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Aldehyde Dehydrogenase-2 Transgene Ameliorates Chronic Alcohol Ingestion-Induced Apoptosis in Cerebral Cortex

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Abstract

Chronic intake of alcohol results in multiple organ damage including brain. This study was designed to examine the impact of facilitated acetaldehyde breakdown via transgenic overexpression of mitochondrial aldehyde dehydrogenase-2 (ALDH2) on alcohol-induced cerebral cortical injury. ALDH2 transgenic mice were produced using the chicken β -actin promoter. Wild-type FVB and ALDH2 mice were placed on a 4% alcohol or control diet for 12 wks. Protein damage and apoptosis were evaluated with carbonyl formation, caspase and TUNEL assays. Western blot was performed to examine expression (or its activation) of ALDH2, the pro- and anti-apoptotic proteins Caspase-8, Bax, Bcl-2, Omi/HtrA2, apoptosis repressor with caspase recruitment domain (ARC), FLICE-like Inhibitory Protein (FLIP), X-linked inhibitor of apoptosis protein (XIAP), Akt, glycogen synthase kinase-3 β (GSK-3 β), p38, c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). Chronic alcohol intake led to elevated apoptosis in the absence of overt protein damage, the effect of which was ablated by the overexpression of ALDH2 transgene. Consistently, ALDH2 transgene significantly attenuated alcohol-induced upregulation of Bax, Omi/HtrA2 and XIAP as well as downregulation of Bcl-2 and ARC without affecting alcohol-induced increase of FLIP in cerebral cortex. Phosphorylation of Akt and GSK-3 β was dampened while total/phosphorylated JNK and p38 phosphorylation were elevated following chronic alcohol intake, the effects of which were abrogated by ALDH2 transgene. Expression of total Akt, GSK-3 β , p38 and ERK (total or phosphorylated) was not affected by either chronic alcohol intake or ALDH2 transgene. Our results suggested that transgenic overexpression of ALDH2 rescues chronic alcoholism-elicited cerebral injury possibly via a mechanism associated with Akt, GSK-3 β , p38 and JNK signaling.

Keywords

Alcohol; ALDH2 transgene; cerebral cortex; cell injury

INTRODUCTION

Chronic alcohol intake leads to alcoholic organ damage including alcoholic neuropathy, which is manifested by impaired neuronal survival, growth, neurotransmitter function and intracellular adhesion in alcoholics (Harper and Matsumoto 2005). A number of hypotheses have been speculated for the alcohol-induced brain tissue damage including direct toxicity of

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alcohol or its metabolite acetaldehyde, accumulation of reactive oxygen species and fatty acid ethyl esters, modifications of lipoprotein and apolipoprotein particles, insulin resistance, metabolic and excitotoxic changes as well as genetic predisposition (Hannuksela et al. 2002; Li and Ren 2007a; Zhang et al. 2004; Zimatkin et al. 2006). However, none of these theories has been fully validated by either clinical or experimental data. An ample of clinical, epidemiological and experimental evidence over the last two decades has demonstrated a high morbidity associated with elevated levels of acetaldehyde, the first oxidized metabolite of ethanol, in individuals with chronic alcohol ingestion, especially in Asian and African American populations with defective aldehyde dehydrogenase (ALDH) (Ren 2007a; Tsukamoto et al. 1989). These observations have prompted to the “acetaldehyde toxicity” theory in alcoholic brain complications. Nonetheless, precise mechanism is still lacking to fully validate the role of acetaldehyde in alcoholic neuropathy. To better elucidate the role of acetaldehyde in alcoholic brain damage, a transgenic mouse line was generated to overexpress the human mitochondrial ALDH type 2 (ALDH2) in order to facilitate acetaldehyde detoxification. Apoptosis and protein damage were evaluated along with the expression of pro- and anti-apoptotic proteins Bax, Bcl-2, apoptosis repressor with caspase recruitment domain (ARC), the serine protease Omi/HtrA2, FLICE-like inhibitory protein (FLIP) and X-linked inhibitor of apoptosis (XIAP). We also evaluated the cell survival factors Akt and glycogen synthase kinase-3 β (GSK-3 β), as well as stress signaling molecules mitogene-activated protein kinase (MAPK) family including p38, c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinases (ERK) in cerebral cortex from ALDH and wild-type FVB mice following chronic alcohol intake.

