PS 1549f Multiplex Blood Biomarker Approach to Determine Drug-Induced Vascular Injury in Rat

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Vascular injury is a common finding during the pre-clinical safety study of drugs. A lack of understanding of mechanisms of drug-induced vascular injury (DIVI) in animals and the absence of qualified biomarkers have become significant barriers in the development of new therapeutic agents. Recently, a number of promising candidate biomarkers for DIVI have been nominated. However, any effort to further validate and evaluate these markers is impeded by the lack of qualified high-throughput assays.
Based on Luminex xMAP® technology, we developed three panels of multiplexed immunoassays to quantify 17 potential DIVI biomarkers in rat serum and plasma samples. These biomarker candidates included caveolin-1, CTGF, PAI-1, IL-6, MCP-1, CINC-1, TIMP-1, VEGF, TNF-alpha, adiponectin, sICAM-1, sE-selectin, von Willebrand Factor (vWF), alpha-2-macroglobulin (A2M), alpha-1-acid glycoprotein (AGP), fibrinogen and haptoglobin. Furthermore, fenoldopam induced rat DIVI models were used to validate the sensitivity and specificity of these assays.
To induce vascular injury, rats were treated with the vasodilator compound fenoldopam mesylate (FM). FM was administered by continuous intravenous infusion over 24 hours with a dose of 6 mg/kg/h. Subsequently to the infusion, serum and plasma samples were collected from each group of three rats on post-infusion day 1, 3, and 7. Sterile 0.9% saline was used in the vehicle control cohorts. MILLIPLEX® MAP Rat Vascular Injury Magnetic Bead Assays were used to measure 17 candidate protein biomarkers simultaneously from the rat serum and plasma samples. Fenoldopam treatment resulted in significant increase in blood biomarkers, consistent with its well described vasotoxic effect. Several biomarkers were found promising in this study, including A2M, sICAM-1, CTGF, vWF and TIMP-1. The simultaneous measurement of these proteins with multiplex technology offered a robust and convenient method to study these biomarkers. Taken together, our data demonstrate the feasibility of using the multiplex biomarker approach to detect the onset and progression of DIVI in pre-clinical drug safety studies.

PS 1549g Conjugate Formation of 1-Bromopropane with Glutathione *In Vitro*

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Glutathione (GSH) is a ubiquitous tripeptide in mammalian systems for playing critical roles, including defense against many electrophiles. 1-Bromopropane (1-BP) is widely used as a substitute for chlorofluorocarbons because of its lower ozone-depleting potency, high volatility and non-flammability. It has been known that 1-BP could produce GSH conjugates so that the homeostasis would be interrupted. In the present study, the formation of GSH conjugates of 1-BP was characterized in vitro by using mass spectrometry. Rat liver homogenates and cell cultures including MCF-7 and HepG2 cells were compared for their ability to form S-propyl GSH. In rat liver homogenates, S-propyl GSH was also produced concentration-dependently from 1 mM 1-BP. A time-course study indicated that Tmax for the formation of S-propyl conjugates would be 1 hr after the treatment with 1-BP. In addition, the production of S-propyl conjugates was associated with the decrease in the level of GSH in liver homogenates. In cell cultures, S-propyl GSH was only produced in MCF-7 cells, but not in HepG2 cells. In addition, the conjugates were mostly detected in the cultured media, indicating that conjugates are excreted from MCF-7 cells when produced. A time-course study indicated that Tmax for the excretion of S-propyl conjugates would be 8 hr after the treatment with 1-BP in MCF-7 cells. The present results indicated that S-propyl conjugates of 1-BP could be produced in vitro and that toxicologically 1-BP in MCF-7 cells might act differently from HepG2 cells. Supported by a grant from National Research Foundation of Korea (2010-0026220).

