Wednesday, August 3, 2016

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

Animal Clinical Chemistry

B-001

Mouse models of chronic and chronic-binge ethanol-induced alcoholic liver disease: effects of dietary fat

I. Kirpich, W. Feng, Y. Wang, Y. Liu, M. Miller, H. Liu, C. McClain. *University of Louisville, Louisville, KY*

Aim: The use of animal models contributes to understanding of the molecular mechanisms underlying various human diseases, including alcoholic liver disease (ALD). Animal models allow to examine different factors involved in the ALD development and progression other than alcohol, such as nutrition. Accumulating evidence suggests that dietary fat, along with alcohol consumption, play critical roles in ALD pathogenesis. Given that there are several animal models of ALD, it is important to ensure reproducibility and applicability of these models to human ALD. The aim of the present study was to examine and compare the effects of different types of dietary fat in two different animal models of ALD: ethanol (EtOH)-mediated liver injury caused by chronic or chronic-binge ethanol administration. **Methods:** C57BL/6 male mice were fed either an USF (corn oil) or a SF (medium chain triglycerides [MCTs] and beef tallow enriched) Lieber-DeCarli diet (40%E from fat). Animals were fed control or EtOH containing diets (5% EtOH [vol/vol], 35%E from EtOH) *ad libitum* for 8 weeks (*chronic model*), for 10 days, followed by a single dose of EtOH (5 g/kg body weight) by gavage (*chronic-binge model*). Control mice were pair-fed on an isocaloric basis. Liver triglyceride (TG) accumulation was assessed by biochemical assay and Oil Red O staining. Liver injury was evaluated by plasma ALT activity. Plasma LPS levels were measured as a marker of endotoxemia. Hepatic macrophage and neutrophil infiltration were determined by F4/80 and chloroacetate esterase staining, respectively. Hepatic inflammation was examined by pro-inflammatory cytokine/chemokine expression as assessed by qRT-PCR. **Results**: Examination of livers from experimental animals demonstrated noticeable fat accumulation in USF+EtOH compared to control and SF+EtOH fed animals in both chronic and chronic-binge ALD models. Moreover, there was extensive microvesicular and macrovesicular liver steatosis in USF+EtOH compared to SF+EtOH in the chronic ALD model. In the chronic model, ALT activity was similar in SF+EtOH and control fed mice; significantly elevated ALT levels were observed in USF+EtOH fed mice compared to control as well as SF+EtOH fed animals (44.9+2.8 *vs* 21.4+1.2 and 27.3+1.9 U/L, respectively). In the chronic-binge model, ALT levels were elevated in response to EtOH regardless dietary fat; however, SF+EtOH fed mice had significantly lower ALT levels compared to USF+EtOH group. Chronic consumption of SF+EtOH did not affect endotoxemia, a 4.5-fold increase in plasma LPS levels was observed in USF+EtOH fed mice compared to controls. There were no noticeable changes in endotoxemia in chronic-binge model in response to either SF+EtOH or USF+EtOH administration. Compared to controls, hepatic macrophage and neutrophil infiltration was significantly elevated in USF+EtOH but not in SF+EtOH fed mice in both ALD models. Liver inflammation, characterized by up-regulation of pro-inflammatory cytokines and chemokines, was more prominent in the chronic USF+EtOH fed animals compared to control and SF+EtOH fed mice. **Conclusions**: Dietary SF attenuated EtOH-induced hepatic steatosis and injury caused by either chronic or chronic-binge EtOH administration regardless the type of animal model of ALD. The molecular mechanisms underlying these protective effects might be distinct between the models and need to be further investigated.

B-002

Histopathological Changes in Some Organs of Diabetic Rats Administered Aqueous or Ethanolic Root Extract of Uvaria Chamae

F. E. Olumese[1], I. O. Onoagbe[1], G. I. Eze[1], F. O. Omoruyi[2]. *[1]University of Benin, Benin City . Nigeria, Benin, Nigeria, [2]Texas A & M,, Corpus Christi, TX*

