

Tuesday, July 28, 2015

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

Animal Clinical Chemistry

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The Inhibitory Effect of DENND1B Overexpression on the Release of Cytokine IL-4 in a Murine Ovalbumin-induced Asthma ModelA. Hei, Z. Jiang, W. Wang, Z. Wang, J. Li, J. Cai. *Beijing Hospital & Beijing Institute of Geriatrics, Ministry of Health, Beijing, China*

Background: The role of asthma-associated gene DENND1B has not been defined in the pathogenesis of asthma. This study aimed at evaluating the effects of DENND1B overexpression in vivo on the release of inflammatory cytokines in a murine model of asthma induced by ovalbumin (OVA).

Methods: Female C57/BL6 mice at 5-6 weeks of age were sensitized on days 0 and 7 and 14 by intraperitoneal injection of 20 mg OVA added in 2 mg of aluminum hydroxide. On days 21 and 22 and 23, mice were exposed to aerosolized OVA once a day for 3 days. Lentivirus containing DENND1B coding sequence vector (OVA + DENND1B vector) or empty vector (OVA + Empty vector) was intravenously injected on day 11. The mice were sacrificed and evaluated 24hrs after the last OVA challenge. Total Immunoglobulin E (IgE) in plasma and the levels of interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) in bronchoalveolar lavage fluid (BALF) were measured by ELISA kits. The number of leucocyte in BALF was counted on an automatic blood cell analyzer.

Results: Systemic sensitization with OVA significantly increased the plasma level of IgE, but the levels were not significantly changed in any of OVA-treated groups. The number of leucocyte significantly increased in the BALF of OVA and OVA +Empty vector groups. By contrast, the number of leucocyte in the BALF significantly decreased in OVA+DENND1B vector group. The levels of IL-4, IL-5, IL-6 and TNF- α in BALF were significantly increased in three OVA-treated groups. However, the level of IL-4 in BALF was significantly lower in the OVA+DENND1B vector group than in OVA group mice.

Conclusions: The release of cytokines IL-4 was suppressed by the overexpression of DENND1B in the lung. The overexpression of DENND1B could play partial protective effect against inflammatory damage in the OVA-induced murine model.

Table. The level of IgE in plasma, leucocyte number and cytokines levels in BALF

Analytes	Normal Control	OVA	OVA + Empty vector	OVA + DENND1B vector
IgE in plasma (ng/ml)	244.01±53.44	1161.56±62.04	1048.67±84.74	1159.01±39.80
Leucocyte number in BALF ($\times 10^6$ /ml)	1.67±0.54	48.70±15.23	38.52±14.78	24.81±9.23*
IL-4 in BALF (pg/ml)	51.89±18.61	215.22±72.75	160.67±20.71	91.13±42.73*
IL-5 in BALF (pg/ml)	91.59±22.98	184.72±23.48	179.56±15.20	143.63±27.57
IL-6 in BALF (pg/ml)	170.5±62.22	385.54±50.86	359.25±72.12	318.08±34.12
TNF- α in BALF (pg/ml)	231.42±88.27	449.50±119.67	473.47±84.51	409.71±99.10

* P<0.05 vs. OVA mice

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Organ Function and Oxidative Stress Indices in Streptozotocin-induced Diabetic Rats Administered Aqueous or Ethanolic Extract of Uvaria Chamae RootsF. E. Olumese¹, I. O. Onoagbe¹, F. O. Omoruyi². ¹University of Benin, Benin City, Nigeria, Benin, Nigeria, ²Texas A&M, Corpus Christi, TX

Background: *Uvaria chamae* is a medicinal plant that is used in some regions of the world in the treatment of many diseases including diabetes, cough and gastroenteritis. The chemical constituents include C-benzylated monoterpenes, aromatic oils, flavanones, C-benzylated flavanones, and C-benzylated dihydrochalcones. However, the scientific basis for the traditional use of this plant extracts in the management of diabetes is not well understood. In this study, we evaluated organ function and oxidative stress indices in the blood of normal and streptozotocin-induced diabetic rats administered aqueous or ethanolic extract of *Uvaria chamae* roots. **Method:** Thirty

