CYP2E1 is not involved in early alcohol-induced liver injury

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CYP2E1 is not involved in early alcohol-induced liver injury. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G1259–G1267, 1999.—The continuous intragastric enteral feeding protocol in the rat was a major development in alcohol-induced liver injury (ALI) research. Much of what has been learned to date involves inhibitors or nutritional manipulations that may not be specific. Knockout technology avoids these potential problems. Therefore, we used long-term intragastric cannulation in mice to study early ALI. Reactive oxygen species are involved in mechanisms of early ALI; however, their key source remains unclear. Cytochrome P-450 (CYP)2E1 is induced predominantly in hepatocytes by ethanol and could be one source of reactive oxygen species leading to liver injury. We aimed to determine if CYP2E1 was involved in ALI by adapting the enteral alcohol (EA) feeding model to CYP2E1 knockout mice. Female CYP2E1 wild-type (+/+) or −/− mice were given a high-fat liquid diet with either ethanol or isocaloric maltose-dextrin as control continuously for 4 wk. All mice gained weight steadily over 4 wk, and there were no significant differences between groups. Groups were also no differences in ethanol elimination rates between CYP2E1 +/+ and −/− mice after acute ethanol administration to naive mice or mice receiving EA for 4 wk. However, EA-stimulated rates 1.4-fold in both groups. EA elevated serum aspartate aminotransferase levels threefold to similar levels over control in both CYP2E1 +/+ and −/− mice. Liver histology was normal in control groups. In contrast, mice given ethanol developed mild steatosis, slight inflammation, and necrosis; however, there were no differences between the CYP2E1 +/+ and −/− groups. Chronic EA induced other CYP families (CYP3A, CYP2A12, CYP1A, and CYP2B) to the same extent in CYP2E1 +/+ and −/− mice. Furthermore, POBN radical adducts were also similar in both groups. Data presented here are consistent with the hypothesis that oxidants from CYP2E1 play only a small role in mechanisms of early ALI in mice. Moreover, this new mouse model illustrates the utility of knockout technology in ALI research.

CYP2E1 knockout mouse; intragastric feeding

THE ESTABLISHMENT OF A continuous intragastric enteral feeding protocol in the rat by Tsukamoto and French (32) was a major development in research in alcohol-induced liver injury. With this model, not only is steatosis observed, which is characteristic of several animal models, but inflammation and necrosis also occur in ~2–4 wk and fibrosis begins to develop in 8–10 wk. Inactivation of Kupffer cells with gadolinium chloride (GdCl3) diminished free radical formation (17) and prevented early alcohol-induced liver injury in the enteral model (1). Furthermore, intestinal sterilization with antibiotics (2) or lactobacillus feeding (24) diminished endotoxin and minimized liver injury. These results are consistent with the hypothesis that Kupffer cells activated by gut-derived endotoxin play an important role in the mechanism of early alcohol-induced liver injury.

Ethanol administration in vivo is associated with the formation of free radicals due to oxidant stress (17). Cytochrome P-450 (CYP)2E1 is induced predominantly in the hepatocyte by ethanol and could be a source of reactive oxygen species, leading to liver injury (26). French and his collaborators (3) have shown that a correlation between blood levels of alcohol and induction of CYP2E1 as alcohol cycles in the enteral model exists. Importantly, the level of CYP2E1 correlates with the degree of pathology, leading to the idea that it is involved in alcohol-induced liver disease. Moreover, inhibitors of CYP2E1 partially reduced hepatic pathology caused by enteral ethanol (3). Alternatively, NADPH oxidase in the Kupffer cell, which is activated by endotoxin, could also be a source of reactive oxygen species. Thus whether oxidant stress is from CYP2E1 in the hepatocyte or NADPH oxidase in the Kupffer cell remains unclear.

Recently, it was reported that CYP2E1 was induced similarly by ethanol and ethanol + GdCl3 in the enteral model; however, liver pathology was prevented by GdCl3 (19). These data suggested that oxidant stress from Kupffer cells rather than from CYP2E1 predominates in the initiation of alcohol-induced liver injury. However, much of what has been learned to date involves inhibitors or nutritional manipulations that may not be specific. Knockout technology could be useful to avoid these potential problems.
French et al. (35) delivered alcohol enterally to mice in his study with Mallory bodies, demonstrating that successful surgery was possible in the mouse; however, mortality was high due to drug priming. Therefore, here we established a long-term intragastric feeding model to study alcohol-induced liver injury in mice and adapted it to knockout technology. The specific purpose of this study was to determine the role of CYP2E1 in early alcohol-induced liver injury using CYP2E1 knockout (−/−) mice.

