Antidiabetic Activity of Aqueous Kalanchoe pinnata Preparation: Potential Mechanism of Action

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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 hematomatological 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 eight 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 control 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 blood white 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.

Evaluation of oxidized linoleic acid metabolites in rodent models of alcoholic liver disease

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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 dextrose 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 (Tgfβ and Arg-1) cytokine gene expression. These changes may be a direct effect of HODEs on macrophage gene expression because RAW264.7 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.
Effect of Neutral Sphingomyelinase Inhibition on ER Stress and Apoptosis in Liver Ischemia-Reperfusion Injury


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: Spingomyelin 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.

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 ≥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 ±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.