six (eighteen adult normal and eighteen streptozotocin-induced diabetic rats) Sprague Dawley rats were assigned by weight into six groups [6 rats per group, average body weight 265.23 \pm 7.20 g]. The six groups were composed as follows: Healthy rats receiving de-ionized water (Normal Control); Normal rats receiving aqueous extract (Normal plus Aqueous Extract); Normal rats 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/day) for 35 days. Animals were then euthanized by decapitation and blood was collected for assays. **Results:** We noted a significant ($p<0.05$) decrease in blood glucose levels in the diabetic groups treated with each extract compared to the diabetic control group. The levels of serum total cholesterol, triglycerides, LDL- cholesterol and VLDL- cholesterol were not significantly reduced by each extract. However, there was a significant ($p<0.05$) increase in HDL- cholesterol levels in the diabetic groups administered each extract compared to the diabetic control group. Serum uric acid, blood urea nitrogen and creatinine levels in the diabetic groups were not significantly altered by the treatments. We also noted non-significant changes in serum amino transferase activities, and total protein, albumin and globulin levels in the treated diabetic groups compared to diabetic control group. The consumption of each extract resulted in elevated serum total antioxidant capacity and superoxide dismutase (SOD) activity compared to the diabetic control group. The administration of each extract to normal rats resulted in elevated serum uric acid levels and decreased SOD activity. **Conclusion:** The observed reduced blood glucose and elevated HDL-cholesterol levels are indicative of the beneficial effects of each extract in the management of diabetes.

The administration of each extract may be protective against oxidative stress that is often associated with the development of diabetic complications as evidenced in the observed improved antioxidant defense system. However, the consumption of each extract in non-diabetic states should be done with caution because of the observed elevated serum uric acid levels and decreased SOD activity in the normal treated groups.

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The role of ethanol and dietary fat in the disruption of intestinal barrier integrity and liver injury in an animal model of alcoholic liver diseaseL. Kirpich, W. Feng, C. McClain. *University of Louisville, Louisville, KY*

Introduction/Aim: Alcoholic liver disease (ALD) ranks among major causes of morbidity and mortality in the United States and worldwide. ALD includes a spectrum of pathologies from simple steatosis to steatohepatitis characterized by inflammation with the potential progression to fibrosis and cirrhosis over time. Although, animal models of ALD do not recapitulate all components of human ALD, they are important tools in understanding the molecular mechanisms underlying alcohol-induced liver steatosis, inflammation and injury. Dietary fat and alcohol both play important roles in the pathogenesis of ALD. Diets enriched in saturated fatty acids protect against ALD, whereas linoleic acid (LA), a major unsaturated fatty acid in the American diet, is known to exacerbate alcohol-induced liver injury. However, the underlying molecular mechanism(s) are not completely understood. It is well documented that ethanol-induced endotoxemia due to the disruption of intestinal barrier integrity plays an important role in the ALD development. The aim of the present study was to examine the effects of different types of dietary fat on intestinal barrier integrity and consequent liver injury in an animal model of ALD.

Materials and Methods: In this study we employed a Lieber-DeCarli ad libitum EtOH feeding model, a widely accepted animal model of ALD. C57BL/6N mice were fed either an unsaturated fat (USF, LA enriched) or a saturated fat (SF, medium triglycerides enriched [MCT]) control or EtOH-containing diets for 8 weeks. Control mice were pair-fed on an isocaloric basis. Initially, all mice were given the control liquid maltose dextrin diets (SF or USF, no EtOH) for one week. Ethanol was gradually increased every 3-4 days from 11.2% to 35% of total calories. Liver injury and steatosis; intestinal morphology and inflammation; intestinal permeability and blood endotoxin levels were evaluated.

Results: After 8 weeks of EtOH feeding significant liver injury and steatosis were observed in USF+EtOH compared to pair-fed group. These effects of EtOH were blunted by SF diet containing MCT. Serum ALT levels, as a marker of liver injury, was significantly higher ($p<0.05$) in USF+EtOH group (44.91+2.81 IU/L) compared to SF+EtOH fed animals (27.27+1.92 IU/L). Hepatic triglyceride content was also higher in USF+EtOH compared to SF+EtOH fed animals (100.2+8.1 vs 68.76+7.96

mg/g liver, $p < 0.05$). The effects of ethanol on intestinal integrity associated with different types of fat were evaluated by measuring the intestinal permeability to FD-4. Significantly increased gut permeability was observed in the ileum segments of the mice fed USF+EtOH, this event was accompanied by a 4.5-fold ($p < 0.05$) increase of blood LPS levels compared to control animals. A moderate, but not significant, increase of LPS levels was found in SFD+EtOH compared to pair-fed group. Significantly elevated intestinal TNF- α and MCP1 mRNA levels as a markers of inflammation were detected only in USF+EtOH group.