Background: *Uvaria chamae* is a medicinal plant that is used in many parts of the world in the treatment of diabetes, and other diseases. The chemical constituents of *Uvaria chamae*, include C-benzylated monoterpenes, aromatic oils, flavanones, C-benzylated flavanones, and C-benzylated dihydrochalcones. Traditionally, the root extracts are used in the treatment of many diseases, including diabetes. However, the use of this plant extracts in the treatment of diabetes have not been scientifically validated. In this study, we evaluated the histopathological changes in the heart, liver and pancreas of normal and diabetic rats administered aqueous or ethanolic extract of *Uvaria chamae* roots. **Methods:** Thirty six Sprague Dawley rats were assigned by weight into six groups [6 rats per group, average body weight 265.23 ± 7.20 g] as follows: Healthy rats receiving de-ionized water (Normal Control); Normal rats receiving aqueous extract (Normal plus Aqueous Extract); Normal receiving ethanolic extract (Normal plus Ethanolic Extract); Diabetic rats receiving de-ionized water (Diabetic Control); Diabetic rats receiving aqueous extract (Diabetic plus Aqueous Extract); and Diabetic rats receiving ethanolic extract (Diabetic plus Ethanolic Extract). Diabetes was induced using a single injection of streptozotocin (Sigma-Aldrich, 60 mg/kg body weight in 0.05 M-citrate buffer, pH 4.5) intraperitoneally. Normal and diabetic rats were then administered the aqueous or ethanolic extract (300 mg/kg body weight per day) for 35 days. Animals were euthanized by decapitation and blood collected for glucose assay. Organs were collected and preserved in buffered formalin for histopathological evaluation. **Results**: There was a significant ($p<0.05$) decrease in blood glucose levels in the diabetic groups treated with aqueous or ethanolic extract compared to the diabetic control. We observed intimal ulceration, medial thickening and luminal stenosis of the coronary artery in the heart of diabetic control rats compared to the normal control. Diabetic rats treated with ethanolic extract showed good luminal patency. We also observed mild vascular congestion and dilatation in the diabetic rats treated with the aqueous extract with well delineated luminal patency. The liver of the diabetic control group showed portal congestion and infiltrates of chronic inflammatory cells(portal hepatitis) when compared to the normal control. However, treatment of the diabetic groups with aqueous or ethanolic extract showed dilatation of blood vessels and activation of the kupffer cells (local immune system) when compared to the diabetic control. The normal control groups treated with aqueous or ethanolic extract showed exuberant pancreatic islet cells compared to the untreated normal control. Similarly, the diabetic groups treated with aqueous or ethanolic extract showed resurgent pancreatic islet cells compared to the diabetic control group. **Conclusion:** The observed activation of hepatic kupffer cells and the resurgence of pancreatic islet cells due to aqueous or ethanolic extract consumption are indicative of the potential benefits of each extract in the effective management of diabetes. The observed good luminal patency of the coronary artery by either extract supplementation may prevent lipid deposition in the arteries that is often associated with the development of diabetic complications.

B-003

Anti-diabetic properties of combined inositol hexakisphosphate and inositol in streptozotocin-induced type 2 diabetes mellitus Sprague-Dawley rats

S. R. Foster[1], R. L. Alexander Lindo[1], L. L. Dilworth[1], J. Bustamante[2], F. O. Omoruyi[3]. *[1]The University of the West Indies, Mona Campus, Kingston, Jamaica, [2]Texas A&M University, Kingsville, TX, [3]Texas A&M University, Corpus Christi, TX*

Background: Diabetes mellitus is ranked among the major causes of morbidity and mortality associated with non-communicable diseases worldwide. Type 2 diabetic patients struggle with classic early symptoms of increased body weight, dyslipidemia, polyphagia, polydipsia and polyuria. Inositol hexakisphosphate (IP6) is a plant constituent found in appreciable quantities especially in grains and seeds while inositol is a carbohydrate found in plants and animals. Inositol and IP6 are thought to individually exhibit hypoglycemic activities but the full mechanism is not yet known. Therefore the anti-diabetic effects of an IP6 and inositol combination in a type 2 diabetes mellitus (T2DM) rat model was evaluated in this study. **Methods:** Thirty male Sprague-Dawley rats with average body weight of 168 ± 5.9 g were used in this 8 week study. Type 2 diabetes mellitus was induced in 18 of these rats by feeding them high-fat diet for 4 weeks followed by intravenous administration of a low dose of streptozotocin (Sigma-Aldrich, 35 mg/kg/body weight in 0.1 M-citrate buffer, pH 4.5) after two weeks. Type 2 diabetes mellitus was confirmed by hyperglycemia (blood glucose ≥ 300 mg/dL) and a positive response to an anti-diabetic drug response test. Diabetic rats were placed into three groups (6 rats per group) namely; IP6 and inositol combination (IP6+INO; 650 mg/kg body weight/day), glibenclamide (Glib; 10 mg/kg body weight/day) and diabetic control (DC). In addition, two groups of non-diabetic control rats were fed normal diet (NC) and high fat diet (HFC) during the initial 4 weeks of the experiment. However, for the final four weeks, all rats were fed normal diet and given their respective treatment regimes. The rats were fasted overnight, euthanized by decapitation and blood samples were collected at the end of the 8 week period. **Results**: Treatment with IP6 & inositol combination significantly reduced blood glucose concentration (306 ± 53 mg/dL) and insulin resistance score

