both strains in urinary L-FABP excretion. Histological observation revealed that foamy eosinophilic globules slightly existed in proximal tubule on Day 4 and those urinary L-FABP excretion elevated on Day 1 in correspondent rats.
These results suggest that the urinary L-FABP may be a very useful sensitive biomarker of nephrotoxicity not only in humans but also in experiment animals.

PS 114 Preclinical Nephrotoxicity Biomarker Analysis Using Toxicoproteomics Approaches

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Increased application of proteomics approaches to toxicological studies has led to the development of a new discipline that is being called "toxicoproteomics". Major research areas of toxicoproteomics include development of biomarkers of toxicity, identification of molecular targets of toxicants, and elucidation of toxicity mechanisms. To understand the molecular mechanisms of nephrotoxicity and identify kidney injury biomarkers, quantitative proteomic analyses were conducted on kidneys from aristolochic acid (AA)-treated rats. Animal treatment was conducted according to the protocol approved by the NCTR Institutional Animal Care and Use Committee. Big Blue rats were treated with 0.9% sodium chloride as the control or AA at 10 mg/kg body weight, five times/week, for 12 weeks. Kidneys were collected one day after the last treatment. Kidney tissues from the control and the AA-treated rats were processed for proteomic analysis using stable isotope labeling and two-dimensional liquid chromatography coupled online with tandem mass spectrometry (LC-MS/MS). More than 100 proteins changed their abundance as a result of AA treatment, which may be related to the toxicity and carcinogenicity of AA. Intriguingly, several FDA-qualified preclinical kidney injury biomarkers were identified in this study, and their abundance showed significant changes. A mass spectrometry-based multiplex kidney injury biomarker assay was developed for targeted quantitative measurement of the biomarker candidates discovered in this study, and many of the expression-changed proteins were confirmed. The combined open discovery and targeted proteomics approaches facilitated the identification of kidney injury biomarkers. Additional studies need to be performed in animals and humans to investigate their translational nature.
The views presented do not necessarily reflect those of the U. S. Food and Drug Administration.

PS 115 Detailing the Human Exposome: Mass Spectrometry-Based Chemical Profiling of Human Mitochondria

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While most studies have utilized easily accessible bio-fluids to monitor environmental agents and associated metabolic response, few have measured exogenous compounds within sub-cellular compartments. The purpose of this study was to profile the endogenous and exogenous mitochondrial metabolome of human organ tissue, with an emphasis on identifying the presence of environmental agents. Viable mitochondria were obtained from human adrenal glands (n=5) within 36 hours of removal using a differential centrifugation isolation procedure. Isolates were normalized on a per protein basis (250 ug), and analyzed in tandem with blood plasma using liquid chromatography followed by dual polarity electrospray ionization and high resolution mass spectrometric detection. Mitochondria specific metabolites and environmental agents were tentatively identified by matching (mass error ≤ 10 ppm) the mass-to-charge (m/z) ratio to the Kyoto Encyclopedia of Genes and Genome (KEGG) and EPA ToxCast database. Following data extraction and threshold filtering, 5773 unique features were obtained. Pathway mapping indicated that the adrenal mitochondria metabolome was well covered, with metabolites corresponding to mitochondrial specific pathways such as cysteine and methionine metabolism (n=27), drug metabolism by cytochrome P450 (n=60), and steroid hormone biosynthesis (n=54). Ions corresponding to exogenous chemicals were also present, which matched m/z's corresponding to fungicides, carbamate insecticides and herbicides. Compounds with known endocrine disrupting tendencies where similarly matched (n=6), including di-n-propylpthalate, di-n-hexylphthalate and styrene. Future work will include confirmation of chemical identities and absolute quantification. These results indicate that high-resolution chemical profiling of cellular organelles can be utilized to both identify exogenous agents within mitochondria and their associated metabolic response, providing relevant, in vivo toxicological information.

PS 116 Proteomic Analysis of Dried Blood Spots for Biomonitoring Organophosphorus Exposures

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Organophosphorus (OP) compounds are involved in most of the occupational and environmental intoxications reported worldwide. They inhibit cholinesterases and other serine hydrolases, leading to neurological damage and serious health effects. The standard methods used for biomonitoring OP exposures are the Ellman assays, which measure the enzymatic activity of either red blood cell (RBC) acetylcholinesterase or plasma butyrylcholinesterase (BChE). Despite being fast and inexpensive, these assays suffer from several drawbacks.
We have developed a rapid immunomagnetic bead (IMB) purification for biomarkers of OP exposure, followed by OP-adduct identification on active site serines by high-resolution mass spectrometry (MS). The IMB-MS methods have been adapted to identify OP insecticide adducts on both plasma BChE and RBC acylpeptide hydrolase (APH), two known biomarkers of OP exposure. Plasma, RBCs or dried blood spots (DBS) are used for our IMB-MS methods. The methods have been designed for adaptation to high-throughput protocols to facilitate their transfer to clinical laboratories.
These methods will be validated using a cohort of Washington State agricultural workers with characterized OP exposures to chlorpyrifos or azinphos-methyl. The IMB protocols purify BChE and APH from as low as 25 μL of plasma or RBCs, respectively, or from DBS.
The use of DBS will facilitate sample collection, shipping, and archiving as well as the analysis of already archived samples. The IMB-MS protocols have significant advantages: they do not require a pre-exposure measurement, they can detect low levels of exposure, they can be automated, and they provide information on both the OP and percentage modification of the active site serines.
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PS 117 Toxicometabolomics Approach to Prediction of Hepatotoxicity by Troglitazone/LPS in Rats

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Troglitazone (TGZ) is a thiazolidinedione antidabetic agent which is the synthetic ligands for the peroxisome proliferator-activated receptor γ (PPAR γ). However, it was withdrawn from the market in 2000 due to liver injury in humans. In this study, we endeavored to discover surrogate biomarkers which are correlated with hepatotoxicity induced by TGZ using urinary proton nuclear magnetic resonance (1H NMR) spectral data. A procedure of 1H NMR urinary and serum analysis using pattern recognition was proposed for early screening of the hepatotoxicity of TGZ with lipopolysaccharide (LPS) in rats. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were the highest in 6 hours and hepatic inflammation, in histopathology, was observed in 2 hours. All treated animals were divided into two groups of responder and non-responder based on the ALT and AST levels because the levels of ALT and AST were not changed dose-dependently after treatment of TGZ (2 g/kg, p.o). In urinary analysis, 1H NMR spectroscopy did not show different clustering between responder and non-responder in global metabolic profiling through principal component analysis (PCA). However, it was significantly separated in orthogonal projections to latent structures-squares discriminant analysis (OPLS-DA). In targeted profiling, endogenous metabolites of phenylacetylglycine, glucose, acetate, 2-oxoglutarate, formate, creatine, citrate, 3-indoxysulfate, hippurate, acetone, phenylalanine, glycine, betaine, cis-aconitate, and tyrosine were selected as putative biomarkers for hepatotoxicity by TGZ. In serum analysis, pattern recognition was similar to the results of the urinary analysis, and then we could select putative 6 endogenous metabolites such as ethanol, glucose, alanine, glutamine, lactate, and 3-hydroxybutyrate. According to these results, toxicometabolomics can be used to predict or screen hepatotoxicity caused by TGZ in urine and serum of rats.

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