Conclusion: The current data demonstrated that EtOH and unsaturated, but not saturated, fat promote liver injury associated with increased gut permeability. Intestinal inflammation caused by USF+EtOH likely play a critical role in the gut leakiness, elevated blood endotoxemia and consequent liver injury in an animal model of ALD.

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Effects of hypoxia time on the extracellular matrix accumulation and development of renal fibrosis in the adriamycin-treated rats

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Background: Tubulointerstitial fibrosis (TIF) is characterized by the accumulation of interstitial fibroblasts and the deposition of extracellular matrix (ECM), which lead to end-stage renal failure. Its presence correlates with impaired excretory function and the degree of fibrosis is a strong pathologic marker of progression. The histopathology of TIF indicates the deposition of ECM in association with inflammatory cells, tubular cell loss, and fibroblast accumulation. Recent experimental evidence suggests that hypoxia and prolonged activation of hypoxia-inducible factor (HIF) are associated with tubulointerstitial injury, which leads to fibrosis and further tissue damage. In this study, the effects of hypoxia time on the ECM accumulation and the development of fibrosis are investigated in experimental nephropathy model.

Methods: Adriamycin-injected nineteen male Wistar Hannover rats were assigned into three groups as sham operation, 15 min ischemia-reperfusion (IR), and 45 min IR (n: 6, 7, and 6, respectively) in the left kidney. Tissue sections were stained with Masson's Trichrome stain, and immunohistochemical (IHC) staining was performed for fibronectin, integrin, laminin, and TGF- β 1, and the levels of these parameters were also measured by ELISA in serum samples. Additionally, connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), α -SMA, and HIF-1 α tissue expressions were examined through IHC method.

Results: When compared to the sham group (Masson's Tricrom), there were seen periglomerular fibrosis, segmental sclerosis and thin interstitial fibrosis in the 15 min IR group. Also, when compared to 15 min IR group; a marked fibrosis in glomerules and interstitium in the 45 min IR group was seen. Dense staining fibronectin, integrin, laminin, TGF- β 1, and α -SMA were seen in the evaluation of the IHC staining. Moreover, the serum levels of fibronectin, integrin, laminin, TGF- β 1 and VEGF were found to be higher both in the 15 min IR and in the 45 min group than the sham group.

Conclusion: The current study shows that hypoxia and increased renal HIF-1 α expression are associated with tubulointerstitial injury, which lead to further tissue damage and fibrosis. The levels of fibronectin, integrin, laminin and TGF- β 1 can be used as biomarkers in the diagnosis of renal fibrosis. Hence, understanding the changes in ECM-associated proteins and biomarkers in the progressive renal injury will facilitate in the design of novel strategies to treat chronic kidney disease, such as hypoxia-induced TIF and glomerulosclerosis.

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Role of RNase L in Kidney

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Objective: Investigate the role of RNaseL in kidney function and investigate the mechanism by which RNaseL regulates the EGF excretion in urine.

Clinical Relevance: Renal diseases have been continuing to be a prevalent problem. Current data indicate that 1% of patients admitted to the hospital are diagnosed initially with acute kidney injury (AKI), while about 2-5% of hospitalized patients develop AKI secondarily. It has been reported that epidermal growth factor (EGF)/EGFR activation contributes to the development and progression of renal diseases such as obstructive nephropathy, diabetic nephropathy, hypertensive nephropathy, and glomerulonephritis through mechanisms involved in induction of tubular atrophy,

overproduction of inflammatory factors, and/or promotion of glomerular and vascular injury.

Methodology: In this study, we used an animal model of wild type and RNaseL knockout mice to show that 2-5A dependent RNaseL (RNaseL), one of the key enzymes playing an important role in the molecular mechanisms of interferon functions against microbial infection and cell proliferation, mediated EGF/EGFR activation. Interestingly, we found that the dissected kidney from aged (18months) RNaseL deficient mice was significantly smaller than that in wild type mice under the same condition. Histological staining revealed that there were remarkably a higher number of vacuoles in the kidney of RNaseL deficient mice than that in wild type mice although the biological significance of the observation is largely unknown. Using Westernblot analysis and proteomic analyses of urine protein excretion discovered that lack of RNase L exclusively blocked EGF excretion. Further investigation of the molecular mechanism showed that RNaseL regulated the shedding of EGF precursor through inhibiting some specific proteases responsible for the event.

