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Original Contribution

Chronic ethanol ingestion induces oxidative kidney injury through taurine-inhibitable inflammation

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ABSTRACT

Chronic ethanol ingestion mildly damages liver through oxidative stress and lipid oxidation, which is ameliorated by dietary supplementation with the anti-inflammatory β-amino acid taurine. Kidney, like liver, expresses cytochrome P450 2E1 that catabolizes ethanol with free radical formation, and so also may be damaged by ethanol catabolism. Sudden loss of kidney function, and not liver disease itself, foreshadows mortality in patients with alcoholic hepatitis [J. Altamirano, Clin. Gastroenterol. Hepatol. 2012, 10:65]. We found that ethanol ingestion in the Lieber-deCarli rat model increased kidney lipid oxidation, 4-hydroxynonenal protein adduction, and oxidatively truncated phospholipids that attract and activate leukocytes. Chronic ethanol ingestion increased myeloperoxidase-expressing cells in kidney and induced an inflammatory cell infiltrate. Apoptotic terminal deoxynucleotidyl transferase nick-end labeling-positive cells and active caspase-3 increased in kidney after ethanol ingestion, with reduced filtration with increased circulating blood urea nitrogen (BUN) and creatinine. These events were accompanied by release of albumin, myeloperoxidase, and the acute kidney injury biomarkers kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin, and cystatin c into urine. Taurine sequesters HOCl from myeloperoxidase of activated leukocytes, and taurine supplementation reduced renal lipid oxidation, reduced leukocyte infiltration, and reduced the increase in myeloperoxidase-positive cells during ethanol feeding. Taurine supplementation also normalized circulating BUN and creatinine levels and suppressed enhanced myeloperoxidase, albumin, KIM-1, and cystatin c in urine. Thus, chronic ethanol ingestion oxidatively damages kidney lipids and proteins, damages renal function, and induces acute kidney injury through an inflammatory cell infiltrate. The anti-inflammatory nutraceutical taurine effectively interrupts this ethanol-induced inflammatory cycle in kidney.

Excessive alcohol use is common, with an estimated 8% of adult Americans abusing or depending on alcohol [1]. A significant subset of these individuals progress to alcoholic steatohepatitis and then to severe alcoholic hepatitis, of which the 6-month mortality is nearly 40% [2].

Ethanol is oxidized in the liver by three pathways, but metabolism by cytochrome P450 2E1 (CYP2E1)[1] monooxygenase, which forms superoxide ($O_2^{\bullet -}$) and then H_2O_2, is the path that instigates tissue oxidative stress and damage [3]. Genetic ablation and transgenic rescue [4] show that CYP2E1 participates in ethanol toxicity, whereas inhibition and genetic targeting to reduce reactive oxygen species (ROS) formation from type 2 NADPH oxidase (NOX2) show this enzyme also is critical in ethanol toxicity [5,6]. Thus, both tissue-produced ROS from CYP2E1 and ROS from the NOX2 of invading inflammatory cells contribute to liver damage from ethanol catabolism.

The syndrome of acute kidney injury (AKI) describes the sudden loss of kidney filtration with the retention of nitrogenous waste products in blood (i.e., blood urea nitrogen, BUN). Kidney expresses 5 to 10% of total body CYP2E1 [7–10] and whereas chronic alcoholism associates with chronic kidney disease [11–13], it is the development of AKI that associates with the mortality of alcoholic hepatitis [14]. This chronic ethanol use is modeled in rats by feeding relevant amounts of ethanol for 28 days [15]. Ethanol feeding well beyond this time structurally damages the kidney [16,17], but little is known about ethanol damage to this organ.

Free radical oxidation of cellular phospholipids generates a host of oxidatively truncated phospholipids. Leukocytes express the single receptor for the inflammatory phospholipid platelet-activating factor (PAF) [18], and some oxidized phospholipids sufficiently resemble the phospholipid PAF that they are ligands and agonists of this G-protein-coupled receptor [19]. This is relevant because biologic

Abbreviations: AKI, acute kidney injury; CYP2E1, cytochrome P450 isotype 2E1; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; PAF, platelet-activating factor

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PAF metabolism is controlled [20], whereas formation of oxidatively truncated phospholipids is not [21], so oxidation can induce unregulated inflammation and leukocyte activation.

PAF attracts and activates neutrophils [22] that are the primary repository of myeloperoxidase. Myeloperoxidase uses H_2O_2 to form HOCl [23] that is primarily responsible for oxygen-dependent bacterial killing [24], but also damages host tissue.

The nonmetabolizable β-amino acid taurine suppresses inflammation [25] and reduces hepatic lipid oxidative stress [26], protecting liver function during ethanol metabolism [27,28]. Because it is nonmetabolizable, the way taurine is protective is ill-defined, but its anti-inflammatory and antioxidative activities primarily [25] result from its sequestration of HOCl and HOBr [29]. However, taurine also regulates osmolarity and membrane stability, aids Ca^{2+} homeostasis [25], and, in neurons, protects mitochondria and endoplasmic reticulum from stress [30].

We hypothesized that ethanol catabolism imposes damaging oxidative stress in kidney because CYP2E1 is present in this organ. Because taurine protects against several kidney diseases [31] and has anti-inflammatory/antioxidant activities, we also determined whether this β-amino acid would protect kidney from potential damage imposed by ethanol catabolism.

