Research Article

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Pharmacological activation of aldehyde dehydrogenase 2 by Alda-1 reverses alcohol-induced hepatic steatosis and cell death in mice

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Background & Aims: Effective therapies for alcoholic liver disease are currently unavailable. The present study tested the efficacy of Alda-1, a specific aldehyde dehydrogenase 2 (ALDH2) activator, in treating alcoholic liver disease.

Methods: Male C57BL/6J mice were exposed to alcohol for a time-course study on aldehyde metabolism. The specificity and efficacy of Alda-1 on activating hepatic ALDH2 and aldehyde clearance were determined by acute treatments. Then, mice were fed alcohol for 8 weeks with Alda-1 administration for the last 10 days to test the therapeutic potential of Alda-1. Lastly, H4IIEC3 cells were treated with ethanol, acetaldehyde, or 4-hydroxynonenal to define the link between aldehydes and hepatotoxicity.

Results: Alcohol feeding for 8 weeks induced hepatic ALDH2 dysfunction and aldehyde accumulation. One dose of Alda-1 administration elevated hepatic ALDH activity, which was blocked by the specific ALDH2 inhibitor, daidzin. Alda-1 accelerated acetaldehyde clearance after acute alcohol intoxication. Alda-1 treatment in the 8-week alcohol feeding model reversed liver damage along with reduction of hepatic aldehydes. Alda-1 re-activated transcription factors, upregulated fatty acid oxidation enzymes, and reversed steatosis. Alcohol-induced endoplasmic reticulum stress and apoptotic cell death were also attenuated by Alda-1. Acetaldehyde or 4-hydroxynonenal treatment to H4IIEC3 cells inactivated transcription factors and induced endoplasmic reticulum stress and apoptosis, while ethanol *per se* showed limited effects.

Conclusions: Pharmacological activation of ALDH2 by Alda-1 reversed alcoholic steatosis and apoptosis through accelerating aldehyde clearance. This study indicates that ALDH2 is a promising molecular target and Alda-1 has therapeutic potential for treating alcoholic liver disease.

Introduction

Alcoholic liver disease (ALD) is a major cause of morbidity and mortality worldwide [1]. Although great efforts to explore potential therapeutic targets for ALD have been made for decades, effective therapies for any stage of ALD are currently lacking.

Ethanol is metabolized mainly in the liver through alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1), and catalase pathways [2], which all generate acetaldehyde. Ethanol metabolism also generates reactive oxygen species which, in turn, cause lipid peroxidation, leading to formation of lipid aldehydes such as 4-hydroxynonenol (4-HNE) and malondialdehyde (MDA). Acetaldehyde is known to be detoxified to acetic acid by the aldehyde dehydrogenase (ALDH) family, of which the mitochondrial ALDH2 is the most potent isoenzyme. Furthermore, ALDH2 is also involved in lipid aldehyde detoxification [3].

The vital role of ALDH2 in aldehyde detoxification and ALD progression has been reported both in humans and experimental models. Individuals carrying ALDH2 mutant alleles showed a high blood acetaldehyde level after alcohol consumption [4], thereby being more susceptible to alcohol-induced organ injury. ALDH2 knockout mice showed an increased acetaldehyde accumulation in the liver after alcohol exposure [5,6] or acetaldehyde inhalation [7] along with exaggerated liver inflammation and fibrosis [6]. In contrast, ALDH2 over-expressing mice exhibited

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Abbreviations: ALDH2, aldehyde dehydrogenase 2; ALD, Alcoholic liver disease; ADH, alcohol dehydrogenase; 4-HNE, 4-hydroxynonenol; MDA, malondialdehyde; AF, alcohol-fed; PF, pair-fed; BW, body weight; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

a significant reduction in acetaldehyde as well as apoptosis after chronic alcohol feeding [8]. These findings provide a strong rational of targeting ALDH2 for treating ALD. Our previous study showed that upregulation of hepatic ALDH2 by zinc supplementation is associated with attenuation of MDA accumulation in the liver of alcohol-fed mice [9].

The present study was designed to test if pharmacological activation of ALDH2 by Alda-1 (*N*-[1,3-benzodioxol-5-ylmethyl]-2,6-dichlorobenzamide), a specific ALDH2 activator, can reverse liver damage pre-established by chronic alcohol exposure, and to determine how Alda-1 modulates alcoholic hepatotoxicity.

Materials and methods

Animals and treatments

Male C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal experiments were performed according to the protocol approved by the North Carolina Research Campus Institutional Animal Care and Use Committee (project number 13017).

Experiment I

To determine the effects of ethanol exposure on hepatic ALDH2 and aldehyde accumulation, mice at 10 weeks of age were fed the Lieber-DeCarli alcohol (alcohol-fed, AF; n = 8) or control (pair-fed, PF; n = 6) liquid diet (Dyets Inc., Bethlehem, PA) for 2, 4, or 8 weeks. The ethanol content (%, w/v) in alcohol diet was 4.00%, 4.14%, 4.28%, and 4.42% for every 2 weeks, respectively. The AF mice were fed *ad libitum*, while PF mice were fed the control diet in the same amount consumed by AF mice in the previous day.