MATERIALS AND METHODS

Generation of ALDH2 transgenic mice

All animal procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee. The human ALDH2 gene was amplified by PCR from pT7-7-hpALDH2 (kindly provided by Dr. Henry Weiner from Purdue University, Lafayette, IN) using the following primers: ALDH-F (5'-tcgaattctatgttgcgctgccgccc) and ALDH-R (3'-cacggagtcttcttgattcttaaggc). The amplified ALDH2 fragment was digested with EcoRI and cloned into the EcoRI site of vector pBsCAG-2 under the CAG cassette, where ALDH activity was increased using the chicken β -actin promoter as reported previously (Li et al. 2004). This promoter has been widely used to produce a systemic overexpression of the gene of interest (Valera et al. 1994). Elevated ALDH activity reduces circulating acetaldehyde by metabolizing it to acetate. The full length of the promoter portion of the CAG-ALDH gene was sequenced to confirm accuracy. The transgene can be removed from the plasmid by digestion with Kpn I and Sst I, which was shown to drive expression in many mammalian cells (Li et al. 2004; Li et al. 2006). The ALDH2 insert was excised and separated from the plasmid by Kpn I/Sst I restriction digestion and agarose gel electrophoresis. The insert was purified on Qiagen 20 columns, followed by the spin gel chromatography and filtration through 0.22 μ m filters. A concentration of 1 μ g/ μ l of purified transgene insert DNA was microinjected into a one-cell embryo of the inbred strain FVB. Around 20–30 microinjected embryos were implanted into each pseudopregnant female and allowed to come to term. After weaning, mice tail clips were collected for genotyping of DNA insertion of ALDH2 (Fig. 1A). Further breeding was conducted with the same background wild-type FVB. All mice were housed in a temperature-controlled room under a 12hr/12hr-light/dark and allowed access to tap water *ad libitum*. Four month-old adult male FVB and ALDH2 transgenic (F8) mice were placed on a nutritionally complete liquid diet (Shake & Pour Bioserv Inc., Frenchtown, NJ) for a one-week acclimation period. The use of a liquid diet is based on the scenario that ethanol self-administration resulted in less nutritional deficiencies and less stress to the animals in comparison to forced-feeding regimens, intravenous administration, or aerosolized inhalation (Keane and Leonard 1989).

Upon completion of the acclimation period, half of the FVB and ALDH2 transgenic mice were maintained on the regular liquid diet (without ethanol), and the remaining half began a 12-week period of isocaloric 4% (vol/vol) ethanol diet feeding. An isocaloric pair-feeding regimen was employed to eliminate the possibility of nutritional deficits. Control mice were offered the same quantity of diet ethanol-consuming mice drank the previous day. Body weight was monitored weekly (Hintz et al. 2003).

Measurement of blood ethanol levels

On the last day of diet feeding (0800 AM), mice were sacrificed under anesthesia (ketamine/xylazine: 3:1, 1.32 mg/kg, i.p.). Blood was collected and was stored in sealed vials. A volume of 100 μ l plasma from each sample was put into an autosampler vial. Six microliter of n-propanol and 194 μ l H₂O were then added to the vial. Following a 20-min incubation at 50°C, 50 μ l aliquot of headspace gas was removed and transferred to an Agilent 6890 Gas Chromatograph (Agilent Technologies, Inc, Wilmington, DE) equipped with a flame ionization detector. Ethanol, n-propanol and other components such as acetaldehyde were separated on a 60 m VOCOL capillary column (Supelco Inc., Bellefonte, PA) with film of 1.8 μ m in thickness and an inner diameter of 320 μ m. The carrier gas was helium at a flow rate of 18.0 ml/min. Quantitation was achieved by calibrating peak areas against those from headspace samples of known ethanol standards (Li and Ren 2006).