(1.93 ± 0.45) compared to the diabetic control group (522 ± 24 mg/dL and 5.10 ± 0.69 respectively; $p < 0.05$). A similar effect was observed with Glibenclamide. However, the combined supplement was more effective in lowering serum total cholesterol and triglycerides by 26% and 31% respectively compared to the glibenclamide treated group ($p < 0.05$). Body weight, fluid intake and food consumption were also significantly reduced in the IP6+INO group by 8%, 16% and 10% respectively compared to the Glibenclamide treated group ($p < 0.05$). Food and fluid intake were reduced by 52% and 28% respectively, whereas serum leptin concentration was increased by 34% in rats treated with the combined supplement compared to the diabetic control group ($p < 0.05$).**Conclusion:** Treatment of T2DM rats withIP6 and inositol combination significantly improved blood glucose concentration and ameliorated insulin resistance, dyslipidemia, polyphagia and polydipsia. This study shows that a combined IP6 and inositol supplementation may be effective in the management of T2DM and associated metabolic disorders.

B-004

Evaluation of Hematology and Flow Cytometry Parameter Stability in Non-Human Primate, Canine, and Rodent K_2EDTA Whole Blood Samples to support Pre-clinical Toxicology Studies

S. Wildeboer, C. Phanthalangsy, C. Drupa, R. Giovanelli, S. Sokolowski. *Pfizer, Groton, CT*

Background: Historically, our laboratory has determined K_2EDTA sample stability to be 24-30 hours post collection when stored at 2-8°C for all hematology parameters. Based on literature, there are several variables that may affect pre-analytical stability, such as processing delays and sample storage conditions. Therefore, an evaluation of stability beyond 30 hours was needed to support multi-site collection and evaluation of hematology and flow cytometry parameters. Extended pre-analytical stability of various species in K_2EDTA whole blood provided our laboratory with a more robust methodology and consistency in eliminating variation and bias from reported results of whole blood samples.

Methods: At least 10 samples from normal colony animals (both male and female) of non-human primate (NHP) Cynomolgus monkey, Beagle canine, Sprague-Dawley(SD) and Wistar rat, and CD1 and C57BL/6 mouse K_2EDTA whole blood samples were analyzed for either hematology or flow cytometry parameters. Samples for hematology or flow cytometry were analyzed within 2 hours of collection and at various time points between 6 and 168 hours post collection to establish stability. Samples were stored at 2-8°C between analysis time points and were allowed to come to room temperature prior to analysis. Hematology whole blood samples were analyzed for a complete blood count (CBC), automated white cell differential (Auto DIFF), and reticulocyte (RETIC) using the Siemens Advia 2120 hematology analyzer. Additionally, lymphocyte subset stability was assessed using the FACSCanto flow cytometer and FACSDiva software. Total T cell, helper T cell, cytotoxic T cell, B cell and natural killer (NK) cell populations were measured using lineage specific antibodies and reported as a percentage of lymphocytes. Stability values showing acceptable recovery or actual difference were considered stable.

Results: NHP blood was stable up to 96 hours for CBC, RETIC, total T cells, helper T cells, cytotoxic T cells, B cells and NK cells. However, the automated differential was only stable up to 72 hours for NHP. Canine blood was stable up to 48 hours for CBC, 96 hours for Auto DIFF, RETIC, white blood cell count (WBC), and platelet count (PLT), and 168 hours for all flow cytometry parameters assessed. SD rat blood was stable up to 28 hours for CBC, 32 hours for RETIC, 72 hours for Auto DIFF and WBC, and 54 hours for all flow cytometry parameters. Lymphocyte subsets were stable in Wistar rat blood for 30 hours. CD-1 and C57BL/6 mouse blood was stable for 96 and 72 hours, respectively, for lymphocyte subsets.

Conclusion: All outlined criteria for the validation of extended sample stability in NHP, canine, rat and mouse whole blood samples for hematology as well flow cytometry parameters were met and have been used to support discovery and multispecies pre-clinical studies. Therefore, with the evaluation of extended sample stability we have been able to provide support across multiple sites, and offer a more robust methodology and confidence in reported results of whole blood samples.

