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Cytochrome P450 2E1 (CYP2E1)-Dependent Production of a 37-kDa Acetaldehyde–Protein Adduct in the Rat Liver

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Ethanol-inducible cytochrome P450 2E1 (CYP2E1) has been shown to be involved in the metabolism of both ethanol and acetaldehyde. Acetaldehyde, produced from ethanol metabolism, is highly reactive and can form various protein adducts. In this study, we investigated the role of CYP2E1 in the production of a 37-kDa acetaldehyde–protein adduct. Rats were pair-fed an isocaloric control or an alcohol liquid diet with and without cotreatment of YH439, an inhibitor of *CYP2E1* gene transcription, for 4 weeks. The soluble proteins from rat livers of each group were separated on SDS–polyacrylamide gels followed by immunoblot analysis using specific antibodies against the 37-kDa protein acetaldehyde adduct. In addition, catalytic activities of the enzymes involved in alcohol and acetaldehyde metabolism were measured and compared with the adduct level. Immunoblot analysis revealed that the 37-kDa adduct, absent in the pair-fed control, was evident in alcohol-fed rats but markedly reduced by YH439 treatment. Immunohistochemical analysis also showed that the 37-kDa adduct is predominantly localized in the pericentral region of the liver where CYP2E1 protein is mainly expressed. This staining disappeared in the pericentral region after YH439 treatment. The levels of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase isozymes were unchanged after YH439 treatment. However, the level of the 37-kDa protein adduct positively correlated with the hepatic content of P4502E1. These data indicate that the 37-kDa adduct could be produced by CYP2E1-mediated ethanol metabolism in addition to the ADH-dependent formation.

Key Words: **acetaldehyde; acetaldehyde–protein adduct; alcohol metabolism; alcohol dehydrogenase; CYP2E1 inhibitor.**

It is well established that ethanol (alcohol) is mainly metabolized in the liver, and the liver is one of the primary sites of ethanol-mediated tissue damage. Under normal circumstances, ethanol is predominantly metabolized by the cytosolic alcohol dehydrogenase (ADH)[5] to acetaldehyde, which is further metabolized to acetate by the mitochondrial aldehyde dehydrogenase (ALDH) in the presence of NAD^+ as a cofactor. However, ethanol can be metabolized to acetaldehyde and subsequently to acetate by the microsomal ethanol-inducible cytochrome P450 2E1 (CYP2E1) or peroxisomal catalase. The role of catalase in ethanol metabolism in the liver seems restricted due to limited availability of H_2O_2 (1). Chronic alcohol intake induces CYP2E1 mainly by protein stabilization and leads to

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[5] Abbreviation used: ABC, avidin–biotin complex; ADH, Alcohol dehydrogenase; ALDH1, cytosolic aldehyde dehydrogenase class 1 isozyme; ALDH2, mitochondrial aldehyde dehydrogenase class 2 isozyme; ALDH3, cytosolic aldehyde dehydrogenase class 3 isozyme; BAC, blood alcohol concentration; CYP1A1/2, aryl hydrocarbon-inducible cytochrome P450 1A1/2; CYP2E1, ethanol-inducible cytochrome P450 2E1; CYP3A, cytochrome P450 3A; DMSO, dimethyl sulfoxide; KLH, keyhole limpet hemocyanin; PBS, phosphate-buffered solution; SDS–PAGE, SDS–polyacrylamide gel electrophoresis; YH439, isopropyl 2-(1,3-dithioetane-2-ylidene)-2-[*N*-(4-methylthiazol-2-yl) carbamoyl]acetate.