Tissue collection and protein carbonyl assay

To collect cerebral cortex, a pair of sturdy dissecting scissors was used to cut open the scalp along the longitudinal fissure. After the brain was carefully opened with the membranous tissues removed, cerebellum located at the posterior dorsal region of brain was gently lifted to isolate the two hemispheres of cerebral cortex (Li and Ren 2007b). To assess oxidative protein damage, the carbonyl content of protein extracted from the isolated cerebral cortex was determined as described (Ren 2007b). Briefly, proteins were extracted and minced to prevent proteolytic degradation. Nucleic acids were eliminated by treating the samples with 1% streptomycin sulfate for 15 min, followed by a 10 min centrifugation (11,000 $\times$ g). Protein was precipitated by adding an equal volume of 20% TCA to protein (0.5 mg) and centrifuged for 1 min. The TCA solution was removed and the sample resuspended in 10 mM 2,4-dinitrophenylhydrazine (2,4-DNPH) solution. Samples were incubated at room temperature for 15–30 min. Following a 500 μ l of 20% TCA addition, samples were centrifuged for 3 min. The supernatant was discarded, the pellet washed in ethanol:ethyl acetate and allowed to incubate at room temperature for 10 min. The samples were centrifuged again for 3 min and the ethanol:ethyl acetate steps repeated 2 more times. The precipitate was resuspended in 6 M guanidine solution, centrifuged for 3 min and insoluble debris removed. The maximum absorbance (360–390 nm) of the supernatant was read against appropriate blanks (water, 2 M HCl) and the carbonyl content was calculated using the molar absorption coefficient of 22,000 M⁻¹cm⁻¹.

Caspase-3 assay

The caspase-3 activity was determined according to the published method (Li et al. 2004). Briefly, 1 ml PBS was added to a flask containing cerebral cortex homogenates prior to centrifugation at 10,000 g at 4°C for 10 min. The supernatant was discarded and homogenates were lysed in 100 μ l of ice-cold cell lysis buffer [50 mM HEPES, pH 7.4, 0.1% CHAPS, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.1% NP40]. The assay was carried out in a 96-well plate with each well containing 30 μ l of cell lysate, 70 μ l of assay buffer (50 mM HEPES, 0.1% CHAPS, 100 mM NaCl, 10 mM DTT and 1 mM EDTA) and 20 μ l of caspase-3 colorimetric substrate Ac-DEVD-pNA (Sigma). The 96-well plate was incubated at 37°C for 1 hr, during which time the caspase in the sample was allowed to cleave the chromophore p-NA from the

substrate molecule. Absorbency was detected at 405 nm with caspase-3 activity being proportional to color reaction. Protein content was determined using the Bradford method. The caspase-3 activity was expressed as picomoles of pNA released per μg of protein per minute.

Caspase- 3/7 assay

The caspase-3/7 activity was determined using an Apo-ONE homogeneous caspase-3/7 assay kit (Promega Corporation, Madison, WI). Caspase-3 and -7 are members of the cysteine aspartic acid-specific protease (caspase) family which play key roles in apoptosis in mammalian cells. In brief, activity of caspase-3 and caspase-7 activities were detected in cells undergoing apoptosis via cleavage of a rhodamine 110, bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide (Z-DEVD-R110) substrate, which exists as a profluorescent substrate prior to the assay. To perform the Apo-ONE caspase-3/7 assay, a caspase-3/7 buffer and the Z-DEVD-R110 substrate were mixed and added to the cerebral cortex sample. Upon sequential cleavage and removal of the DEVD peptides by caspase-3/7 activity, the R110 leaving group becomes intensely fluorescent at an excitation wavelength of 499 nm and an emission wavelength of 521 nm. The caspase-3/7 activity was directly proportional to R110 fluorescence and was expressed as the net fluorescence (Alnemri et al. 1996).

TUNEL staining

TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) assessment of myonuclei positive for DNA strand breaks was determined using a fluorescence detection kit (Roche Applied Science) and fluorescence microscopy. After perfusion, brains from four groups were removed and fixed in 4% paraformaldehyde overnight at room temperature. Cross sections (5 μm) from brains were placed in a cryostat (-23°C) and fixed in 4% paraformaldehyde 20 mins and then fixed. Sections were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. TUNEL reaction mixture containing terminal deoxynucleotidyl transferase (TdT), fluorescein-dUTP was added to the sections in 50- μl drops and incubated for 60 min at 37°C in a humidified chamber in the dark. The sections were rinsed three times in PBS for 5 min each. Following embedding, sections were visualized with an Olympus BX-51 microscope equipped with an Olympus MaguaFire SP digital camera. DNase I and label solution were used as positive and negative controls. To determine the percentage of apoptotic cells, the TUNEL-positive nuclei and TUNEL-negative cells were counted using the ImagePro image analysis software (Media Cybernetics) (Li Q et al. 2007).

