2236 Effects of Repeated Nanomaterial Exposure and Recovery on Circulating Mediators and Neurotoxicity

E. Mostovenko[1], S. A. Saunders[1], A. Vucetic[1], K. Fraser[2], M. J. Campen[3], A. Erdely[2], and A. K. Ottens[1]. *[1]Virginia Commonwealth University, Richmond, VA; [2]NIOSH, Morgantown, WV; and [3]University of New Mexico, Albuquerque, NM.*

We recently reported that modeled acute exposure to multi-walled carbon nanotubes (MWCNT) increased systemic inflammation, induced vascular dysfunction, disrupted the blood-brain barrier (BBB) and induced neuroinflammation. We further identified a dramatic shift in circulating peptides, found to be mediators of systemic bioactivity and a promising source of health-effect biomarkers. Here we assessed the burden of repeated MWCNT exposure in inciting a peptidomic response, its association with MMP-9 proteolysis, and longer term neuroinflammatory ramifications. Male C57BL/6 wild-type (WT) and MMP-9$^{-/-}$ knockout (KO) mice were exposed to MWCNT-7 by oropharyngeal aspiration (n=7/grp): 0 µg control vehicle (0.6 mg/ml albumin, 0.01 mg/ml DPPC) once per week, 10 µg once per week, and 40 µg at week-1 followed by 0 µg once per week until collecting serum and brains at 28 days after the initial treatment (7 days after the last aspiration). An enriched-peptide fraction was extracted and assessed by untargeted data-independent mass spectrometry while brains were assessed by immunofluorescence microscopy. In WT mice, 1613 (34%) of 4759 reproducibly quantified serum peptide factors were significantly responsive across all exposures (5% FDR) with half (785) directly dependent on MMP-9, speaking to the sizeable, though non-exclusive, role of MMP-9 in production of the MWCNT-responsive peptidome. Repeated 10 µg exposure significantly induced 957 (59%) of the peptidomic response, highlighting the significance of repeated lower dose exposures. Separately, 1119 (69%) peptide factors were significantly shifted a full 4-weeks after the larger 40 µg bolus dose, supporting a prolonged impact to circulating factors. Likewise, albumin leakage across the BBB and pronounced microglial activation proximal to impacted vessels were observed after repeated low dose as well as after 4-week recovery from high dose MWCNT. These effects were strikingly muted in brains of MMP-9 KO animals. Overall findings affirm significant MWCNT effects on the circulating peptidome, a sustained neurotoxicological burden of exposure, and a dependence on MMP-9 proteolytic function, together substantiating a prolonged impact of exposure.

2237 Systematic Comparative Assessment of the Cellular Stress Response Pathway Activation by Various Valproic Acid Analogues to Support Biological Read Across

N. G. Vrijenhoek[1], M. J. Moné[1], R. Gräpel[1], S. E. Escher[2], and B. van de Water[1]. *[1]LACDR, Leiden, Netherlands; and [2]ITEM, Hannover, Germany.*

Chemical read across is commonly evaluated without considering mechanistic biological knowledge. Here we used a large panel of valproic acid (VPA) analogues to include detailed mode-of-action (MoA) data as a proof-of-concept for read across. VPA is used as an anticonvulsant, but can induce liver steatosis. In rodents, VPA and some of its analogues cause hepatic steatosis, whereas other analogues do not. Since hepatotoxicity is linked to cellular stress pathway activation, we anticipated that VPA analogues that cause steatosis would activate similar stress response pathways, whereas *in vivo* negative compounds do not. Therefore, we deployed a panel of cellular stress response HepG2 BAC-GFP reporter cell lines, and performed high throughput transcriptomics analysis of cellular stress pathways. We measured stress response pathway activity at high throughput in the reporter cell lines with SRXN1 as target protein for oxidative stress, BiP for unfolded protein response, and p21 for DNA damage response. Cells were treated with a concentration range of 18 different VPA analogues for 24 h, and then imaged by confocal microscopy followed by quantitative image analysis. We were able to largely predict the steatosis *in vivo* potential of VPA analogues based on the expression of SRXN1-GFP and P21-GFP. Subsequently, we checked whether VPA analogues that cause liver steatosis yield similar transcriptional perturbations. TempO-Seq analysis of ~3000 genes involved in stress pathway responses for different VPA analogues in a concentration range over 24 h confirmed the concentration response activation of both oxidative stress and p53 downstream targets as well as additional stress pathways, supporting the application of TempO-Seq technology. Further comparative analysis among all VPA analogues in both HepG2 and primary human hepatocytes will be discussed. Thus, by monitoring adaptive stress response pathways at both the protein and the transcription level, we anticipate to support risk assessment by providing quantitative and mechanistic biological information to corroborate a robust read-across approach. *This work was part of the EU-ToxRisk project and received funding of the European Union's Horizon 2020 research and innovation program under grant agreement No 681002.*