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increased metabolism of ethanol via CYP2E1, from which acetaldehyde can be produced (1–3). Acetaldehyde's carbonyl group is highly reactive and thus can interact with free amino group of various cellular macromolecules including DNA and various proteins to produce both stable and unstable acetaldehyde adducts (3–6). Interaction of acetaldehyde with DNA produces several DNA adducts (7). The acetaldehyde modified proteins include various serum or cellular proteins such as hemoglobin, low density lipoproteins, CYP2E1, collagen, tubulin, etc. (3–6). The physiological function of these proteins are usually altered by the adduct formation (3–6). A recently suggested hypothesis is that acetaldehyde modified proteins are considered as neo-antigens that initiate autoimmune reactions often associated with alcohol mediated liver injury (4, 6). Further data showed an excellent correlation between the levels of an acetaldehyde–protein adduct (37 kDa) and the severity of alcoholic liver disease (8). A 37-kDa adduct protein was recently determined to be Δ^4-3-ketosteroid-5β-reductase (9). CYP2E1 plays a key role (10, 11) in this model of ethanol mediated liver damage caused by intragastric feeding of ethanol (8). These data suggest a possible role of CYP2E1 in the production of acetaldehyde adducts and the initiation of alcohol induced tissue injury. This hypothesis is contrary to the previous report of a negative role of CYP2E1 in the production of the 37-kDa adduct protein, where ADH plays a key role (12). Furthermore, the identity of the acetaldehyde protein adducts, elevated in the pericentral region of the liver during early alcoholic liver injury (11), was not fully verified. In this study, we reinvestigated the role of CYP2E1 in the production of acetaldehyde adduct. To identify the role of CYP2E1-mediated ethanol metabolism in acetaldehyde adduct formation, we used a synthetic compound, YH439, isopropyl 2-(1,3-dithioetane-2-ylidene)-2-[*N*-(4-methylthiazol-2-yl) carbamoyl]acetate, which transcriptionally inhibits the expression of the *CYP2E1* gene (13). Although YH439 was shown to induce other P450 isozymes, namely *CYP1A1/2* genes (14), its effects on the activities of the enzymes involved in ethanol and acetaldehyde metabolism are unknown. To reinvestigate the role of the enzymes involved in ethanol and acetaldehyde metabolism in the production of acetaldehyde adduct protein(s), we determined the catalytic activities of ADH, CYP2E1, and ALDH isozymes and correlated their levels with that of the 37-kDa acetaldehyde protein adduct in the absence and presence of YH439.

MATERIALS AND EXPERIMENTAL PROCEDURES

Materials. YH439 was kindly provided by Dr. Jong-Wook Lee (Yuhan Research Center, Yuhan Corporation, Gunpo-si, Kyunggi-Do, Korea). Keyhole limpet hemocyanin (KLH), acetaldehyde, propionaldehyde, pyrazol, benzaldehyde, and a kit for measuring blood alcohol concentration were purchased from Sigma Aldrich Chemicals. Bradford protein assay kit was from Biorad Inc. (Richmond, CA). Polyclonal antibody against ADH was provided by Dr. William F. Bosron (University of Indiana, Indianapolis, IN), while antibodies against ALDH1 and ALDH3 were obtained from Dr. Ronald G. Lindahl (University of South Dakota, Vermillion, SD). Other materials not mentioned here was the same as described (13–15).

Animal treatment. Male Wistar rats (6 per group) from Charles River Breeding company (Raleigh, NC) were maintained on a 12-h light–dark cycle with autoclavable rat diet and water *ad libitum* in accordance with the NIH guideline for small laboratory animals. Individually housed rats were fed a dextrose control diet in the absence and presence of YH439 (150 mg/kg, oral administration, every other day). The other half rats were fed a Lieber–DeCarli alcohol liquid diet (15; providing 35% daily calories, BioServ, Frenchtown, NJ) with and without YH439 for 4 weeks. Weight and food consumption of each animal was measured every morning. The liquid diets were freshly prepared every day during the treatment. At the end of 4 weeks of feeding, animals were sacrificed. Rat livers from different treatments were rapidly excised and subjected to either immunohistochemistry (16) or pathological analyses (10), as described below. The rest of the livers were immediately frozen in dry ice methanol bath and stored at −80°C.