Western blot analysis

The total protein was prepared as described (Dong et al. 2006). In brief, tissue samples from cerebral cortex were removed and homogenized in a lysis buffer containing 20 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 0.1% SDS and 1% protease inhibitor cocktail. Samples were then sonicated for 15 sec and centrifuged at $12,000\times g$ for 20 min at 4°C . The protein concentration of the supernatant was evaluated using Protein Assay Reagent (Bio-Rad, Hercules, CA). Equal amounts (50 μg protein/lane) of the protein from the tissue extraction, or prestained molecular weight markers (SeeBlue® Plus2, Invitrogen, Carlsbad, CA) were separated on 10% or 15% SDS-polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II, Bio-Rad); then were transferred electrophoretically to Nitrocellulose membranes (0.2 μm pore size, Bio-Rad Laboratories, Inc, Hercules, CA). Membranes were incubated for 1 hr in a blocking solution containing 5% milk in Tris-buffered saline (TBS), then membranes were washed briefly in TBS and incubated overnight at 4°C with anti-ALDH2 (1:1,000, provided by Dr. Henry Weiner from Purdue University), anti-Caspase-8 (1:1,000, Cell Signaling, Beverly, MA), anti-Bax (1:1,000, Cell Signaling), anti-Bcl-2 (1:1,000, Santa Cruz. Biotech, Santa Cruz, CA), anti-Omi/HtrA2 (1:1,000, Abcam Inc, Cambridge, MA), anti-ARC (1:1,000, Santa Cruz), anti-FLIP_{S/L}, anti-HILP (XIAP, 1:1,000, BD Biosciences, San

Jose, CA), anti-Akt (1:1,000, Cell Signaling), anti-pAkt (1:1,000, Thr308, Cell Signaling), anti-GSK-3 β (1:1,000, Cell Signaling), anti-phospho-GSK-3 β (Ser9, 1:1,000, Cell Signaling), anti-p38 (1:1,000, Cell Signaling), anti-pp38 (Thr180/Tyr182, 1:1,000, Cell Signaling), anti-JNK (1:1,000, Cell Signaling), anti-pJNK (Thr183/Tyr185, 1:1,000, Cell Signaling), anti-ERK (1:1,000, Santa Cruz), anti-pERK (Tyr204, 1:1,000, Santa Cruz), and anti- β -actin (as loading control, 1:5,000, Cell Signaling) antibodies. After washing blots to remove excessive primary antibody binding, blots were incubated for 1 hr with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5,000). Antibody binding was detected using enhanced chemiluminescence (Amersham Pharmacia, Piscataway, NJ), and film was scanned and the intensity of immunoblot bands was detected with a Bio-Rad Calibrated Densitometer (Model: GS-800).

Data analysis

Data were presented as mean $\pm$ SEM. Statistical significance ($p < 0.05$) for each variable was estimated by analysis of variance (ANOVA) followed by a Tukey's *post hoc* analysis.

RESULTS

General feature of mice, cortical ALDH2 expression and cerebral cortex injury

Alcohol intake did not significantly affect body weight gain. Heart but not liver, kidney and brain weights were overtly increased compared with non-alcohol consuming mice. ALDH2 transgene did not affect body or organ weights in the absence of alcohol intake although it significantly alleviated alcohol-induced increase in heart weight. Blood alcohol levels were elevated in a comparable fashion in alcohol consuming FVB and ALDH2 transgenic mice. The levels of blood alcohol were minimal in non-alcohol consuming mice (Table 1). Cortical protein expression of ALDH2 was significantly elevated in the ALDH2 transgenic mice, validating the ALDH2 transgenic model. Cortical expression of ALDH2 was not affected by chronic alcohol intake in either wild-type FVB or ALDH2 transgenic group (Fig. 1B). Chronic alcohol intake is associated with cerebral cortex apoptosis (Caspase-3, Caspase-3/7 and Caspase-8) in the absence of protein damage evaluated by carbonyl formation. ALDH2 transgene itself did not affect apoptosis in cerebral cortex in the absence of alcohol intake although it significantly attenuated the alcohol ingestion-induced apoptosis. ALDH2 transgene did not affect protein carbonyl formation in the presence or absence of alcohol intake (Fig. 2). The chronic alcohol intake- and ALDH2 transgene-elicited effect on apoptosis in cerebral cortex was further supported by TUNEL staining analysis. The TUNEL-positive nuclei visualized in fluorescein green as a percentage of all nuclei stained with DAPI (blue) was significantly elevated in cortex from chronic alcohol-fed FVB mice, the effect of which was ablated by the ALDH2 transgene. ALDH2 transgene itself did not affect the TUNEL-positive nuclei in the absence of alcohol exposure (Fig. 3).