MATERIALS AND METHODS

Animals. Female CYP2E1 wild-type (+/+ ) or CYP2E1 −/− (129/Sv ter substrain) mice (18–20 g body wt) were obtained from a colony maintained at the University of North Carolina at Chapel Hill. Animals were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care. All animals received humane care in compliance with institutional guidelines. Mice had free access to a chow diet and water ad libitum before the study.

Operative procedures and gastric cannulation. Mice were fasted for 24 h before surgery. A 45-cm PE-90 polyethylene tube (Becton Dickinson, Sparks, MD) with a small silicon tip (1.5 mm) on one end was used for gastric cannulation using aseptic surgical techniques. Briefly, animals were anesthetized by inhalation of methoxyflurane and a vertical midline incision was made in the skin of the abdomen from the xiphoid cartilage extending to the midabdomen. A second small incision was made in the dorsal cervical area. A subcutaneous tunnel was exposed and 7-0 polypropylene sutures were passed 1-mm apart through the serosa and muscular layer of the stomach. After a small opening was made in the forearm stomach between sutures, the tip of the cannula was inserted 0.8 cm into the stomach. The tube was anchored to the stomach wall with Dacron felt, and the stomach was replaced in the abdominal cavity. The small incision where the cannula exits through the abdominal wall was closed with 5-0 silk and tied around the cannula, resulting in tight fixation of the cannula to the abdominal wall. The abdominal wall and skin were closed with 5-0 silk and tied around the cannula, anchoring button. The use of a spring coil and swivel (Lomir Biomedical Quebec), was connected to the cannula exited through a flanged button (Instech Laboratories, Plymouth Meeting, PA). A protective spring col (Instech Laboratories), through which the cannula passes to the swivel (Lomir Biomedical Quebec), was connected to the anchoring button. The use of a spring col and swivel protected the cannula and allowed free movement of animals in individual metabolic cages. The mouse was placed in a metabolic cage, and the end of the gastric cannula was connected to a swivel and infusion pump.

Diet. A liquid diet described first by Thompson and Reitz (29) supplemented with lipotropes as described by Morimoto et al. (23) was used. It contained corn oil as fat (37% of total calories), protein (23%), carbohydrate (5%), minerals, and vitamins, plus ethanol or isocaloric dextrin-maltose (control diet) as described elsewhere (31). Throughout the enteral feeding period, mice had free access to cellulose pellets as a source of fiber (Harlan Teklad, Madison, WI).

Experimental protocol. Mice were randomly divided into two experimental groups and fed either high-fat control or high-fat ethanol-containing diet continuously for up to 4 wk via intragastric feeding. The diet (1.29–1.31 kcal/ml) was infused at a rate of 0.44 ml·g body wt−1·day−1 with an infusion pump (Harvard Apparatus, Natic, MA). All animals received humane care in compliance with institutional guidelines, and severe alcohol intoxication was assessed carefully to evaluate development of tolerance using a 0–3 scoring system (0 = normal, 1 = sluggish movement, 2 = loss of motor activity but still moving if stimulated, 3 = loss of consciousness). The amount of ethanol in the diet was varied from 4.0% to 8.0% to obtain optimal delivery of calories without compromising growth or survival. Ethanol initially was delivered at 14 g·kg−1·day−1 (27% of total calories) and was increased 1 g/kg for 2 days until the end of the first week and then 1 g/kg for 4 days until the end of the experiment (final alcohol delivered = 28 g·kg−1·day−1; 40% of total calories). Animals had free access to water.

Clinical chemistry. Ethanol concentration in urine, which was shown to be representative of blood alcohol levels in the rat (5), was measured daily. Mice were housed in metabolic cages that separated urine from feces, and urine was collected over 24 h in bottles containing mineral oil to prevent evaporation. Each day at 9 AM, urine collection bottles were changed and a 1-ml sample was stored at −20°C in a microtube for later ethanol analysis. Ethanol concentration was determined by measuring absorbance at 366 nm resulting from the reduction of NAD+ to NADH by alcohol dehydrogenase (8).

