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 Wednesday, August 2, 2017
 

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Poster Session: 9:30 AM - 5:00 PM

Animal Clinical Chemistry

**B-001****Antidiabetic Activity of Aqueous *Kalanchoe pinnata* Preparation: Potential Mechanism of Action**L. E. Rios, F. Omoruyi, J. Sparks. *Texas A&M University Corpus Christi, Corpus Christi, TX*

**Background:** The aqueous preparation of *Kalanchoe pinnata* is traditionally used in the management of Type 2 diabetes mellitus, but the effectiveness in curtailing the indices of Type 2 diabetes is not clear. In this study, we evaluated hematological and oxidative stress indices, and enzymes involved in carbohydrate metabolism in the liver of Type 2 diabetic rats treated with aqueous preparation of *K. pinnata*. **Methods:** Six rats were fed a normal diet, while 24 rats were fed a high fat diet (HFD) for twenty-one days. Diabetes was induced in eighteen of the rats fed HFD by a low dose of streptozotocin administration on day fourteen and diabetes was confirmed on day 21. Animals were then divided into five groups (n = 6) as follows: non-diabetic group; non-diabetic control group fed HFD; diabetic group; diabetic plus *K. pinnata* (0.14 g/kg body weight/day); diabetic plus metformin (300 mg/kg body weight/day). Animals were euthanized by decapitation after treatment for 28 days and blood and liver were collected for assays. **Results:** Type 2 diabetic rats treated with *K. pinnata* preparation lost significant (P < 0.05) weight. *Kalanchoe pinnata* consumption resulted in decreased serum glucose. There were also significant (P < 0.05) increases in white blood cell count and hemoglobin levels. Serum reduced glutathione (GSH) levels, superoxide dismutase and hepatic pyruvate kinase activities were significantly (P < 0.05) elevated. Hepatic malic enzyme and glucose-6-phosphate dehydrogenase activities were not significantly (P > 0.05) altered in Type 2 diabetic rats treated with aqueous *K. pinnata* preparation. **Conclusion:** Overall, our data showed that the consumption of aqueous preparation of *K. pinnata* in Type 2 diabetic rats decreased body weight and serum glucose levels. Similarly, the observed increase in superoxide dismutase activity and GSH levels in the diabetic rats treated with *K. pinnata* preparation may be protective against oxidative stress associated with the disease. The observed increase in hepatic pyruvate kinase activity in diabetic rats treated with *K. pinnata* preparation may be indicative of improved glucose metabolism via the glycolytic pathway with subsequent decrease in blood glucose.

**B-002****Evaluation of oxidized linoleic acid metabolites in rodent models of alcoholic liver disease**D. Warner<sup>1</sup>, A. Feldstein<sup>2</sup>, C. McClain<sup>1</sup>, I. Kirpich<sup>1</sup>. <sup>1</sup>*University of Louisville, Louisville, KY*, <sup>2</sup>*University of California San Diego, San Diego, CA*

**Introduction/Goal:** Liver dysfunction as a result of alcohol consumption is a significant health problem for which there are no current FDA-approved therapies. Alcoholic liver disease (ALD) encompasses a range of severities from steatosis to steatohepatitis, and further to irreversible damage, fibrosis and cirrhosis. Progression from early to late stages may be influenced by dietary factors such as the types of dietary fatty acids and therefore present an opportunity for intervention. It has been previously shown that rodents placed on diets high in unsaturated fat (USF, enriched predominantly in dietary polyunsaturated fatty acid, linoleic acid [LA]) when combined with ethanol showed an increase in the production of oxidized LA metabolites (OXLAMs). These OXLAMs may lead to the enhanced liver injury by mechanisms that remain to be determined. Therefore, a determination of plasma OXLAM burden may be a beneficial diagnostic tool in the assessment of ALD severity. The goal of the present study was to test the hypothesis that ethanol-induced oxidation of LA and subsequent increase in hepatic and circulating OXLAMs exacerbate liver inflammation and injury via shifting hepatic macrophages toward the pro-inflammatory (M1) phenotype. **Materials/Methods:** Two animal models of ALD (chronic and chronic-binge ethanol administration) were used in this study. Male mice (C57BL/6) were fed a liquid diet that was enriched in USF (primarily corn oil/LA-enriched) or saturated fat (SF, medium chain triglyceride and beef tallow-enriched) and supplemented with 5% (v/v) ethanol or isocaloric maltose dextrin for 10 days followed by a single "binge" of 5g/kg ethanol administered by oral gavage.