Material and methods

Materials

Adult male Wistar rats (~170–180 g) were purchased from Harlan Sprague–Dawley (Indianapolis, IN, USA). Lieber-deCarli liquid ethanol diet was from Dyets (Bethlehem, PA, USA) and taurine from Sigma (St. Louis, MO, USA). Anti-albumin antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA), anti-4-hydroxynonenal antibody was from Alpha Diagnostics (San Antonio, TX, USA), and anti-myeloperoxidase and anti-neutrophil gelatinase-associated lipocalin (NGAL) antibodies were from Abcam (Cambridge, MA, USA). Kits for BUN and creatinine were purchased from Arbor Assays (Ann Arbor, MI, USA). Anti-KIM-1 antibody, terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay kits, KIM-1 ELISA kits, and cystatin c ELISA kits were from R&D Systems (Minneapolis, MN, USA). Caspase-3 Apo-ONE Homogeneous Caspase-3/7 fluorescence assay kits were the product of Promega (Madison, WI, USA) and myeloperoxidase activity assay kits were from Cell Technology (Mountain View, CA, USA).

Animal model and ethanol feeding

The chronic ethanol feeding model used in this study has been previously described [28] using animals that received humane care in a protocol approved by the Cleveland Clinic Institutional Animal Care and Use Committee. Briefly, age-matched rat litters were randomly assigned to be ethanol fed or pair fed a control diet in which maltose dextrin isocalorically substituted for ethanol in the liquid diet to maintain equal body weights. For the first 2 days of the protocol, rats in the ethanol group were fed a liquid diet with 17% of the calories supplied as ethanol and then were provided an ad libitum liquid diet for 4 weeks containing 6.4% (v/v) ethanol, which constitutes 36% of the total caloric content. The amount of ethanol in the circulation of these animals is not excessive, being in the clinically relevant 0.1–0.15% range. Rats were anesthetized and exsanguinated, with serum isolated and stored at −80 °C. Kidneys were isolated and stored in RNAlater for RNA quantification or at −80 ° until homogenization. A small portion of the kidney was immediately fixed in 10% formalin for histology. For taurine supplementation, Lieber-deCarli ethanol diets or pair-fed diets were supplemented with 30 g of taurine per liter of diet [32].

Collection of urine

Urine was collected by syringe withdrawal from the urinary bladder after anesthesia injection just before sacrifice. Samples were immediately centrifuged (300 *g*, 5 min) to remove debris or casts before storage at −80 °C.

Western blots

Excised rat kidneys were washed with phosphate-buffered saline (PBS) and homogenized 10 times in a Potter–Elvehjem glass homogenizer with a tight pestle containing 1× lysis buffer (Cell Signaling Technology) with protease inhibitor (Sigma) on ice before centrifugation (30 min, 14,000 *g*). The resulting supernatants were mixed with Laemmli gel loading buffer containing 10% SDS and 200 mM dithiothreitol (DTT), followed by boiling. For Western blotting of urine samples, equal volumes of urine samples were mixed with 6× Laemmli buffer containing 10% SDS and 200 mM DTT, followed by boiling. SDS–PAGE, unless otherwise stated, occurred in 10–12% gels that were blotted onto nitrocellulose membranes (Bio-Rad) and blocked with 5% nonfat dry milk (Bio-Rad). Detection used anti-CYP2E1 (Abcam, 1:2000), anti-myeloperoxidase (Abcam, 1:2000), anti-KIM-1 (R&D Systems, 1:2000), anti-NGAL (Abcam, 1:2000), or anti-albumin (Santa Cruz Biotechnology, 1:10000) antibodies incubated overnight at 4 °C. The conjugates were then ligated by horseradish peroxidase (HRP)-conjugated anti-rabbit (1:5000) or anti-mouse (1:10,000) or anti-goat (1:20,000) antibody before detection with Amersham Biosciences ECL Prime. Blots were reprobed with anti-β-actin (Santa Cruz Biotechnology).

TUNEL assay

Kidneys were fixed in 10% buffered formalin and embedded in paraffin. Kidney sections (5 μm) were deparaffinized in Safeclear II xylene substitute and consecutively hydrated in 100, 95, 85, and 70% ethanol followed by two washes in PBS. TUNEL staining was performed according to the manufacturer's (R&D Systems) protocol. Briefly, kidney sections were treated with proteinase K (30 min) for antigen retrieval before peroxidase activity was blocked with methanol and hydrogen peroxide. The sections were washed with PBS and incubated with labeling buffer followed by the reaction mix containing TdT, dNTP, and TdT enzyme with Mn^{2+} for 60 min at 37 °C. Sections were again washed with PBS before incubation with Strep–HRP solution for 10 min at 37 °C. After being washed in PBS, the sections were treated with diaminobenzidine chromogen solution for 5 min at room temperature, washed, and immersed in 1% methyl green for 1 min. The sections were air dried and mounted with mounting medium. Images were acquired with a 60× objective.

Caspase-3 activity

Caspase-3 activity was measured with Apo-ONE Homogeneous Caspase-3/7 assay kit (Promega) using crude kidney homogenates according to the manufacturer's protocol. Briefly, 50 μl of Apo-ONE Caspase-3/7 reagent and 50 μl of kidney homogenate were co-incubated for 1 h at 37 °C before the fluorescence intensity was measured (SpectraMax 100) with excitation at 499 nm and emission at 515 nm. The background fluorescence was corrected by subtracting the value derived from the no-enzyme control.