Experiment II

Mice were given 5 mg/kg body weight (BW) Alda-1 (dissolved in dimethyl sulfoxide, DMSO) at 9:00 am *via* three routes, intraperitoneal injection (*i.p.*), femoral vein injection, or gavage (n = 4 for each group). Livers were collected 3 hours after treatments, and hepatic ALDH activity was immediately measured. To assess the specificity of Alda-1 on activating ALDH2, mice were given an ALDH2 specific inhibitor, daidzin (Cayman Chemical, Ann Arbor, MI), at 50 mg/kg BW (dissolved in DMSO) via *i.p.* along with 5 mg/kg BW Alda-1 administration at 9:00 am (n = 4). Three hours later, liver and ileum were collected, mitochondrial and cytosolic fractions from the liver were isolated, and ALDH activity was immediately measured.

Experiment III

To test the effects of ALDH2 activation on acetaldehyde clearance, mice were given a single intragastric dose of ethanol (5 g/kg BW) right after an *i.p.* injection of Alda-1 at 5 mg/kg BW at 9:00 am (n = 4). Blood and liver samples were collected at 1, 3, or 24 hours after ethanol gavage.

Experiment IV

Mice were fed the Lieber-DeCarli alcohol or control diet for 8 weeks as described in Experiment I. Alda-1 was given via *i.p.* injection at 5 mg/kg BW every other day for the last 10 days (n = 6).

Cell culture and treatments

H4IIEC3 rat hepatoma cells (American Type Culture Collection, Rockville, MD) were treated with 100 mmol/L ethanol, 100 μmol/L acetaldehyde, or 10 μmol/L 4-HNE for 3 days, respectively, with or without Alda-1 at 20 μmol/L.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). To compare values between two groups, Student's *t* test was used. To compare values obtained from four groups, one-way analysis of variance (ANOVA) was performed, followed by Student-Newman-Keuls post hoc test. Differences between groups were considered significant at $p < 0.05$.

Results

Chronic alcohol feeding caused hepatic ALDH2 dysfunction and aldehyde accumulation

To determine hepatic ALDH2 responses to chronic alcohol exposure, mice were fed alcohol for 2, 4, or 8 weeks. Compared to the PF mice, the AF mice showed reduced *ALDH2* mRNA levels at 2 and 8 weeks (Fig. 1A). An increased ALDH protein level was found in AF mice at both 4 and 8 weeks, and also in PF mice at 8 weeks (Fig. 1B, C). Compared to a uniform distribution in PF mice, the port vein and central vein area showed more ALDH2 distribution in AF mice (Fig. 1C). Although the ALDH2 protein levels at 8 weeks are comparable in the PF and AF mice, the ALDH

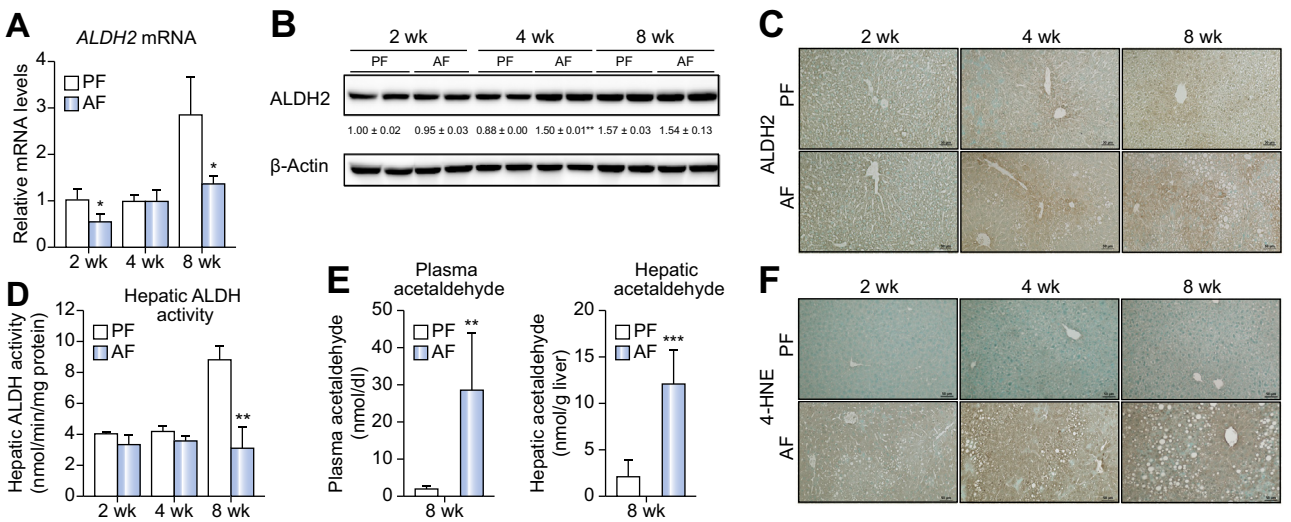

Fig. 1. Chronic alcohol exposure caused hepatic ALDH2 dysfunction and aldehyde accumulation. Mice were fed the Lieber-DeCarli liquid diets for 2, 4, and 8 weeks. (A) mRNA levels, (B) protein levels, and (C) immunohistochemistry of hepatic ALDH2. (D) Activities of hepatic ALDH. (E) Plasma and hepatic acetaldehyde concentrations. (F) Immunohistochemical staining of hepatic 4-HNE. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* PF. PF, pair-fed; AF, alcohol-fed. Scale bar: 50 μm.