Immunohistochemistry. The major lobe of each rat liver was cut into 2–3 pieces with a razor blade, fixed in 10% formalin solution neutralized to pH 7.4 for at least 24 h, embedded into a paraffin block, serially cut into 4-μm thickness, placed on charged glass slides (Probe On Plus Microscope Slides, Fisher Scientific, Cincinnati, OH), deparaffinized in xylene twice for 5 min and rehydrated with a series of graded alcohol. Endogenous peroxidase was blocked by incubation with 0.03% hydrogen peroxide-methanol for 40 min. Tissue sections were washed with 1× phosphate-buffered saline (PBS) containing 0.03% nonfat milk and 0.01% Tween 20, and then immunostained with affinity-purified rabbit antibodies to acetaldehyde protein adducts overnight at 4°C. The antigen–antibody complex was visualized using the avidin–biotin complex (ABC) method, using the ABC kit (Vector Laboratories) with 3′, 3′-diaminobenzidine (Zymed, San Francisco, CA) as the chromogen. Tissue sections were then rinsed in distilled water and counter-stained with Mayer's hematoxylin.

Blood collection and determination of blood alcohol concentration. Trunk blood (1 ml) from each animal was collected at the time of sacrifice (at 10 a.m. with 5-min interval per animal) and transferred into EDTA containing tubes. Plasma was prepared and blood alcohol concentration (BAC) determined spectrophotometrically using a kit purchased from Sigma Chemicals (St. Louis, MO).

Subcellular fractionation and measurement of catalytic activities. Frozen rat livers were homogenized in a buffer containing 50 mM Tris–HCl (pH 7.4), 0.15 M KCl, 100 μM phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, 100 munit/ml α2-macroglobulin, and 1 mM EDTA. Mitochondria, microsomes and cytosolic fractions were prepared by differential centrifugation as described previously (13–15). Protein concentration was measured by Bradford method. The activity of cytosolic ADH was measured by the production of NADH at 340 nm by the method (17) with a slight modification using 3 mM NAD^+ and 10 mM ethanol as a substrate. The activity of microsomal CYP2E1 was measured by *N*-nitrosodimethylamine demethylase activity (13, 15). Activity of cytosolic ALDH1 was measured by using 1 mM NAD^+ and 1 mM propionaldehyde as a substrate, as described (17), while 1 mM NAD^+ and 10 μM propionaldehyde were used for mitochondrial ALDH2 activity (18). The ALDH3 activity was measured by the

method (19) using 1 mM $NADP^+$ and 1 mM benzaldehyde as a substrate. Direct effects of YH439 (dissolved in dimethyl sulfoxide, DMSO) on the catalytic activities of ADH, CYP2E1, and ALDH isozymes were determined at two protein concentrations (0.5 or 1.0 mg protein of the respective subcellular fractions per assay). DMSO was used as a negative vehicle control. Because of interferences with the assay systems at higher concentrations of YH439, direct effects of YH439 on the catalytic activities were accurately determined at lower concentrations (up to 10 μM).

Preparation of antibody to acetaldehyde–protein adduct and immunoblot analyses. Acetaldehyde modified protein was prepared by the method previously described (8). About 100 mg of KLH was mixed with 250 mM acetaldehyde in 50 mM sodium phosphate buffer (pH 7.0) for 2 h at room temperature followed by the addition of 100 mM sodium cyanoborohydride. Following overnight incubation, acetaldehyde–KLH adduct was purified by gel-filtration chromatography over PD-10 Sephadex column. The pooled fractions containing acetaldehyde-KLH adduct were emulsified with an equal volume of Freund's incomplete adjuvant and used as immunogen (300 μg protein/intradermal injection) to raise antibody in rabbit over an 8-week period. Mono-specific antibody to acetaldehyde adduct protein was purified from rabbit serum by affinity column chromatography using acetaldehyde–KLH adduct as a ligand. The purified antibody was neutralized to pH 7.5 with 0.1 M NaOH and then used for immunoblot and immunohistochemical analyses. A polyclonal antibody against the purified recombinant protein of rat ALDH2 protein (20) was also raised in rabbit. Rat liver proteins from different subcellular fractions (cytosol, mitochondria, and microsomes) were separated by 10% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and then electrically transferred to nitrocellulose membranes and subjected to immunoblot analyses, using the specific polyclonal antibodies against the respective protein of ADH, ALDH isozymes or CYP2E1 (13).

