Activities of Antioxidant Enzymes Induced by Ethanol Exposure in *Aldehyde Dehydrogenase 2* Knockout Mice

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Acetaldehyde production during ethanol metabolism has been implicated as an important link between oxidative stress and cell damage, which suggests that oxidative stress caused by ethanol exposure may be more severe in aldehyde dehydrogenase 2 (ALDH2)-deficient individuals than in those with wild-type ALDH2. We evaluated the activities of the major antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), in liver tissue isolated from *Aldh2* +/+ and *Aldh2* –/– mice that were exposed to ethanol. The activities of CAT and GPx were significantly increased by ethanol treatment in *Aldh2* +/+ mice (3.33-fold and 1.65-fold, respectively). The mean activity of SOD in *Aldh2* +/+ mice was 1.46-fold that in the *Aldh2* +/+ control group, but the difference was not statistically significant. In *Aldh2* –/– mice, the activities of SOD and CAT were decreased and that of GPx was slightly increased after ethanol exposure, but the differences were not significant. We postulate that antioxidant enzyme expression after ethanol consumption may differ according to the intracellular level of acetaldehyde or free radicals, which in turn depends on the activity of ALDH2. These results suggest that the greater toxicity of ethanol in *Aldh2* –/– mice than in *Aldh2* +/+ mice may be due to decreased antioxidant enzyme expression.

Key words —— aldehyde dehydrogenase 2, knockout mice, ethanol, oxidative stress, antioxidant enzyme

INTRODUCTION

Ethanol-induced health hazards are one of the world's major public-health concerns. Ingested ethanol is oxidized by cytosolic class I alcohol dehydrogenase 2 (ADH2) to acetaldehyde, which is subsequently oxidized by mitochondrial aldehyde dehydrogenase 2 (ALDH2) to acetic acid.[1,2] During ethanol metabolism, biochemical changes occur in hepatocytes, causing accumulation of acetaldehyde, a potent toxicant.[3] Acetaldehyde production has been implicated as an important link between oxidative stress and cell damage during ethanol toxicity.[4] Previous research produced strong evidence that reactive oxygen species (ROS) are important in the genesis of alcoholic liver disease.[5–8] These results suggest that oxidative stress and genetic damage caused by ethanol exposure may be more severe in ALDH2-deficient individuals than in those with wild-type ALDH2.

Ethanol exposure of rats increases the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in liver tissue.[9] However, data in the literature concerning alcohol-related variations in antioxidant enzyme (AOE) activities are conflicting.[10] Moreover, there have been few reports on the interaction between the effects of ethanol on AOE activities in liver tissue and ALDH2 activity. In this study, we evaluated the activities of SOD, CAT, and GPx in liver tissue isolated from *Aldh2* +/+ and *Aldh2* –/– mice that were exposed to ethanol.

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MATERIALS AND METHODS

Animals —— *Aldh2* –/– mice were generated as described previously.[11] We used 12-week-old male *Aldh2* +/+ and *Aldh2* –/– mice (C57BL/6J strains). The mice were housed individually in plastic mouse cages and had free access to standard rodent chow and water throughout the experiments. The colony room was maintained at 23–25°C under a 12 hr light (07:00–19:00), 12 hr dark (19:00–07:00) cycle. All procedures were performed according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (Bethesda, MD, U.S.A.).

ALDH2 Genotyping and Ethanol Treatment —— Genomic DNA extracted from the tip of the tail was tested for the presence of mutated and wild-type *Aldh2* alleles by PCR amplification as described previously.[12] Seven *Aldh2* +/+ and seven *Aldh2* –/– mice in each group received 40% ethanol (2 g/kg) everyday in saline solution by gavage for 7 day. It is better to delete volume of saline solution (0.5 mL/kg) The control group, which consisted of seven *Aldh2* +/+ and seven *Aldh2* –/– mice, received saline alone.

