AWARDS PRESENTED AT THE 2011 EUROTOX PARIS CONGRESS

Pictured from left to right: Dr. Sebastien Antherieu (recipient G-Z YSA), Dr. Sibel Ozden (recipient SITOX YSA), Prof. Marina Marinovich (President SITOX), Dr. Nancy Claude (President EUROTOX), Prof. Ruth Roberts (President-elect EUROTOX) during the award presentation at the 2011 EUROTOX Paris Congress, August 31, 2011.

2011 EUROTOX GERHARD ZBINDEN YOUNG SCIENTISTS AWARD

Supported by EUROTOX for drug-oriented toxicological research

Dr. Sebastien Antherieu, University of Rennes (France)

P1302

DECREASE OF BSEP EXPRESSION IS ASSOCIATED WITH OXIDATIVE STRESS IN CHLORPROMAZINE-INDUCED CHOLESTASIS IN HEPARG CELLS

S. Anthérieu, A. Guillouzo
Faculté De Pharmacie, INSERM U991 - Université de Rennes 1, Rennes, France

Purpose: Cholestasis is a clinical syndrome induced by various factors, including a number of drugs such as chlorpromazine (CPZ). Unfortunately, the mechanisms of drug-induced cholestasis are varied and poorly understood, and current models used for safety assessment in drug development do not accurately predict cholestasis in humans. We have investigated hepatic effects of CPZ using the well differentiated human hepatoma HepaRG cell line. Methods: Bile acid transport was analysed using [H^3]-taurocholic acid, mRNA quantification by RT-qPCR analysis, CYP3A4 activity by testosterone 6β-hydroxylation measurement and oxidative stress by the glutathione assay. All analyses were carried out after CPZ treatment of differentiated HepaRG cells. Results: CPZ induced an accumulation of bile acids, the hallmark of cholestasis, in HepaRG cells, that was associated with a decreased expression of canalicular efflux transporters BSEP and MDR3. Moreover, increased basolateral transport (MRP4 overexpression), decreased bile acid synthesis (CYP8B1 inhibition) and enchanced bile acid detoxication (CYP3A4 up-regulation) could represent hepatoprotective mechanisms aimed at reducing intrahepatic accumulation of toxic bile acids. Furthermore CPZ was shown to generate reactive oxygen species and blocking this oxidative stress by N-acetyl cysteine strongly prevented bile acid accumulation and BSEP expression decrease but did not affect MDR3 expression. The present study provides the first in vitro analysis of mechanisms involved in CPZ-induced cholestasis in a human model and support the view that the HepaRG cell line is an appropriate cell model for a better understanding of drug-induced liver lesions in human.

2011 ECETOC YOUNG SCIENTISTS AWARD

Supported by ECETOC for toxicological research into mechanisms and risk assessment

Dr. Amy Zmarowski, NOTOX (The Netherlands)

P2317

DIFFERENTIAL EFFECTS OF METHYLAZOXYMETHANOL AND MK-801 ADMINISTRATION ON LEARNING AND MEMORY IMPAIRMENT IN SPRAGUE DAWLEY AND WISTAR HAN RATS

A. Zmarowski, M. Beekhuijzen, M. Teunissen, H. Emmen
Toxicology, NOTOX B.V., 's-Hertogenbosch, The Netherlands

Wistar Han (WH) and Sprague Dawley (SD) rats are the preferred strains for Europe and the US, respectively, though both are acceptable for EU and US test guidelines. Potential differences in behavioral performance between the strains have not been extensively investigated, though unexpected differences between strains could complicate risk assessment. Learning and memory testing was conducted within a developmental neurotoxicity study. Three groups were used for each strain. F0-females received saline or 20 mg/kg Methylazoxymethanol (MAM; known to produce neuropathic and behavioral alterations in offspring) intra-peritoneally on gestation Day15. Animals littered, and offspring were tested in the Biel maze at weaning (PNDs 22-28) or adulthood (PNDs 62-68). On days of testing, animals were given saline or 0.1mg/kg MK-801, which impairs learning and memory performance. Animals swam 'path A,' were then switched to an alternative 'path B,' and were last switched back to 'path A' for the memory test. Time to escape the maze and number of errors were quantified. Both strains learned each path, both remembered 'path A' when tested, and both were impaired with MK-801 administration. However, strain differences were evident: in general, SD rats learned better than WHs (taking less time and making fewer errors). They were also more impaired with MAM treatment, with more time and errors than WHs. Conversely, WH rats were more impaired with MK-801 administration, especially over 'path B'. In conclusion, these data highlight differences in general test performance and in drug sensitivity between the strains, which could ultimately require consideration in risk assessment.