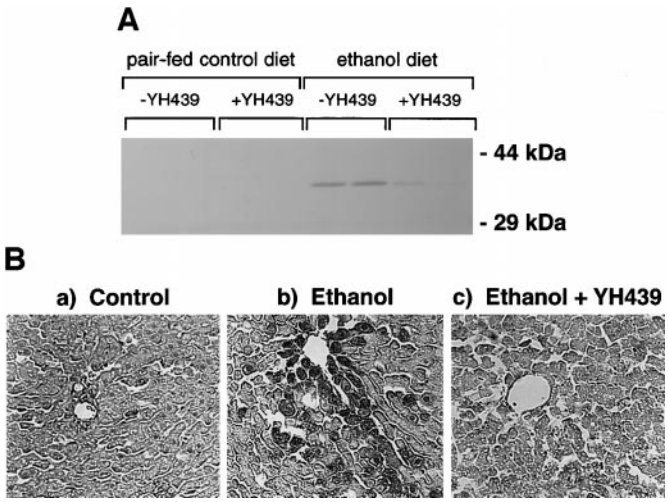

FIG. 1. Immunoblot and immunohistochemical analyses of the 37-kDa acetaldehyde-protein adduct. (A) Cytosolic proteins (100 μg/lane), from each group as indicated (n = 3 per lane), were analyzed by 10% SDS–PAGE followed by immunoblot analysis using the monospecific antibody to the acetaldehyde-modified protein. Marker proteins were: ovalbumin (44 kDa) and carbonic anhydrase (29 kDa). (B) Paraffin sections of each rat liver from different treatments were subjected to immunohistochemical analysis (×200) using the same antibody as described above. Figures show typical immunohistogram of pericentral regions of each group: (a) pair fed control; (b) ethanol-fed without YH439; and (c) ethanol fed with YH439.

RESULTS

Changes in Body Weight and Blood Alcohol Concentration

Animals appeared healthy and normal during the course of treatment with the liquid diets. No apparent hepatic damage, except minor hepatic steatosis, was observed at the end of treatment in these animals. This was probably due to a low BAC, compared to the intragastric feeding model (10, 11). Rats from both control diet and ethanol diet without YH439 gained body weight about 110 g over the 4-week period. Rats on the control diet and ethanol diet gained weight, about 62 and 60 g, respectively, in the presence of YH439. Thus, YH439 treatment, three times a week, reduced the body weight gain by about 50 g, compared to the untreated groups. The BAC of each animal at the time of sacrifice varied widely. The average values were 63.2 ± 4.3 and 99.0 ± 7.6 mg/dl for the rats with and without YH439 treatment, respectively. The similar body weight gain and a higher level of BAC in the presence of YH439 compared to that in the absence of YH439 suggested that the apparent detection of the protein–acetaldehyde adduct was due to direct effect of alcohol metabolism through ADH or CYP2E1 after YH439 treatment. Furthermore, these data indicate that CYP2E1 may play a role in alcohol oxidation after chronic alcohol treatment, as suggested (1).

Immunoblot and Immunohistochemical Analyses of the 37-kDa Protein Adduct

Three rat livers (1 g from each liver) were combined and processed together for further analyses. As previously reported by Lin *et al.* (8, 9), our polyclonal antibody recognized a single protein–acetaldehyde adduct (apparent M_r 37-kDa) that was absent in the livers of rats fed the control diet with and without YH439. This adduct was observed only in the cytosol from the alcohol treated rat liver. The amount of this adduct was markedly reduced by simultaneous treatment of rats with YH439 (Fig. 1A).

The 37-kDa protein adduct determined by immunohistochemistry was not evident in the pericentral region in the liver of rats fed control diet regardless of YH439 treatment (Fig. 1B, a). However, the amount of this adduct protein was elevated in the pericentral region (Fig. 1B, b) while a small amount was also observed in the periportal area (data not shown) in the alcohol fed rat. The amount of the 37-kDa protein adduct was markedly reduced by cotreatment with YH439 (Fig. 1B, c), despite the high level of ethanol in these animals, as described above.