Alternatively, mice were placed on SF- or USF-enriched liquid diets (control or ethanol-containing) for 8 weeks. Plasma and hepatic concentrations of OXLAMs were determined by LC-MS and hepatic gene expression was assessed by qRT-PCR. **Results:** Lipidomic analysis by mass spectrometry demonstrated that plasma and hepatic concentrations of LA and OXLAMs (9- and 13-hydroxyoctadecadienoic acids [9-HODE and 13-HODE]) were significantly higher in mice fed USF+ethanol compared to controls and those mice fed SF+ethanol in both models. This was correlated with enhanced liver damage as determined by plasma ALT activity and increased hepatic neutrophil/macrophage infiltration. qRT-PCR analysis for macrophage type M1 and M2 cytokine gene expression revealed that M1-associated proinflammatory cytokines (*Tnf-α* and *Il-1β*) were elevated in mice provided the USF+ethanol diet but showed no changes in M2-associated (*Tgfb* and *Arg-1*) cytokine gene expression. These changes may be a direct effect of HODEs on macrophage gene expression because RAW246.7 cells (a mouse macrophage cell line) expressed more *Tnf-α* and *Il-1β* following incubation with 9-HODE. **Conclusions:** Increased plasma OXLAM levels were found in both experimental animal models of ALD and were correlated with greater liver injury in mice fed ethanol and a diet high in LA. Furthermore, increased macrophage polarization to a pro-inflammatory state may be one mechanism by which LA metabolites lead to greater ethanol-induced liver injury. Therefore, plasma OXLAM concentrations may be a predictor of liver inflammation resulting from ethanol-induced oxidation of dietary fatty acid, LA.

**B-003****Developing a policy/protocol for testing of non-human and forensic samples by a clinical core laboratory**G. Pomper<sup>1</sup>, S. Appt<sup>2</sup>, T. Register<sup>3</sup>, E. Palavecino<sup>1</sup>, M. Beatty<sup>1</sup>, P. Lantz<sup>4</sup>, J. McLemore<sup>4</sup>, J. Hausman<sup>5</sup>, E. Wilson<sup>5</sup>, D. Dennard<sup>5</sup>, E. Oliphant<sup>5</sup>, S. H. Wong<sup>1</sup>. <sup>1</sup>*Wake Forest School of Medicine, Pathology, Winston-Salem, NC*, <sup>2</sup>*Wake Forest School of Medicine, Pathology, Comparative Medicine, Preclinical Translational Services/Center for Industry Research Collaboration, Winston-Salem, NC*, <sup>3</sup>*Wake Forest School of Medicine, Pathology, Comparative Medicine, Preclinical Translational Services/Center for Industry Research Collaboration, Winston-Salem, NC*, <sup>4</sup>*Wake Forest School of Medicine, Pathology and Forensic Pathology, Winston-Salem, NC*, <sup>5</sup>*Wake Forest Baptist Health, Winston-Salem, NC*

**Background:** Testing of non-human samples provides data for translational studies using various animal models. Analysis of postmortem forensic autopsy samples serves as an important adjunct for death certification. If these tests are performed in a clinical laboratory for patients' samples, safeguards must be considered so that patient testing is not adversely affected by inadvertent contamination. The Comparative Medicine and Forensic Pathology sections of our Department and a few investigators had occasional test requests. Consequently, we conducted multiple discussions and planning sessions. We consulted with colleagues from other medical centers and diagnostic companies. Consequently, we developed an initial policy/procedure for testing these samples with focus on avoiding cross contamination. **Methods:** Chemistry and Hematology Sections of our Core Laboratory accept veterinary and postmortem samples with the full understanding that validation studies for analysis of non-human or human cadaveric specimens had not been established. The Microbiology section does not accept veterinary samples for culture. However, serology testing for infectious diseases could be accepted for testing by the chemistry analyzers. Feline samples were unacceptable due to viscosity. After internal and external consultations, we classified two test groups : A) Established veterinary testing based on tests/analyzers used previously by veterinary lab customers; B) No previously established testing for veterinary and forensic postmortem samples. The following protocol was developed with sequential steps/considerations. Investigators contact the central processing department to obtain an approval form, indicating the projected volume, frequency of testing, desired turn-around-time, and funding information. Section managers use this information to schedule testing, and to increase reagent and disposable orders. Acceptable samples include blood, urine and cerebrospinal fluid. Group A veterinary samples are tested without further review. Group B tests are reviewed by the section director who makes the final decision. Upon approval, the investigators notify central processing about tests, sample size and delivery. Samples are batched and analyzed during the weekend on a non-STAT basis. These steps safeguard against mix-up with human clinical samples and avoid cross contamination. After testing, five aliquots of water are used to rinse the instrument, followed by testing of quality control samples and 5 previously tested patient samples. These steps ascertain acceptable QC values and non-significant differences of the 5 patient samples.