2238 Benchmark Dose Modeling of Genomic Data to Assess the Functional Effects of Cigarette Smoke Exposure on Peripheral Blood Mononuclear Cells

G. Liu, and G. Prasad. *Reynolds American Inc., Winston-Salem, NC.*

Benchmark dose (BMD) modeling of toxicogenomics data has gained interest from regulatory agencies for chemical risk and toxicity assessment. To date, limited studies have used this tool for assessing toxicological effects associated with the use of tobacco products. In this study, we applied an integrative approach combining BMD modeling, gene ontology (GO) classification, and gene set enrichment analysis to assess dose-dependent microarray data for quantitative assessment of the biological effects of cigarette smoke exposure. Gene expression changes in peripheral blood mononuclear cells upon exposure to three nicotine equivalent doses of whole smoke-conditioned medium were used as a case study. Our approach consists of three steps. First, "BMDExpress" software was used to model gene expression data, and the BMDs of every dose-responsive genes and their associated GO categories were estimated. Second, dose-sensitive biological pathways were identified based on their BMD values and the percentage of differentially expressed genes in the pathway. Third, dose-response gene set enrichment analyses were performed to identify the key pathways that are significantly enriched with genes showing significant monotonic dose response. Our analysis identified 64 dose-sensitive pathways, which are mainly associated with three key cellular processes including inflammatory response, cellular response to stress, and Toll-like receptor signaling. For example, a biological pathway named "response to nitrosative stress" was observed to be dose-dependent and its BMD was estimated. In addition, our enrichment analysis identified seven significantly enriched pathways such as amino acid transport across the plasma membrane and small cell lung cancer pathway. Together, we present a case study using BMD modeling to identify dose-responsive genes and pathways associated with smoke exposure, and calculate the reference doses at which cellular processes are altered.

2239 N-Acetylcysteine, Silibinin and Benzylpenicillin Do Not Confer Protection against α-Amanitin Cytotoxicity in HepG2 and HepaRG Cells

D. R. Rodrigues, V. M. Costa, M. L. Bastos, and F. Carvalho. *University of Porto, Porto, Portugal.*

Mushroom poisoning is a worldwide public health problem. *Amanita phalloides* is one of the most poisonous mushrooms, and amatoxins, specially α-amanitin, are responsible for its major deleterious effects. Nowadays, there is no fully effective procedure or antidote for amatoxin intoxicated patients and *Amanita phalloides* intoxications remain highly lethal. Since the liver is a major target for α-amanitin toxicity, the identification of α-amanitin toxicity mechanisms in hepatic cells and search for effective antidotes are crucial in this field. Therefore, α-amanitin cytotoxic effects were evaluated in two hepatic cell lines, HepG2, and in confluent and differentiated HepaRG cells. HepG2, confluent and differentiated HepaRG cells showed a concentration and time dependent mitochondrial and lysosomal dysfunction. Nevertheless, confluent and differentiated HepaRG cells were more sensitive towards α-amanitin cytotoxic effects 48-h following incubation, possibly reflecting the presence of the sodium-taurocholate cotransporting polypeptide uptake transporter, previously reported to play an important role in α-amanitin uptake and therefore in its cytotoxicity. Additionally, the effects of previously identified antidotes for amatoxin-intoxicated patients but with low clinical efficiency such as N-acetylcysteine, silibinin and benzylpenicillin were assessed in HepG2, confluent and differentiated HepaRG cells. None of the antidotes conferred protection against α-amanitin cytotoxicity in these cell lines. Therefore, the identification of effective antidotes for α-amanitin poisoning is highly needed. *VMC thanks FCT for grant (SFRH/BPD/110001/2015). Work supported by FEDER funds through the Operational Programme for Competitiveness Factors - COMPETE and by national funds by FCT ("PTDC/DTP-FTO/4973/2014- POCI-01-0145-FEDER- 016545").*

58TH ANNUAL MEETING & ToxExpo · MARCH 10–14, 2019

The Toxicologist

Supplement to *Toxicological Sciences*

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 168, Issue 1
March 2019

www.academic.oup.com/toxsci

The Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

www.toxicology.org

Publication Date: February 18, 2019