TABLE I

Catalytic Activities of the Enzymes Involved in Alcohol and Acetaldehyde Metabolism

	Pair-fed controls		Ethanol-fed	
Activity	(−)YH439	(+)YH439	(−)YH439	(+)YH439
ADH[a]	7.3 ± 1.0	9.4 ± 1.4	7.1 ± 0.7	6.9 ± 0.8
ALDH1[a]	7.5 ± 0.4	7.6 ± 0.3	7.6 ± 0.2	7.6 ± 0.4
ALDH2[a]	15.8 ± 0.5	14.9 ± 0.6	20.6 ± 0.8	16.8 ± 0.6
ALDH3[a]	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
CYP2E1[b]	2.0 ± 0.1[c]	1.4 ± 0.1	5.3 ± 0.3[c,d]	1.9 ± 0.2[d]

Note. Each rat liver from different treatment (n = 6 per group) was homogenized and subjected to sub-cellular fractions. Catalytic activities of the following enzymes were determined at least three times by the published methods (13–19).

[a] One unit represents a reduction of one μmole NAD^+ or $NADP^+$/min/mg protein at 23°C.

[b] One unit represents a production of one nmole formaldehyde/min/mg protein at 37°C.

[c] Significantly different ($P < 0.01$).

[d] Significantly different ($P < 0.001$).

Changes in the Levels of Enzymes Involved in Alcohol and Acetaldehyde Metabolism

To correlate the level of the 37-kDa protein adduct and that of the major enzymes involved in alcohol and acetaldehyde metabolism, we determined the catalytic activities and the content of ADH, CYP2E1, and ALDH isozymes. As summarized in Table I, ADH activities in ethanol-treated rats were 7.1 ± 0.7 and 6.9 ± 0.8 unit in the absence and presence of YH439, respectively, suggesting that YH439 treatment does not significantly change the ADH activity. In addition, the ADH activity was not inhibited by the direct addition of YH439 (up to 10 μM) into the assay mixture (data not shown). The catalytic activities of cytosolic ALDH1, mitochondrial ALDH2, and cytosolic ALDH3 isozymes in the control rats were 7.5, 15.8, and 0.3 unit, respectively. Neither ethanol nor YH439 treatment caused significant changes in the catalytic activities of ADH or ALDH isozymes (Table I), where addition of YH439 (up to 10 μM) into the assay systems did not influence the activities of ALDH1 and ALDH2. This conclusion was further supported by the immunoblot analyses using specific antibodies to the respective ADH or ALDH isozymes (Figs. 2 and 3). The levels of ADH (M_r 40 kDa), ALDH1 (M_r 54 kDa), ALDH2 (M_r 54 kDa), or ALDH3 (M_r 55 kDa) were slightly changed or unchanged by either ethanol with or without YH439 treatment. The amount of ALDH3 protein was very low or absent in the liver cytosol, as was expected in the apparently normal animals (19).

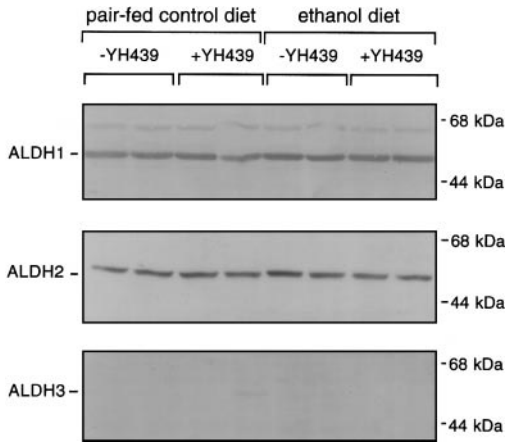

FIG. 2. Immunoblot analyses of ALDH isozymes. Cytosolic or mitochondrial proteins were subjected to SDS–PAGE followed by immunoblot analyses using the specific antibody to the respective protein as indicated: ALDH1 and ALDH3 (cytosols, 30 μg/lane); and ALDH2 (mitochondria, 30 μg/lane).