After 4 wk of ethanol treatment, blood was collected via the aorta and centrifuged. Serum was stored at −20°C in a microtube until it was assayed for aspartate aminotransferase (AST) by standard enzymatic procedures (8).

Pathological evaluation. After 4 wk of ethanol treatment, livers were formalin-fixed, embedded in paraffin, and stained with hematoxylin-eosin to assess steatosis, inflammation, and necrosis. Liver pathology was scored as described for the rat by Nanji et al. (25) as follows: for steatosis (the percentage of liver cells containing fat), <25% = 1+, <50% = 2+, <75% = 3+, >75% = 4+; for inflammation and necrosis at one focus per low-power field = 1+ and at two or more foci = 2+. Pathology was scored in a blinded manner by one of the authors and by an expert in rodent liver pathology.

Alcohol metabolism. In naive mice, ethanol (3 g/kg ip) was given acutely before experiments. In cannulated mice, after 4 wk, ethanol-containing diet was removed immediately before experiments (13). Mice were forced to breathe into a closed, heated chamber (37°C) for 15 s, and 1 ml of breath was collected with a gas-tight syringe. The concentration of ethanol in breath was determined by gas chromatography, and rates of alcohol metabolism were calculated from linear decreases in blood alcohol concentration per unit time using Widmark's formula as described elsewhere (13).

Western blotting for CYP2E1. Mice livers were immediately excised after death, placed into liquid nitrogen, and stored at −70°C. Tissue was subsequently homogenized in three volumes of 10 mM Tris chloride buffer, pH 7.4, containing 150 mM potassium chloride, 1 mM EDTA, and 0.25 M sucrose with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) and centrifuged at 10,000 g for 30 min, and then 9 µg of supernatant were immunoblotted after separation of proteins on a 9% polyacrylamide gel. Electrophoretic transfer of protein to nitrocellulose sheets was performed as previously described (27). Nitrocellulose sheets were blocked overnight in 3% (wt/vol) nonfat dry milk in Tris-buffered saline (10 mM Tris chloride, pH 7.4, with 150 mM NaCl) at 4°C. CYP2E1 was detected using sheep anti-
rabbit CYP2E1 antiserum (1:100,000), with rabbit anti-sheep horseradish peroxidase (HRP, 1:5,000), using enhanced chemiluminescence (ECL) detection. The P-450 reductase was a rabbit anti-pig IgG (1:2,500) provided by Dr. B. S. Masters (University of Texas at San Antonio); detection with goat anti-rabbit HRP and ECL detection followed. Staining intensity of the complex was determined with a Bio-Rad GS-363 molecular imager (Bio-Rad, Richmond, CA) with a CH-1 imaging screen and Bio-Rad molecular analyst software.

Measurement of P-450 subfamilies. Testosterone hydroxylation was assayed as described by Brunner et al. (9) except that 400 µg of the S9 fraction from liver homogenates were used in place of microsomes. The dealkylation of ethoxyresorufin and pentoxyresorufin was determined as described by Lubet et al. (21) and adapted to microtitre plates as described by Kennedy and Johns (16). Reactions in a final volume of 250 µl contained 5 µM alkylresorufin added in 5 µl of DMSO, 100 mM sodium phosphate buffer, pH 8.0, 125 µg of S9 protein, 5 µl of dicumarol, and 250 µM NADPH to initiate the reaction. Reactions were terminated by the addition of 100 µl of acetonitrile, and resorufin was determined spectrofluorometrically using a Fluorostar microplate reader with an excitation filter of 544 nm and an emission filter of 590 nm.