Conclusions: Lack of RNaseL may affect the kidney function and development, and altered protein production. Our findings provide new insight into the pathogenesis of renal diseases and RNaseL may be considered as a target molecule for therapeutic treatment of the diseases.

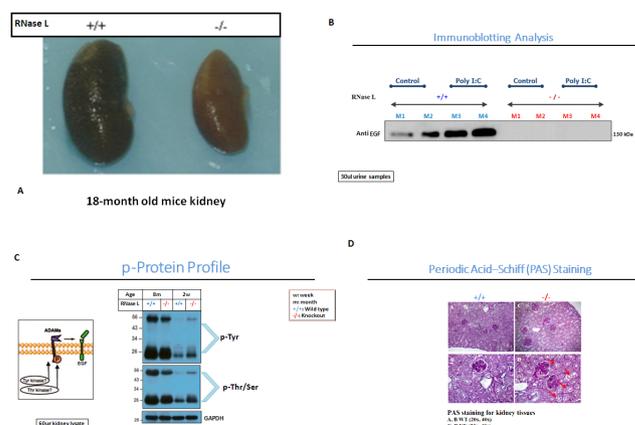


Fig 1: A: Aged mice kidneys of Wild type and knockout RNase L show differences in size and color. B: urine samples obtained from Wild type mice (M1-M4 - blue) and RNaseL knockout mice (M1-M4 - red), subjected to SDS-PAGE analysis then immunoblotted with anti-EGF antibody (ABCAM). C: protein extracted from kidney of wild type and RNase L knockout mice by NP-40 lysis buffer subjected to SDS-PAGE immunoblotted with anti-phosphotyrosin and phosphoserin and phosphothreonin antibodies from (Santa Cruz). D: PAS staining was performed on formaldehyde fixed kidney tissues. A, B: wild type (20x, 40x); C, D: Knockout type (20x, 40x)

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A comparison of the Jaffee creatinine and Enzymatic creatinine in pre-clinical species

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This study was designed to compare the non specific colorimetric (Jaffe reaction) and the enzymatic methods of creatinine measurements in monkey, dog, rat, and mouse serum and urine samples. Siemens Healthcare Diagnostics manufacturers both the Jaffe creatinine (CREA_2), and Enzymatic creatinine (ECRE_2) which are optimized for the Siemens Advia® 1800 automated clinical chemistry system. These assays utilize liquid-ready –to– use reagents and require the same Siemens chemistry calibrator. The Jaffe reaction of creatinine and picric acid is the oldest clinical methodology still in use for the measurement of creatinine. There are many analytical problems associated with the use of the Jaffe's reaction, in particular those relating to positive and negative interference by non-creatinine chromogens. Common interfering substances of the Jaffe's based methods include glucose and bilirubin. Glucose and bilirubin both inhibit the reaction between creatinine and alkaline picrate which is the principle method of the Siemens CREA_2 method. Enzymatic creatinine assay is the most accurate routine method available at present. The enzymatic method exhibits several advantages over Jaffe based methods. The enzymatic method has greater specificity. Glucose does not interfere with the enzymatic method however bilirubin may have a negative interference depending on the concentration. The correlation of the CREA_2 and the ECRE_2 methods showed no difference in the results for monkey, dog and rat in serum and urine samples exhibiting an overall average R² value of 0.978. When comparing mice urine samples the two methods correlated well with an R² of 0.994 however, mice serum results showed no correlation exhibiting an, R² value of 0.2995. Since non-creatinine chromogens are a known interference in the Jaffe method, it is

the hypothesis that chromogens, specific in mouse serum, are interfering with this assay, and that the enzymatic method is the method of choice when analyzing mouse serum.

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Changes in the specific extracellular matrix protein levels are related with the renal fibrosis in rats with adriamycin-induced nephropathy

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Background: Adriamycin nephropathy is characterized by the reduction in glomerular filtration rate, proteinuria, glomerulosclerosis and tubulointerstitial fibrosis (TIF). Proposed stimuli for TIF include proteinuric glomerulopathy, inflammatory mediators, cytokines and growth factors, and tubulointerstitial ischemia secondary to glomerular capillary injury. In this study, we aimed to investigate the levels and expression of specific ECM proteins (fibronectin, integrin and laminin) and transforming growth factor-beta1 (TGF-β1). In addition to, levels of the other growth factors; connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), and as well as expression of α-smooth muscle actin (α-SMA), and α-Actinin4 in the adriamycin (ADR) induced renal fibrosis.