B-005

Hepatic polyunsaturated fatty acids and inflammatory response in an animal model of endoplasmic reticulum stress

M. Aslan[1], B. Unal[2], F. Ozcan[1], H. Tuzcu[1], E. Kırac[1]. *[1]Department of Medical Biochemistry, Akdeniz University Medical Faculty, Antalya, Turkey, [2]Department of Pathology, Akdeniz University Medical Faculty, Antalya, Turkey*

Background: The presence of endoplasmic reticulum (ER) stress is as an important contributing factor in various diseases, including alcoholic liver disease, nonalcoholic steatohepatitis, diabetes mellitus and hepatocellular carcinoma. Unfolded proteins accumulate in the ER lumen and lead to alterations in glucose homeostasis, lipid metabolism, iron and calcium balance in the liver. Intracellular calcium levels are increased as a consequence of ER stress leading to activation of phospholipase A2 (PLA2). In this study, we sought to evaluate the effect of ER stress on hepatic polyunsaturated fatty acids (PUFAs) and inflammatory response through COX2-mediated pathway.

Methods: Male Wistar rats which were allowed free access to standard rat chow were randomly divided into control, tunicamycin (TM) treated and TM + tauroursodeoxycholic acid (TUDCA) treated groups. Hepatic ER stress was induced by intraperitoneal (i.p.) injection of 1 mg/kg body weight tunicamycin and the ER stress inhibitor tauroursodeoxycholic acid (TUDCA) was injected (250 mg/kg body weight, i.p.) 30 minutes before hepatic induction of ER stress. The presence of ER stress was confirmed by increased intracellular levels of C/EBP-homologous protein (CHOP) and 78-kDa glucose-regulated protein (GRP78). Necroinflammation was evaluated in liver sections stained with hematoxylin-eosin using the Ishak-modified hepatic activity index. Levels of arachidonic acid (AA, C20:4n-6), dihomo-gamma-linolenic acid (DGLA, C20:3n6), eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) in liver tissue were determined by an optimized multiple reaction monitoring method using ultra fast-liquid chromatography coupled with tandem mass spectrometry. Phospholipase A2 (PLA2), cyclooxygenase (COX) and prostaglandin E2 (PGE2) were measured in tissue samples to evaluate changes in the inflammatory pathways.

Results: In the present study, hepatic ER stress was accomplished by TM and was alleviated by TUDCA. Effectiveness of treatments were confirmed by histology and protein biomarkers. Tunicamycin treatment significantly decreased all measured PUFAs and increased AA/EPA ratio in liver tissue compared to controls. Tissue activity of PLA2, COX and PGE2 levels were significantly increased in liver tissue of TM treated rats compared to controls. Tauroursodeoxycholic acid lead to a partial restoration of liver PUFA levels and significantly decreased PLA2, COX and PGE2 levels compared to TM treated rats.

Conclusion: The results of this study reveal the presence of a proinflammatory state in hepatic ER stress as shown by significantly increased AA/EPA ratio. To our best knowledge, this is the first study reporting altered PUFA levels in ER stress and supports the use of omega-3 fatty acids as adjuvant treatment in liver diseases demonstrating ER stress. **Acknowledgement:** This study was supported by a grant from The Scientific and Technological Research Council of Turkey (TUBITAK; # 214S223).

B-006

Evaluation of the Meso Scale Discovery Rat Skeletal Troponin I Assay in Rat and Mouse

T. Lambert, K. Lynch, N. Chau, T. Sellers. *GlaxoSmithKline, King of Prussia, PA*

Skeletal troponin I (sTnI) is a member of the troponin complex of regulatory proteins required for muscle contraction. Recent evidence suggests that skeletal muscle injury results in leakage of sTnI into blood and measurement of serum or plasma levels of sTnI may provide a noninvasive biomarker of muscle injury. Our objective was to evaluate the Meso Scale Discovery® (MSD) Rat Skeletal Troponin I Assay for measurement of skeletal troponin I in rat serum. Acceptable precision (CV ≤10%) and accuracy (RE ±13%) of standard curve values were observed across 10 analytical runs. Precision of sTnI values in rat serum samples was excellent (CVs ≤10%) at concentrations in the range of 5-150µg/L; less precision was observed (CV=21%) in the area of the LLOQ (0.27µg/L). Dilutional linearity ($y=1114.8x – 4782.2$; $r^2=0.99$) and recovery (101-106%) of sTnI in rat serum was demonstrated. Serum sTnI was stable for up to 6 months at -80ºC. Serum sTnI values in clinically healthy Wistar rats (n=20/sex) were below LLOQ to 11.4µg/L for males and at or below LLOQ for females. In two nonclinical safety studies, sTnI increases (up to 1984µg/L) correlated

with histologic evidence of myofiber degeneration and/or necrosis and increases in AST and aldolase. In mice, sTnI increases (474 to 1859µg/L) were observed in plasma of muscular dystrophy mutant mice (C57BL/10ScSn-mdx/J) compared to wild-type C57BL mice (0.38-3.09µg/L). In conclusion, the MSD rat skeletal troponin I assay performed well and sTnI increases correlated with skeletal muscle injury in rats and mice.