AOE Assays —— The enzyme activities of SOD, CAT, and GPx were measured using commercial kits purchased from Cayman Co. (Ann Arbor, MI, U.S.A.) according to the manufacturer's instructions. Briefly, liver tissues were homogenized in 5–10 volumes of the extraction buffers supplied with the kits. The homogenates were centrifuged to remove debris, and the resultant supernatants were used for enzyme assays. Total SOD activity was assayed by detecting superoxide radicals generated by xanthine oxidase and hypoxanthine. The reaction was monitored at 450 nm and 1 U of SOD activity was defined as the amount of enzyme needed to induce 50% dismutation of the superoxide radical. CAT activity was assessed by measuring the reduc-

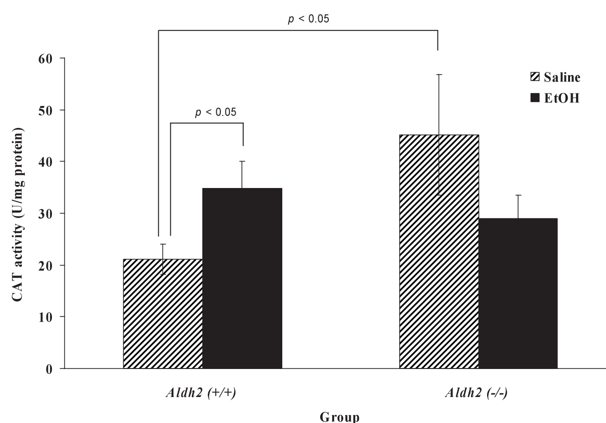

Fig. 2. Enzyme Activity of CAT Enzyme Followed by Ethanol Treatment According to the *Aldh2* Genotype (mean ± S.D. of 7 mice)

Liver tissues were homogenized and supplied with the kits. CAT activity was assessed by measuring the reduction of hydrogen peroxide at 540 nm. Data were adjusted by protein concentration. *p*-value represents statistical significance compared with the *Aldh2* +/+ control mice in the Wilcoxon rank-sum test.

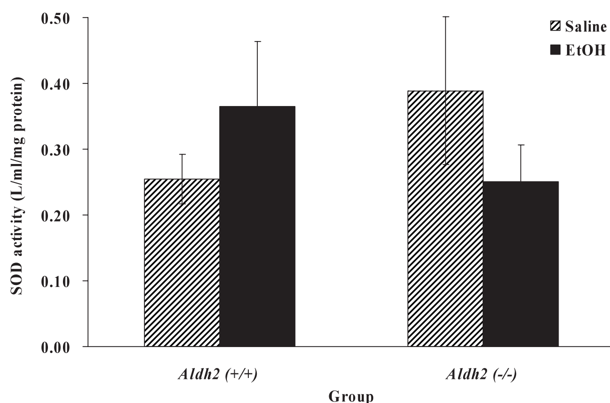

Fig. 1. Enzyme Activity of Superoxide Dismutase Enzyme Followed by Ethanol Treatment According to the *Aldh2* Genotype (mean ± S.D. of 7 mice)

Liver tissues were homogenized and supplied with the kits. The reaction was monitored at 450 nm and 1 U of SOD activity was defined as the amount of enzyme needed to induce 50% dismutation of the superoxide radical. Data were adjusted by protein concentration.

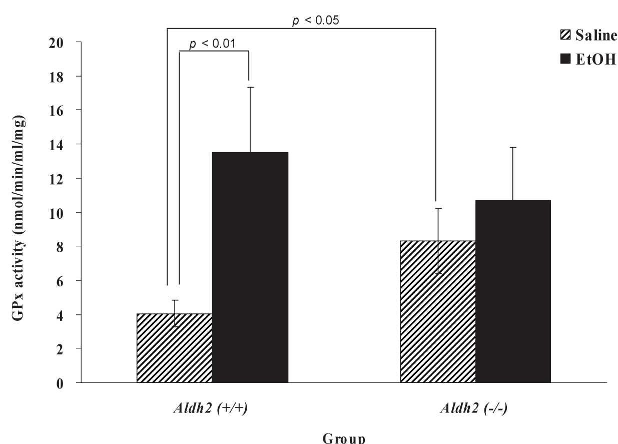

Fig. 3. Enzyme Activity of GPx Enzyme Followed by Ethanol Treatment According to the *Aldh2* Genotype (mean ± S.D. of 7 mice)

Liver tissues were homogenized and supplied with the kits. GPx activity was assayed by measuring the rate of NADPH oxidation by a gluthathione reductase coupled reaction at 340 nm. Data were adjusted by protein concentration. *p*-value represents statistical significance compared with the *Aldh2* +/+ control mice in the Wilcoxon rank-sum test.