2011 SITOX YOUNG SCIENTISTS AWARD

Supported by Italian Society of Toxicology (SITOX) for multi-national collaborative research, results of a collaboration of a minimum of four researchers from different European countries

Dr. Sibel Ozden, University of Istanbul (Turkey)

P1069

PROMOTER DNA METHYLATION PROFILE AS AN EARLY EVENT IN NON-GENOTOXIC CARCINOGENESIS

S. Özden[1], N. Turgut Kara[2], G. Karakuş[1], T. Chen[3], B. Alpertunga[1], W. Dekant[4], J..K. Chipman[3], A. Mally[4]
[1]Department Of Pharmaceutical Toxicology, Istanbul University, Faculty of Pharmacy, Istanbul, Turkey, [2]Faculty Of Science, Department Of Molecular Biology And Genetics, Istanbul University, Istanbul, Turkey, [3]School Of Biosciences, University of Birmingham, Birmingham, UK, [4]Department Of Toxicology, University of Würzburg, Würzburg, Germany

DNA methylation is an epigenetic mechanism associated with regulation of gene expression and it is modulated during chemical carcinogenesis. This may serve as a marker for early detection of non-genotoxic carcinogenesis. Therefore we evaluted the effects of non-genotoxic hepatocarcinogens, tetrachlorodibenzo-p-dioxin (TCDD), hexachlorobenzene (HCB), methapyrilene (MPY) and the male rat kidney carcinogens, d-limonene, p-dichlorobenzene (DCB) and chloroform on promoter CpG island methylation in their respective target tissues of rats. In our previous study, no significant dose-related effects on global DNA methylation were observed in target tissues of rats compared to controls by using LC-MS/MS. In addition, for the investigated genes (connexin-32, E-cadherin, p16, VHL, c-myc, Igfbp2, and p15), only p16 gene was found to be partially methylated in the liver treated with HCB and TCDD by using methylation-spesific PCR (MSP). For further studies, we evaluated CpG island methylation in the promotor region of the p16 gene by bisulfite sequencing (BSP) and/or COBRA assay. Overall, no significantly alterations were observed on DNA methylation in the selected genes in response to liver and kidney non-genotoxic carcinogens. In addition to this targeted profiling approach, an "open" unbiased system of methylated DNA immunoprecipitation (MeDIP) combined with microarrays, which determine the distribution of DNA-methylation within promoters or in the entire genome, might be a useful method to identify genes modulated by treatment with non-genotoxic carcinogens.

2011 BO HOLMSTEDT POSTER AWARD

Supported by the Bo Holmstedt Memorial Foundation for describing a feasible method for the solution of a toxicological problem under maximum respect of the 3R-principle (Reduce, Refine, Replace animal testing)

Dr. Yannick Brunner, SCAHT (Switzerland)

P1307

DEVELOPMENT OF A RAT AND HUMAN IN-VITRO MODEL FOR THE INVESTIGATION OF TESTICULAR TOXICITY

Y. brunner[1], P. Giron[1], L. Geiser[2], P. Antinori[1], D. hochstrasser[3], L. stoppini[4]
[1], SCAHT, Geneva, Switzerland, [2], university of Geneva, Geneva, Switzerland, [3], Hopital Universitaire Genève (HUG), Geneva, Switzerland, [4]Tissue Engineering, hepia Geneva University of Applied Sciences Western Switzerland, Geneva, Switzerland

Purpose: Male fertility has dropped considerably in all industrial countries over the past decades. It is estimated today that male production of spermatozoids decreased by 50 % during the past 50 years, mainly due to environmental factors. Existing models to predict the potential for chemicals to cause reproductive toxicity rely on animal tests that require the use of numerous animals (mainly rodents) which is less and less appreciated and is sometime not predictive enough. In this context, new experimental models are needed to reduce and replace the number of animal used with human-derived models. Methods: We are currently developing an in-vitro rat and human testis model based on the culture of isolated seminiferous tubules (organotypic culture) or on 3D testis engineered tissues from dissociated cells. We are using an air-liquid interface approach that takes advantage from both, the access to nutrients with the contact with culture medium, and proper oxygenation of the growing tissue with the air interface. The organotypic culture aims to maintain the tissue structure as close as possible from in-vivo, while the dissociated culture aims to recreate a tubule-like structure from cell lineage that could be kept frozen before culture. Results: Preliminary results showed a good in-vitro survival of young rat tissue for more than 3 weeks both for the organotypic and dissociation approach. For human tissue, we kept dissociated cells alive for more than 2 weeks. These in-vitro models will be used to perform toxicological studies with reference toxic compounds such as Dioxin, Busulfan and Vinclozolin.