In contrast, the catalytic activity of CYP2E1 (as *N*-nitrosodimethylamine demethylase) was significantly elevated 2.6-fold in ethanol treated rats and varied from 2.0 ± 0.1 to 5.3 ± 0.3 unit/mg protein. The elevated CYP2E1 activity was significantly reduced to 1.9 ± 0.2 unit/mg protein by co-treatment of YH439 (Table I), as expected from our previous study (13). However, the CYP2E1 activity was not affected by direct addition of YH439 (up to 10 μM) in the assay system. As shown in Fig. 3, immunoblot analysis showed that chronic ethanol feeding significantly increased the content of CYP2E1 (M_r 52 kDa), while

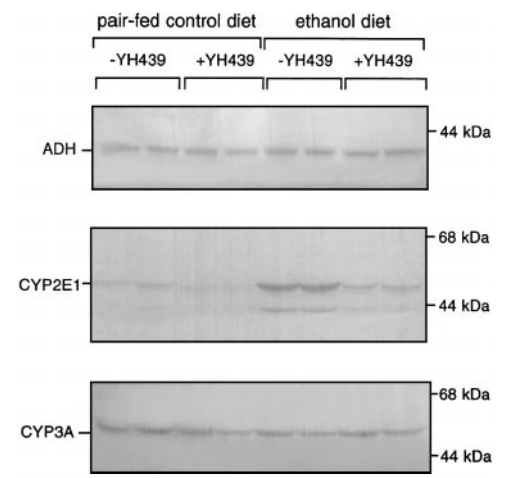

FIG. 3. Immunoblot analyses of ADH, CYP2E1, and CYP3A1. Cytosolic (for ADH, 30 μg/lane) and microsomal proteins (10 μg/lane) were subjected to SDS–PAGE followed by immunoblot analyses using the respective antibody against ADH (top), CYP2E1 (middle), and CYP3A (bottom) as indicated.

YH439 treatment in the absence and presence of ethanol marked reduced the level of CYP2E1 compared to their respective counterpart, consistent with our previous results (13). Unlike the earlier result (11), the level of CYP3A (M_r 53 kDa) was unchanged by ethanol treatment in the absence or presence of YH439. This is most likely due to the low BAC. From the above results, the densitometric level of the 37-kDa protein adduct positively correlated with the level of CYP2E1 activity (r^2 = 0.81) but not with ADH or ALDH isozymes.

DISCUSSION

It is well established that CYP2E1 is involved in the metabolic activation of numerous, small molecules of potentially toxic and carcinogenic potential (1, 2, 21). The substrates of CYP2E1 include various alcohols, aldehydes, organic solvents, anesthetic agents, long chain fatty acids, and the carcinogenic nitrosamines. CYP2E1 mediated catalytic reactions usually result in production of hydroxyethyl radical, cytotoxic aldehydes, including acetaldehyde, reactive oxygen species, lipid peroxides, and other toxic-free radical intermediates (metabolites), while reducing antioxidants such as glutathione (1, 2, 21). Therefore activation or induction of CYP2E1 by alcohol or exposure to other chemical inducers such as isoniazid (22) may render the individual or cell more susceptible to these toxic reaction products. This problem has been demonstrated in both human alcoholics and animal models of alcoholic liver damage (1, 2, 10, 23–25). Although the role of CYP2E1 in alcoholic liver injury model has been recently challenged (26), the contradictory data from various laboratories may result from the different nutritional states (27) or the presence of cotreating agents such as specific composition of fatty acids, iron, acetaminophen, and CCl_4, which were administered together with ethanol. Alternatively, CYP2E1 may be involved in the early state of alcohol mediated liver injury by producing hydroxyethyl radical or acetaldehyde adduct proteins which could initiate autoimmune reactions, as has recently been proposed (6). Nevertheless, the 37-kDa acetaldehyde-protein adduct but not 4-hydroxynonenal-protein adduct appears important in the alcohol mediated liver damage caused by intragastric infusion of ethanol (8).

In this study, we did not observe any apparent pathological damage in the animals, most likely due to insufficient BAC, a short duration of alcohol treatment, and nutrient- and carbohydrate-rich diet, as recently reported (27). However, the 37-kDa protein adduct was clearly observed in the alcohol treated animals, despite a low BAC, which can possibly result from the induced levels of CYP2E1 involved in the alcohol metabolism (1). This protein adduct was primarily expressed in the pericentral region, similar to the pericentral localization of CYP2E1 (28, 29). The reduction in the level of the 37-kDa adduct and its disappearance from the pericentral region by YH439 treatment highly correlated with the changes in CYP2E1 level with and without YH439, suggesting a potentially important role of the CYP2E1 protein in the production of the 37-kDa protein adduct. However, the reduced level of the 37-kDa adduct was not due to changes in the activities of ADH and ALDH isozymes by YH439 treatment, because addition of YH439 up to 10 μM did not interfere with the activities of those enzymes. In addition, concentrations of free (unbound) YH439 and its metabolites were less than 1 μM range in the liver or plasma during the first couple hours after YH439 treatment of animals due to protein binding and rapid metabolism (30), further demonstrating that YH439 is not likely to regulate ADH and ALDH isozymes but does affect CYP2E1 level.