PS 1549h N-Terminal Pro-Natriuretic Peptides and Heart Rate As Cardiovascular Safety Biomarkers

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Functional changes in the cardiovascular (CV) system, such as altered blood pressure, are not usually measured and frequently go undetected in conventional repeat-dose rodent safety studies during drug development. Likewise, in clinical drug development, functional changes in the CV system of humans can go undetected until late stage clinical trials. Sustained changes in blood pressure are important because even small changes in blood pressure have been suspected of producing undesirable CV effects such as cardiac hypertrophy and myocardial degeneration in animals, and Major Adverse Cardiovascular Effects (MACE) in patients. Natriuretic peptides (NP) are cardiac hormones which affect blood pressure and inhibit cardiac hypertrophy by promoting excretion of salt and water through the kidneys, relaxation of vascular smooth muscle, and direct effects on the heart. They have been shown to have utility as prognostic biomarkers of heart failure, cardiac hypertrophy, and left ventricular dysfunction in patients, and may be useful biomarkers in non-clinical species as well. We examined NTproANP and NTproBNP concentrations in plasma of rats administered sunitinib for four days, and nifedipine, fluprostenol, minoxidil, L-NAME, and L-thyroxine for fourteen days. Plasma NTproANP and NTproBNP concentrations were inversely related to blood pressure after administration of sunitinib, nifedipine, minoxidil, and fluprostenol. Administration of L-NAME caused hypertension and increased NTproBNP concentrations, and L-thyroxine caused systolic hypertension and increased heart weight with no change in either NP. Nifedipine and minoxidil caused increased heart weights which were correlated with NTproANP concentrations. Fluprostenol and L-NAME did not cause changes in heart weight, in spite of sustained changes in blood pressure and NTproANP and NTproBNP. Increased heart weights were best correlated with increased heart rates. These studies support further development of NPs as biomarkers of functional changes in the cardiovascular system of veterinary species.

PS 1550 Manganese Exposure Assessment—The Use of Toenails As a Biomarker of Exposure in US Welders

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Previous studies have shown that high exposure to manganese (Mn) in occupational settings may lead to neurological health effects. Currently, there is no established biomarker for Mn exposure. The objective of this study was to evaluate the hypothesis that toenail Mn concentration is reflective of an individual's Mn exposure from occupational and dietary sources in a population of welders.
Toenail clippings of all ten toes were collected from fifteen career welders and nineteen controls. The toenail samples were cleaned and sonicated in Triton X-100 surfactant, digested using microwave acid digestion and analyzed for Mn using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Mn. Respirable Mn exposure was estimated using a model combining air samples, work histories, and modifying factors such as ventilation and use of personal protective equipment. Dietary Mn and Fe were estimated with a food questionnaire.
There was no significant difference between mean Mn concentration in right and left feet (4.89 vs. 4.27 ug/g, p = 0.1856). Toenail Mn levels were higher in welders than in controls (7.14 vs. 3.02 ug/g, p = 0.0003). Toenail Mn was significantly correlated with cumulative exposure to respirable Mn seven to twelve months prior to the toenail clipping date (r2 = 0.31, p = 0.03). When ratio of dietary intake of Mn to Fe, smoking, and age were included as covariates in the statistical model the correlation between respirable Mn exposure and toenail Mn improved (adjusted r2 = 0.56, p = 0.003). With two exceptions, toenail Mn was able to clearly distinguish exposed individuals from controls.
The significant correlation of toenail Mn with the cumulative Mn exposure seven to twelve months prior to the toenail being clipped is consistent with the average growth time of toenails. The results suggest that toenail Mn is a valid, reproducible, easy to acquire biomarker of Mn exposure, which is feasible to use in an industrial welder population. (Supported by NIEHS R01 ES020529 and CDC/NIOSH T03 OH008615)

PS 1551 Elevated Household Dust Mn Levels Are Associated with Children's Hair Mn in Communities Impacted by Ferroalloy Plants in Northern Italy

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Objectives: Determine the association between levels of manganese (Mn) and other metals in household dust samples with levels in exposure biomarkers (hair, blood, saliva, and fingernails) in Italian adolescents in communities with active and historic ferroalloy plant activity.
Rationale: Adolescents living in communities with ferroalloy plant activity exhibit deficits in olfactory and fine motor function. Household dust Mn concentrations have not previously been measured to assess contamination from ferroalloy plant emissions.

The Toxicologist

Supplement to *Toxicological Sciences*

53rd Annual Meeting and ToxExpo™

March 23–27, 2014 • Phoenix, Arizona

An Official Journal of the Society of Toxicology

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OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 138, Issue 1
March 2014

www.toxsci.oxfordjournals.org