Effect of chronic alcohol intake on pro- and anti-apoptotic protein expression

Consistent with the observation of elevated cerebral cortical apoptosis following chronic alcohol intake, results in Fig. 4 indicate that chronic alcohol intake upregulated the expression of the pro-apoptotic proteins Bax and Omi/HtrA2, and downregulated the expression of the anti-apoptotic protein Bcl-2 and ARC. In addition, chronic alcohol intake upregulated the expression of the anti-apoptotic proteins FLIP_{S/L} and XIAP. ALDH2 transgene ablated chronic alcohol ingestion-induced changes in these pro- and anti-apoptotic proteins with the exception of FLIP_{S/L}. ALDH2 transgene itself did not affect the expression of these pro- and anti-apoptotic proteins in the absence of chronic alcohol intake.

Effect of chronic alcohol intake on Akt, GSK-3 β and stress signaling

Our further study revealed that phosphorylation of Akt and GSK-3 β was significantly reduced following chronic alcohol intake, the effect of which was nullified by the ALDH2 transgene. Total protein expression of Akt and GSK-3 β was unaffected by either ethanol or ALDH2 transgene. Phosphorylation of Akt and GSK-3 β was not affected by ALDH2 transgene in the absence of chronic alcohol intake (Fig. 5). Our result also indicated significantly elevated activation of the stress signaling p38 (both absolute p38 activation and pp38-to-p38 ratio), JNK (both total and phosphorylated JNK without affect the pJNK-to-JNK ratio) but not ERK following chronic alcohol ingestion. In line with its effect on apoptosis and apoptotic protein expression, ALDH2 transgene significantly reduced the alcohol intake-induced increase in p38 phosphorylation (pp38 and pp38-to-p38 ratio), total JNK and pJNK. ALDH2 transgene itself did not affect the total protein expression or phosphorylation of p38, JNK and ERK in the absence of alcohol intake (Fig. 6).

DISCUSSION

The major findings of our study demonstrated that ALDH2 transgene significantly attenuated alcohol intake-induced cerebral cortical apoptosis, activation of stress signal molecules p38 and JNK, diminished phosphorylation of Akt and GSK-3 β , indicating a role of acetaldehyde in alcohol ingestion-induced brain damage. These data strongly support an essential role of acetaldehyde in alcoholic brain injury and suggest a therapeutic potential of acetaldehyde detoxification in the management of alcoholic complications. Our current study found that the ALDH2 transgene significantly ameliorated chronic alcohol intake-induced changes in organ weight in the hearts (cardiac hypertrophy) but not other organs. Although it is beyond the scope of the current study, our very recent report revealed enhanced activity of the reactive oxygen species producing enzyme NADPH oxidase p47^{phox} and protein expression of the hypertrophic molecules GATA4 and cAMP-response element binding protein (CREB) in hearts of chronically drunk mice, the effect of which can be alleviated by the ALDH2 transgene (Doser et al. 2009). These observations suggest a role of ALDH2 transgene in antagonizing NADPH oxidase p47^{phox}, GATA4 and CREB in retarding chronic alcohol ingestion-induced cardiac hypertrophy.

