OS1-4

Radio- and chemoprotective effects of Zhu-Ling Mushroom (*Polyporus umbellatus*) in human cultured cells and in mice

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Zhu-Ling Mushroom (*Polyporus umbellatus*) is a commonly used Chinese Medicine in the treatment of renal and liver disease. We examined the radio- and chemoprotective effects of PUPs in human lymphoblastoid TK6 cells and in ICR mice. The pretreatment of PUPs 30 min before irradiation significantly reduced radiation-induced micronuclei (MN) formation and tk mutant frequencies in TK6 cells. Pretreatments of PUPs at a dose of 50 mg/kg by i.p. injection 30 min or 45 min before 6 Gy irradiation caused a significant decrease in the frequencies of MN in the peripheral blood reticulocytes of irradiated mice. Comparative studies showed that PUPs may be a better radioprotective agent with a higher inhibition ratio of radiation-induced micronuclei and tk mutant frequencies than a well-known radioprotective agent amifostine. Mechanistic study showed that administration of PUPs at a dose of 50 mg/kg 30 min before irradiation significantly reduced the Comet tail length in the peripheral blood leucocytes and decreased the formation of the oxidative DNA damage (8-hydroxy-2′-deoxyguanosine) and lipid peroxidation in irradiated mouse liver, implying that the antioxidant activity of PUPs may contribute to its radioprotective effect. Furthermore, PUPs caused a dose-dependent inhibition of cyclophosphamide-induced MN formation in TK6 cells. Pretreatments of PUPs at a dose of 50 mg/kg by i.p. injection 30 min before CP treatment resulted in statistically significant decrease in the frequencies of MN in the peripheral blood reticulocytes in mice. Our results suggest that the potential us of PUPs as a useful radio- and chemoprotective agent.

doi:10.1016/j.toxlet.2011.05.148

OS1-5

Genotoxicity of safrole oxide in HEPG2 cells and in mice

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Safrole oxide (SAFO) is an electrophilic metabolite of the carcinogen safrole, the main constituent of sassafras oil. There is little or no data available on the genotoxicity of SAFO in mammalian systems. We investigated the cytotoxicity and genotoxicity of SAFO by MTT assay, Comet assay and Micronucleus test in HepG2 human hepatoma cells in vitro and in FVB mice in vivo. SAFO exhibited a time- and dose-dependent cytotoxic effect in HepG2 cells. SAFO produced a marked increase in comet tail length and in the frequency of micronucleated binucleated cells at doses of 125 μM and higher. Furthermore, repeated intraperitoneal injections of SAFO to mice caused a significantly increase in mean comet tail length of peripheral blood leukocytes and in the frequency of micronucleated reticulocytes in a dose-dependent manner. Our present data have demonstrated for the first time that SAFO exhibited significant genotoxicity in human cells in vitro and in vivo.

doi:10.1016/j.toxlet.2011.05.149

OS1-6

TiO_2 nanoparticles exhibit genotoxicity and impair both NER and BER DNA repair pathways in A549 cells

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Impact of titania nanoparticles (TiO_2-NPs) is now largely reported, yet published results are often contradictory. A panel of deeply characterized TiO_2-NPs was used to study the influence of physicochemical parameters on their impact on A549 cells. All the tested cytotoxicity assays led to the same conclusion: the cytotoxic impact of TiO_2-NPs is moderate, with a maximum of 25% of cells death after exposure for 48 h to 100 $\mu g\,ml^{-1}$ of NPs. Trypan blue staining and clonogenic assay led to the lowest interference between NPs and the test. NPs were internalized into cells, where they located mostly in the cytoplasm, entrapped in vesicles and vacuoles. Their accumulation caused oxidative stress and oxidative lesions to DNA, mainly 8-oxodGuo. It also induced DNA strand breaks, visualized by Comet assay, which increased between 4 h and 24 h exposure timepoints, then decreased. Conversely the number of gamma-H2AX foci or micronuclei did not increase. This kinetics may be significant of DNA repair processes. However, we also showed that TiO_2-NPs drastically impair DNA repair processes, both nucleotide excision repair (NER) and base excision repair (BER) pathways. These data prove that TiO_2-NPs do not induce severe lethality but cause genotoxic damage to A549 cells, but also impair DNA repair processes, which may preclude their mutagenicity.

doi:10.1016/j.toxlet.2011.05.150

OS1-7

Micronuclei frequency of a pesticide exposed population

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A wide range of chemical products known to be acutely toxic is nowadays used in the agricultural sector – a large number of pesticides with different compositions. Nevertheless, the effects in human health as result of long-term exposure to low levels are not yet completely understood. Human biomonitoring is an extremely useful tool that provides an efficient and effective mean of measuring human exposure to hazardous agents. The methodology for determination of micronuclei in lymphocytes (CBMN) is well validated and accumulating data have shown its relationship to cancer

risk. In opposition, analysis of MN in reticulocytes (MN-RET) in humans is a recent tool on human biomonitoring.