tion of hydrogen peroxide at 540 nm. GPx activity was assayed by measuring the rate of NADPH oxidation by a gluthathione reductase-coupled reaction at 340 nm. Protein content was determined using the Bio-Rad Protein Assay Kit (Heracules, CA, U.S.A.) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

The metabolism of ethanol is believed to result in increased production of ROS, especially superoxide and H_2O_2, and the removal of these toxic species is thought to be a vital initial step in ensuring cell survival during ethanol intoxication.[5–8] The three major AOEs available to the cell during ethanol-induced oxidant stress are SOD, CAT, and GPx.[13] In our study, the enzyme activities of CAT and GPx were significantly increased by ethanol treatment in *Aldh2* +/+ mice (3.33-fold and 1.65-fold, respectively) (Fig. 2 and 3). The mean activity of SOD in ethanol-treated *Aldh2* +/+ mice was 1.46-fold that in the *Aldh2* +/+ control group, although the difference was not statistically significant (Fig. 1). These increases in AOE activity could be due to increased enzyme synthesis induced by elevated generation of free radicals.

On the other hand, in the *Aldh2* –/– mice, the activities of SOD and CAT were decreased and that of GPx was slightly increased by ethanol exposure, but the differences were not significant. This may have been caused by inhibition of protein synthesis by excess acetaldehyde and free radicals.[14] When the production of free radicals or ROS in the tissues exceeds the ability of the antioxidant system to eliminate them, oxidative stress results.[14] Our previous study revealed that murine ALDH2 is important for acetaldehyde metabolism and that *Aldh2* –/– mice have significantly higher acetaldehyde levels after ethanol exposure compared with *Aldh2* +/+ mice.[15] It is also known that CAT and other AOEs may be inactivated by free radicals produced by ethanol metabolism.[16] The GPx enzyme works in tandem with CAT to scavenge excess H_2O_2 and lipid peroxides in response to oxidative stress.[14, 17, 18] However, unlike CAT activity, GPx activity depends on the balance between the levels of glutathione and glutathione disulfide.[19] The difference between the responses of GPx and CAT to ethanol treatment in *Aldh2* –/– mice may be due to differences in precursors, including hepatic glutathione contents. We postulate that AOE expression after ethanol consumption may differ according to the intracellular level of acetaldehyde or free radicals, which in turn depends on the activity of ALDH2. These results suggest that the greater toxicity of ethanol in *Aldh2* –/– mice than in *Aldh2* +/+ mice may be due to decreased AOE expression.

Cytochrome P450 2E1 (CYP2E1), a cytochrome P450 enzyme induced by ethanol, displays high NADPH oxidase activity and generates ROS.[20] CYP2E1 can even generate ROS in the absence of substrate.[21] In a previous study, we found that the *Aldh2* –/– mice showed higher hepatic CYP2E1 expression than *Aldh2* +/+ mice, even in the absence of exposure to ethanol.[22] In addition, oxidative DNA damage was associated with the expression of CYP2E1.[21]

These results are in agreement with the results of the present study, which showed that CAT and GPx expression was significantly greater in the *Aldh2* –/– control group than in the *Aldh2* +/+ control group (Fig. 2 and 3). The expression of SOD was also higher in the *Aldh2* –/– control group than in the *Aldh2* +/+ control group, although the difference was not significant (Fig. 1). We do not know why the activities of AOE were greater in *Aldh2* –/– control mice than in *Aldh2* +/+ control mice. However, it is possible that the induction of these AOE activities may be associated with CYP2E1 enzyme expression, which is highly expressed in *Aldh2* –/– mice to compensate for the absence of the ALDH2 enzyme.

In conclusion, this study showed that the expression of AOEs in liver tissue of mice exposed to ethanol differed according to ALDH2 activity. AOE expression was increased by ethanol exposure in *Aldh2* +/+ mice but was decreased or slightly increased in *Aldh2* –/– mice. These results suggest that ALDH2-deficient individuals may be more susceptible to ethanol-mediated liver disease than wild-type ALDH2 individuals and that this difference may be due to a difference in the activity of AOEs.

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