Methods: Fifteen Wistar Hannover rats were divided into control (n:6), and ADR injected groups (n:9). Kidney tissue sections were stained with the periodic schiff-methenamine (PAS-M) stain. Fibronectin, integrin, laminin, and TGF-β1 levels were measured by ELISA method in the serum samples, and their tissue expressions were also explored immunohistochemical method as well as α-SMA and α-Actinin4, and were examined under a microscope.

Results: Although normal morphology of the kidney in the control group, histopathologic changes were visualized in the renal tissue glomerular and tubulointerstitial areas of rats with ADR-induced nephropathy. The glomerular changes consisted of mild mesangial expansion, glomerular tuft adhesion to the Bowman's space, and glomerulosclerosis. There were dense staining fibronectin, integrin, laminin, TGF-β1, and α-SMA, except for α-actinin4, in the evaluation of the immunohistochemical staining. Moreover, fibronectin, integrin, laminin, TGF-β1 and VEGF serum levels were higher in the ADR group than in the control group.

Conclusion: These results indicate that the levels of TGF-β1, fibronectin, integrin and laminin can be used in the diagnosis of renal fibrosis which is characterised glomerulosclerosis and/or TIF. Hence, understanding the changes in ECM-associated proteins and other biomarkers in the development of fibrosis will facilitate in the design of novel strategies to treat chronic kidney disease and renal failure.

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Investigation of cocaethylene cardiotoxicity in Sprague-Dawley rats

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Background: Cocaine is one of the most abused psychostimulant and leading cause of non-prescription drug related deaths in the United States. It is frequently abused along with alcohol to achieve heightened euphoria. The metabolite of cocaine and alcohol, cocaethylene (CE), has a longer half-life than cocaine; thus the cocaine-induced euphoria is prolonged when alcohol is also present. CE has been associated with an overall increased health risks, especially cardiovascular problems, than either drug alone; however, its mechanism of cardio toxicity is not fully understood. In addition, cocaine abusers tend to have poor diet since it is known to act as an appetite suppressant. Our objective is first to investigate the cardio toxic effects of CE in Sprague-Dawley rats and secondly to determine whether nutrition modulates these effects.

Method: Male Sprague-Dawley rats were divided into three diet groups, normal diet (13.2% of calories from fat, 24.6% protein, 62.3% carbohydrate), low protein diet (10% of calories from fat, 5.2% protein, and 84.7% carbohydrates), and high fat diet (62% of calories from fat, 18% protein, and 20% carbohydrates). Within each diet group (6 animals per group), animals were further divided into three CE dosage groups (0 mg/kg, 15 mg/kg, and 30 mg/kg). All animals received intra-peritoneal injections of either saline or CE daily for 28 days. Last CE dose was administration 24 hours prior to sacrifice. Rat heart tissues were analyzed macroscopically, microscopically, and histologically. Additionally, oxidative toxicity was also investigated. Heart tissue and plasma were analyzed for CE and its metabolite, benzoylecgonine (BE), via

LC-MS/MS. CE was provided by National Institutes on Drug Abuse and the study was approved by the Institutional Animal Care and Use Committee (IACUC) of our institute.

Result: Hearts of animals treated with CE showed histological evidence of myocarditis and inflammation without gross macroscopic changes. Furthermore, CE treatment also resulted in oxidative damages to heart tissues. Analyses of the heart tissues for the presence CE and BE via LC-MS/MS, indicated absence of these analytes in the tissues. However, when plasma was analyzed, BE detected in low protein and high fat diet animals were dose dependent. In contrast, animals on normal diet did not show dose dependent increase of BE in plasma. At the highest CE dose (30 mg/kg), animals on low protein and high fat diet showed increased level of BE in plasma compared to the normal diet animals.

Conclusion: Although CE induced oxidative and inflammatory responses in cardiac cells, neither CE nor BE were found to be deposited in those cells. The rate of CE or BE clearance may be influenced by diet. Thus, it can be inferred that chronic co-abuse of cocaine and alcohol via the formation of cocaethylene may result in oxidative and inflammatory injury to the heart which can be influenced by the nutritional status of abusers.