It is possible that the changes in the 37-kDa acetaldehyde adduct levels could be a consequence of the altered levels of Δ^4-3-ketosteroid 5β-reductase (the 37-kDa target protein) after alcohol and YH439 treatment. However, Lin and her colleagues demonstrated by immunoblot analysis that ethanol feeding did not alter the level of Δ^4-3-ketosteroid 5β-reductase (9). These investigators also reported that chronic ethanol treatment reduced the activity of this enzyme, possibly through the formation of the acetaldehyde protein adduct (31). These earlier results are different or opposite to our current result of the increased level of the 37-kDa acetaldehyde adduct after alcohol feeding. Furthermore, our preliminary result of RT-PCR analysis showed that neither ethanol nor YH439 treatment significantly changed the mRNA level for rat liver Δ^4-3-ketosteroid 5β-reductase (data not shown but provided to the reviewers). All these data indicate that our current results of the changes in the 37-kDa acetaldehyde adduct levels do not reflect the altered levels of Δ^4-3-ketosteroid 5β-reductase, but rather result from the covalent modification of this target protein by fluctuating acetaldehyde concentrations during and after alcohol and YH439 treatment.

Our conclusion is contrary to the previous suggestion that CYP2E1 has a negative role in the 37-kDa adduct formation (12) whereas ADH was proposed to play an apparent key role. The following reasons may account for the different conclusions concerning the role of CYP2E1 in the adduct formation. First, the 37-kDa protein adduct was shown to be localized in the pericentral region (8, 32), where acetaldehyde is produced in large amount (33) and CYP2E1 is expressed and significantly induced by alcohol (28, 29). However, the pericentral distribution of the adduct is different from

that of ADH protein which shows little zonation in the liver acinus (34, 35). Second, despite their careful characterization (12), the catalytic activity of CYP2E1 was not shown in the previous study, especially after pyrazole or 4-methylpyrazole treatment as an inhibitor of ADH. Therefore, it was unknown whether the local production of acetaldehyde by CYP2E1 might be suppressed by pyrazoles through their hydrophobic interaction with critical sites of CYP2E1 (36), resulting in a reduced production of the 37-kDa adduct, despite a twofold elevation in the hepatic content of CYP2E1. Third, CYP2E1 mediated reactions are responsible for the production of CYP2E1-acetaldehyde adduct protein (37), presumably through a local production of reactive acetaldehyde or hydroxyethyl radical (4, 6). Therefore, it may be possible that CYP2E1 can produce another adduct protein such as the 37-kDa adduct. Fourth, the 37-kDa protein adduct was present on the cell surface of hepatocytes (38). Coincidentally, both CYP2E1 and hydroxyethyl radical adduct were shown to be expressed on the plasma membrane of hepatocytes (39, 40), suggesting a potential role of CYP2E1 in the local production of acetaldehyde and the adduct on cell surface. Finally, in comparison to the earlier study (12) where no specific inhibitor of CYP2E1 was used, we employed YH439, which inhibits the transcription of the *CYP2E1* gene and prevents the liver damage caused by CYP2E1 substrates such as CCl_4 (41) or acetaminophen (unpublished observation). In the current study, YH439 treatment significantly reduced the activity and the content of CYP2E1 with little changes in the catalytic activities and protein contents of ADH or ALDH isozymes. Due to a positive correlation between the levels of CYP2E1 and the 37-kDa protein adduct, we conclude that the 37-kDa protein adduct could be produced in a CYP2E1-dependent manner, in addition to the ADH-mediated ethanol metabolism.

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