment. Therefore protection is the main function against miscellaneous environmental impacts like mechanical and chemical stresses. The exposure to different xenobiotic pollutants like polycyclic aromatic hydrocarbons (PAHs) out of the environment is a common feature. Benzo[*a*]pyrene (B[*a*]P) is a well characterized carcinogenic PAH, activated in eukaryotic metabolism via cytochrome P450-dependent monooxygenases (CYP) toward a highly mutagenic and cancerogenic metabolite named benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE).
Although the human body is settled by millions of bacteria, little is known about the microbial degradation of B[*a*]P. This is worrisome, because also the human microbiome is getting in daily contact with such pollutants, hypothesizing that bacteria of the human skin must be able to metabolize xenobiotics.
Previous analyses revealed that the degradation of B[*a*]P on the human skin is a common feature with different underlying degradation pathways leading to complete and partial degradation of this compound. Furthermore, different metabolites are transiently or permanently excreted with cytotoxic potential.
Now, we show that these metabolites have also genotoxic potential in the human skin cell line HaCaT. To investigate the influence on the human phase I metabolism the induction of CYP profiles in HaCaT cells as well as in primary keratinocytes (NHEK) was analyzed. Hence, analytical measurements should identify the responsible cytotoxic and genotoxic metabolites.
We are the first one, reporting about the toxification of substances by bacteria on the human skin. This might contribute to a reconsideration of the bacterial influence on compound-mediated toxicity.

251

Biomarkers of citrinin and ochratoxin A exposure in humans

Nurshad A., Blaszkewicz M., Degen G. H.
IfADo – Leibniz Research Centre for Working Environment and Human Factors,, Dortmund, Germany

Co-occurrence of citrinin (CIT) and ochratoxin A (OTA) as food contaminants may result in combined human exposure to these nephrotoxic mycotoxins. OTA exposure has been assessed by biomarker measurements in human blood, breast milk and urine samples from several countries. Yet, biomonitoring for CIT is still at the beginning, and data on CIT kinetics in humans are lacking. Therefore, CIT metabolism and elimination were now studied in a female volunteer after ingestion of a small dose (45.4 ng/kg bw) with the aim to characterize biomarkers of CIT exposure in urine. Furthermore, in two male individuals on their normal diet, CIT, OTA and their metabolites dihydrocitrinone (HO-CIT) and ochratoxin alpha (OTα) were analyzed repeatedly in blood and urine to obtain profiles over time and to gain more insight into the variability of biomarkers for both mycotoxins. OTA and OTα were determined by a validated method with HPLC-FLD detection (Muñoz K et al. 2010, J Chrom B 878:2623-2629). For analysis of CIT and HO-CIT we used a novel sensitive LC/MS-MS method (Blaszkewicz M et al. 2013, Arch Toxicol 87:1087-1094).
Upon CIT intake, analyte levels in timed urine samples of the female volunteer increased notably, for CIT up to 0.32 ng/mL within 20 h, and HO-CIT up to 0.78 ng/mL, and then decreased to low pre-intake levels 25 h after ingestion. This indicates an efficient conversion of CIT to the less toxic metabolite HO-CIT which represents a major fraction of the total dose excreted: Within a day about 13% of CIT was excreted as parent compound, and about 24% as HO-CIT. This new data provides a first basis for CIT intake estimates based on urine biomarker measurements.
The analyte profiles in blood and urine of one male individual showed small fluctuations, and mean concentrations of OTA, CIT and their metabolites were clearly lower than in the second individual over the period of time. Interestingly, biomarker levels in plasma of both individuals were considerably higher than those measured in urine (plasma/urine ratio for OTA >6, for CIT ≥9 and for HO-CIT ≥3), except for OTα which one person excreted at much higher levels than the other, possibly due to more active detoxification of OTA to OTα. Finally, it can be concluded that interindividual variability for the investigated biomarkers reflects dietary exposure and also conversion of ingested mycotoxins.
Acknowledgement: Nurshad Ali is supported by a stipend from DAAD.

Naunyn-Schmiedeberg´s Arch Pharmacol (2015) 388 (Suppl 1):S1–S98
DOI 10.1007/s00210-015-1087-4

Deutsche Gesellschaft für Experimentelle und Klinische Pharmakologie und Toxikologie e.V.

Abstracts

of the 81th Annual Meeting
March 10 – 12, 2015 Kiel, Germany

This supplement was not sponsored by outside commercial interests.
It was funded entirely by the publisher.

Springer