ALDH2, along with alcohol dehydrogenase (ADH), are key enzymes in the metabolism of ethanol (Duan et al. 2002; Ren 2007a). Although direct ethanol toxicity, oxidative damage, lipid peroxidation, insulin resistance and defective membrane integrity have been suggested to participate in the alcohol-elicited tissue injury (Cederbaum et al. 2001; Bailey et al. 1999), the “acetaldehyde toxicity theory” received more attention recently as acetaldehyde mimics and exaggerates alcohol-elicited cellular and tissue damages (Hintz et al. 2003; Ren 2007a; Cederbaum et al. 2001; Bailey et al. 1999). Data from our current study indicated that alcohol intake-induced brain cerebral tissue damage is associated, at least in part, with apoptosis, reduced activity of the survival factors Akt and GSK-3 β . Activation/upregulation of p38 and JNK following chronic alcohol-intake is also consistent with changes in upregulation of the pro-apoptotic proteins Bax and Omi/HtrA2 as well as downregulation of the anti-apoptotic proteins Bcl-2 and ARC. Upregulation of the anti-apoptotic protein FLIP_{S/L} and XIAP in response to chronic alcohol intake may be a compensatory response to chronic alcohol intake-induced cortical apoptosis evidenced by both caspase expression and TUNEL staining. Interestingly, our data revealed that ALDH2 transgene ablated alcohol-induced changes in these pro- and anti-apoptotic proteins with the exception of FLIP_{S/L}. These data indicated that FLICE-inhibitory protein (FLIP) may not play a major role in ALDH2 transgene-elicited protection against alcoholic brain injury. FLIP, one of the important antiapoptotic proteins, has been shown to be overexpressed in various primary tumor cells. FLIP is known to modulate activation of procaspase-8 and prevents induction of apoptosis mediated by death receptors.

Therefore, FLIP may regulate life and death through inhibiting caspase-8 activity in various types of normal cells and tissues and renders resistance to death receptor-mediated apoptosis in cancer cells (Kataoka 2005). Our finding that ALDH2 transgene reconciled chronic alcohol ingestion-induced upregulation of caspase-8 but not FLIP_{S/L} indicated certain mechanism(s) other than FLIP_{S/L} may be involved in the activation of death receptor. Our results revealed that ALDH2 transgene corrected alcohol intake-induced drop in phosphorylation of Akt and GSK-3 β , indicating a pro-survival effect of ALDH2 transgene against ethanol in cerebral cortex. It is possible that dampened Akt phosphorylation may be responsible for reduction in GSK-3 β phosphorylation. The phosphorylation status of the Akt-GSK-3 β cascade has been deemed as a sensor for oxidative stress (Endo et al. 2007), consistent with the activation of p38 and JNK stress signaling molecules. Our observation was somewhat supported by the previous report that neuronal cultures from ethanol-exposed pups displayed reduced phosphorylation of Akt, GSK-3 β , which suggest that the central nervous system neuronal survival machinery is significantly impaired by chronic exposure to ethanol (de la Monte and Wands 2002). On the other hand, our data did not favor any involvement of ERK activation in the pathogenesis of alcoholic brain injury.

In light of results from the present study, it seems rational to speculate that detoxification of acetaldehyde with ALDH2 transgenic overexpression may alleviate chronic alcohol intake-induced cerebral cortical apoptosis. These data should better our understanding of the mechanism underneath the onset of alcoholic neuropathy and provide further support to potential drug design rationale against alcoholic organ complications. It is worth mentioning that further scrutiny is needed with regards to the impact of the cytosolic isoform of ALDH (ALDH1), which also has a relatively low K_m value (11–18 μ M) for acetaldehyde (Moon et al. 2007), in preventing the acetaldehyde accumulation and cerebral damage following alcohol intake. Although only mitochondrial ALDH (ALDH2) oxidizes acetaldehyde at physiological concentrations in human livers, both ALDH1 and ALDH2 appear to participate in the metabolism of acetaldehyde in rodents (Klyosov et al. 1996).

Acknowledgements

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1 & 4=negative; all others positive

Fig. 1.

(A): PCR identification of ALDH2 transgenic mice using genomic DNA isolated from tail clips of 1-month-old mice. Lanes 1 and 4 are negative and the rest are positive for ALDH2 gene. M: marker. (B): ALDH2 expression in cerebral cortex from FVB and ALDH2 transgenic mice with or without chronic alcohol (ETOH) treatment. Inset: Representative gel blots depicting ALDH2 protein expression using the anti-ALDH2 antibody. Mean $\pm$ SEM, $n = 5-6$ mice per group, * $p < 0.05$ vs. FVB, # $p < 0.05$ vs. FVB+ETOH.

Fig. 2. Effect of ALDH2 transgene on chronic alcohol (ETOH) intake-induced protein damage and apoptosis in mouse cerebral cortex. (A). Protein damage assessed by carbonyl formation; (B): Caspase-3 activity; (C). Caspase-3/7 activity; (D). Caspase-8 expression. Inset: representative gel of Caspase-8. Mean $\pm$ SEM, $n = 6-7$ mice per group, * $p < 0.05$ vs. FVB, # $p < 0.05$ vs. FVB+ETOH.