In this study, we tried to understand the influence of pesticide exposure on MN-RET and CBMN frequencies. Simultaneously, the association between both indicators was studied. A total of 177 individuals were included in this study (93 controls and 84 exposed). All individuals included in exposed group dealt regularly with a great diversity of compounds and therefore it is considered a multiple exposure scenario.

Both MN-RET and CBMN were significantly higher in the exposed subjects when compared to controls ($p < 0.001$). A significant and positive correlation was found between both indicators. Within the exposed group, one can observe that there is a significant correlation between MN-RET and recent exposure (exposure in the previous 10 days) that is it not found when considering CBMN. Likely due to the short life-span of reticulocytes, MN-RET showed to be more useful to characterize recent genetic damage than CBMN.

doi:10.1016/j.toxlet.2011.05.151

OS1-8
Heterocyclic aromatic amines form high levels of DNA adducts in human hepatocytes

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Purpose: Heterocyclic aromatic amines (HAAs), chemicals formed during the cooking of meat and fish and also present in tobacco smoke condensate and diesel exhaust, are suspected to play a major role in liver cancer incidence. In this study, we analyzed the formation of DNA adducts derived from several prevalent HAAs: PhIP, MeIQx, IQ and AαC in human hepatocytes and compared the level of DNA adducts formed with adduct levels of 4-ABP, an aromatic amine, known to be a carcinogen in humans. *Methods*: Primary cultured human hepatocytes were treated with HAA or 4-ABP for 3, 8 or 24 h. DNA adducts and metabolites derived from HAAs or 4-ABP were identified and quantified by liquid chromatography coupled with multistage mass spectrometry. The activities of cytochrome P450 1A2 enzymes were measured by spectrofluorometry and correlated with the levels of adducts observed. *Results*: High levels of deoxyguanosine adducts were formed. The levels of DNA adducts derived from PhIP, MeIQx, IQ and AαC formed in human hepatocytes varied from 0.34 to 14 adducts per 10^6 nucleosides, which was comparable to the level of 4 ABP-derived adducts. The kinetics of DNA adducts elimination were different for each HAA. Results also showed higher levels of adduct formation in human than in rat hepatocytes, signifying important interspecies differences regarding the metabolic activation of these procarcinogens. These data suggest that toxicity studies in rodents underestimate the genotoxic potential of HAAs in humans. These interspecies differences in HAA metabolism therefore should be taken into account for human risk assessment.

doi:10.1016/j.toxlet.2011.05.152

OS2—Reproductive Toxicology

OS2-1
Perinatal programming of obesity later in life by the environmental endocrine disruptor bisphenol A in a mouse model

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Purpose: Human exposure to endocrine disrupting compounds (EDCs) is implicated in the worldwide pandemic of obesity. Following the Developmental Origins of Health and Disease (DOHaD) principle, exposure to EDCs during the sensitive perinatal period can program the developing organism towards increased susceptibility to develop obesity later in life. This programming may occur via epigenetic modulation of energy homeostasis or via altered cell differentiation. In the EU-FP7 project OBELIX, experimental mouse studies are designed to provide supportive evidence for epidemiological associations between exposure to major EDCs and obesity later in life.

Methods: Dams were exposed via the diet to bisphenol-A (BPA) during the perinatal period. Offspring were then assessed for obesity related parameters. Tissues were collected for epigenetic changes, through analysis of DNA methylation. Additionally, serum parameters (e.g. leptin, ghrelin, lipid profile) were measured and pathology on different tissues was performed.

Results: Perinatal exposure to BPA induced a persistent dose-dependent increase of body weight in male, but not in female offspring. This was correlated with fat pad weight and lipid accumulation in adipocytes, and associated with increased food consumption, decreased physical activity and perturbed glucose homeostasis. Further analysis is ongoing to elucidate underlying mechanisms of perinatal programming. In our model, BPA acts as a programming factor resulting in overweight in developmentally exposed male mice. Other EDCs will be tested in the same model.

doi:10.1016/j.toxlet.2011.05.154

OS2-2
Impact of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in adult mouse Leydig cells: An in vitro study

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Purpose: In the present study, we were interested in delving further into the adverse effects caused by TCDD on testis physiology. *Methods:* We developed a model of primary cultures of Leydig cells isolated from 8-week-old mice. We examined the steroidogenic outcome of Leydig cells treated with TCDD as well as the expression levels of the two chemokines Ccl5 and Cxcl4, previously identified as markers of TCDD exposure in the testis. *Results:* We first established that Ccl5 and Cxcl4 were differently upregulated by various cytokines and lipopolysaccharides (LPS). TCDD upregulated Cxcl4 expression levels to a lesser extent than cytokines and LPS. Interestingly, TCDD down-regulated Ccl5 expression levels in TNFalpha-treated but not in control cells. TCDD action on