**Results:** Based on the experience of applying this protocol over the past 4 months, investigators initially had frequent inquiries about account information, sample transport scheduling and testing details. A lead time of 2 - 4 weeks was recommended

for new accounts. Initial testing was limited to basic metabolic panel, comprehensive metabolic panel, lipid profile, automated complete blood count with or without differential, troponin I, and BNP. Results have been acceptable by the two Sections. **Conclusion:** The above policy/protocol has been incorporated into the clinical pathology core laboratories. With weekend testing, occasional short staffing and instrumentation problems can present a challenging but manageable situation without delay to routine patient service. These initial steps would benefit from future reviews and improvements.

**B-004****Effect of Neutral Sphingomyelinase Inhibition on ER Stress and Apoptosis in Liver Ischemia-Reperfusion Injury**

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**Background:** Previous studies have revealed the activation of neutral sphingomyelinase (N-SMase)/ceramide pathway in hepatic tissue following warm liver ischemia reperfusion (IR) injury. Excessive ceramide accumulation is known to potentiate apoptotic stimuli and a link between apoptosis and endoplasmic reticulum (ER) stress has been established in hepatic IR injury. Thus, this study determined the role of selective N-SMase inhibition on ER stress and apoptotic markers in a rat model of liver IR injury.

**Methods:** Selective N-SMase inhibitor was administered via intraperitoneal injections. Liver IR injury was created by clamping blood vessels supplying the median and left lateral hepatic lobes for 60 min, followed by 60 min reperfusion. Levels of sphingomyelin and ceramide in liver tissue were determined by an optimized multiple reaction monitoring (MRM) method using ultra fast-liquid chromatography (UFLC) coupled with tandem mass spectrometry (MS/MS).

**Results:** Sphingomyelin levels were significantly increased in all IR groups compared to controls. Treatment with a specific N-SMase inhibitor significantly decreased all measured ceramides in IR injury. A significant increase was observed in ER stress markers C/EBP-homologous protein (CHOP) and 78 kDa glucose-regulated protein (GRP78) in IR injury, which was not significantly altered by N-SMase inhibition. Inhibition of N-SMase caused a significant reduction in phospho-NF-kB levels, hepatic TUNEL staining, cytosolic cytochrome c and caspase-3, -8 and -9 activities which were significantly increased in IR injury.

**Conclusion:** Data herein confirm the role of ceramide in increased apoptotic cell death and highlight the protective effect of N-SMase inhibition in down-regulation of apoptotic stimuli responses occurring in hepatic IR injury.

**B-005****Comparison of Two Multispecies Hematology Analyzers Used in Nonclinical Drug Safety Studies: Sysmex XT-2000iV vs Siemens Advia 120**

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**Background**

Complete blood counts and white blood cell differentials are standard panels used in the assessment of drug safety. This evaluation was conducted to compare the Sysmex XT-2000iV automated hematology analyzer to the Siemens Advia 120.

**Methods**

Analytical performance of intra-run imprecision and linearity were assessed using quality control material. Blood specimens from laboratory animals originally collected for nonclinical drug discovery and development studies, (Sprague-Dawley Rats n=58, Dogs n=32, NZW Rabbits n=19, Cynos n=45), were analyzed side by side for routine hematology parameters on both platforms. Correlation data generated from these same sample analyses were evaluated using regression statistics and percent bias.

**Results**

Analytical performance data from assessments of within-run imprecision and linearity were comparable between platforms. Regression values were considered satisfactory ( $R \geq 0.90$ ) across all species analyzed, with exceptions that were primarily believed to be related to minor methodology differences. Percent bias data was generally within  $\pm 5\%$ , yet the bias of some parameters (e.g. RETIC%), were greater and attributable to inherently low numeric result values.

**Conclusions**

Comparison of these systems revealed some differences in results, but none were considered significant enough to interfere with the interpretation of nonclinical study data. Our data demonstrated that the analytical performance results of the two platforms, including assessments of intra-run imprecision, linearity, and correlation, were satisfactory. To fully validate a comparison of these two platforms a greater number of sample analyses would be needed. The Sysmex XT2000iV had comparable performance and acceptable correlation to the Siemens Advia 120 when used to support nonclinical studies.