Fig. 3. Photomicrograph showing TUNEL staining assay for cerebral cortex from FVB and ALDH2 transgenic mice with or without chronic alcohol (ETOH) treatment. TUNEL positive nuclei were visualized with fluorescein (green). (A): FVB; (C): FVB ethanol; (E): ALDH2; and (G): ALDH ethanol. All nuclei were stained with DAPI shown in blue color. (B): FVB; (D): FVB ethanol; (F): ALDH2; and (H): ALDH ethanol. Pooled data are shown in panel I. Mean $\pm$ SEM, $n = 6$ mice per group, * $p < 0.05$ vs. FVB, # $p < 0.05$ vs. FVB+ETOH.

Fig. 4.

Effect of ALDH2 transgene on chronic alcohol (ETOH) intake-induced change pro- and anti-apoptotic proteins in mouse cerebral cortex. (A): Bax; (B): Bcl-2; (C): Omi/HtrA2; (D): ARC; (E): FLIP; and (F): XIAP. Inset: representative gels using specific antibodies. Mean $\pm$ SEM, $n = 5-6$ mice per group, * $p < 0.05$ vs. FVB, # $p < 0.05$ vs. FVB+ETOH.

Fig. 5.

Effect of ALDH2 transgene on chronic alcohol (ETOH) intake-induced change in non-phosphorylated and phosphorylated Akt and GSK-3 β in cerebral cortex. (A): Representative gels of Akt, pAkt, GSK-3 β and pGSK-3 β using specific antibodies; (B): Akt and pAkt; and (C): GSK-3 β and pGSK-3 β . Mean $\pm$ SEM, $n = 5-7$ mice per group, * $p < 0.05$ vs. FVB, # $p < 0.05$ vs. FVB+ETOH.

Fig. 6. Effect of ALDH2 transgene on chronic alcohol (ETOH) intake-induced change in non-phosphorylated and phosphorylated p38, JNK and ERK in cerebral cortex. (A): Representative gels of p38, pp38, JNK, pJNK, ERK and pERK using specific antibodies; (B). p38 and pp38; (C): JNK and pJNK; and (D): ERK and pERK. Mean $\pm$ SEM, $n = 5-7$ mice per group, * $p < 0.05$ vs. FVB, # $p < 0.05$ vs. FVB+ETOH.

Table 1

Biometric parameters of mice fed an alcohol diet (4%) for 12 weeks

Parameter	FVB	FVB-ETOH	ALDH	ALDH-ETOH
Body Weight (g)	27.53 ± 0.69	27.71 ± 0.77	28.34 ± 0.54	27.61 ± 0.77
Heart Weight (mg)	183 ± 8	213 ± 7 [*]	180 ± 5	195 ± 5 [#]
Heart/Body Weight (mg/g)	6.75 ± 0.27	7.82 ± 0.23 [*]	6.43 ± 0.20	7.24 ± 0.24 [#]
Liver Weight (g)	1.46 ± 0.04	1.46 ± 0.04	1.42 ± 0.04	1.47 ± 0.05
Liver/Body Weight (mg/g)	54.1 ± 2.2	53.9 ± 2.0	50.7 ± 2.0	54.2 ± 1.7
Kidney Weight (g)	0.39 ± 0.01	0.38 ± 0.01	0.39 ± 0.02	0.39 ± 0.01
Kidney/Body Weight (mg/g)	14.4 ± 0.6	14.2 ± 0.05	14.1 ± 0.8	14.3 ± 0.3
Brain Weight (mg)	462.5 ± 3.6	470.8 ± 3.2	461.0 ± 4.3	464.3 ± 2.4
Brain/Body Weight (mg/g)	18.1 ± 0.2	17.4 ± 1.6	17.1 ± 1.3	17.6 ± 0.2
Blood Alcohol (mg/dl)	Undetectable	45.8 ± 7.4 [*]	Undetectable	52.6 ± 11.2 [*]

ETOH = alcohol consuming; Mean ± SEM, n = 21 mice per group

^{*}
p < 0.05 vs. FVB group[#]
p < 0.05 vs. FVB-ETOH group.