Collection of bile and detection of free radicals. Ethanol concentration in the breath was analyzed by gas chromatography to verify that levels were between 200 and 250 mg/dl when experiments were initiated (13). Mice were anesthetized with pentobarbital (75 mg/kg), the abdomen was opened, and the common bile duct was ligated with a 8-0 polypropylene suture near the liver. After a small opening was made in the fundus of the gallbladder, the tip of a 20-cm length of PE-10 polyethylene tubing was inserted 3 mm into the gallbladder. The opening in the gallbladder was closed with a PE-10 polyethylene suture near the liver. After a small opening was made in the fundus of the gallbladder, the tip of a 20-cm length of PE-10 polyethylene tubing was inserted 3 mm into the gallbladder. The opening in the gallbladder was closed with a PE-10 polyethylene suture around the tube. Spin trap (6-((4-pyridyl-1-oxide)-N-tert-butylnitronate 1 g/kg) was administered intraperitoneally, and bile samples were collected for 3 h into 35 µl of 0.5 mM Desferal (deferoxamine mesylate) to prevent ex vivo radical formation. During bile collection, 4 ml/kg of saline were injected at 30-min intervals to avoid dehydration and ~200–300 µl of bile samples were collected. Samples were stored at −80°C until analysis of free radical adducts by electron spin resonance (ESR) spectroscopy (17).

Samples were thawed and placed in a quartz flat cell, and ESR spectra were obtained using a Varian E-109 spectrometer equipped with a TM110 cavity. Instrument conditions were as follows: 20-mW microwave power, 1.0-G modulation amplitude, 80-G scan width, 16-min scan, and 1-s time constant. Data were collected with an IBM-type computer interfaced to a spectrometer. Simulations and double integrations of spectra to determine amplitude were carried out with a computer program (10).

Statistics. ANOVA or Student’s t-test was used for determination of statistical significance as appropriate. For comparison of pathological scores, the Mann-Whitney rank sum test was used. A P value of <0.05 was selected before the study as the level of significance.

RESULTS

Body weight gain. All mice survived surgery, and diets were initiated after 1 wk to allow for complete recovery from surgery. Steady weight gains were observed during 4 wk of continuous enteral feeding of liquid diets with or without ethanol, indicating adequate nutrition (Fig. 1). Similar weight gains occurred in adult CYP2E1+/+ or −/− mice on chow diet (data not shown). There were no significant differences in weight gains among the groups studied (Fig. 1).

Ethanol concentrations in urine and ethanol metabolism. Representative plots of daily urine alcohol concentrations in mice given ethanol are depicted in Fig. 2. As reported previously by several groups using the Tsukamoto-French enteral protocol in the rat (1, 30), urine alcohol levels in mice also fluctuated in a cyclic pattern from zero to >500 mg/dl, even though ethanol was infused continuously. Similar patterns were observed here in CYP2E1+/+ and CYP2E1−/− mice. The reason for this cyclic pattern is not understood, but, because it occurred in CYP2E1−/− mice, it was concluded that CYP2E1 is not involved in this phenomenon in the mouse. Mean urine alcohol concentrations over 4 wk were 243 ± 17 mg/dl in CYP2E1+/+ and 235 ± 22 mg/dl in CYP2E1−/− mice. There were no significant differences between groups.

After acute ethanol administration (3 g/kg), blood alcohol levels were around 250 mg/dl and not different between wild-type and knockout mice. Under these conditions, rates of ethanol elimination in vivo were 3.2 ± 0.4 mmol·kg−1·h−1 in CYP2E1+/+ and 3.7 ± 1.2 mmol·kg−1·h−1 in CYP2E1−/− mice. After 4 wk of enteral ethanol treatment, rates were increased significantly to 5.1 ± 0.8 mmol·kg−1·h−1 in CYP2E1+/+ and 4.9 ± 0.6 mmol·kg−1·h−1 in CYP2E1−/− mice as expected based on studies with rats (14). Although ethanol increased ethanol elimination rates significantly, there were no significant differences between the wild-type and knockout groups.
Serum transaminase levels. In control groups, serum AST levels were 40 IU/l after 4 wk. Chronic ethanol treatment significantly increased serum AST levels about threefold over control values in both CYP2E1+/+ and CYP2E1−/− mice (Fig. 3); however, there were no significant differences between groups. A macrovesicular pattern of fat accumulation was observed mainly in the pericentral to midzonal regions in both groups (Fig. 4), except for one to three layers of hepatocytes around central veins. This phenomenon appears to be specific to the mouse. Furthermore, infiltrating leukocytes observed in this study were largely lymphocytes with macrophages. In addition, inflammatory foci predominantly containing neutrophils with residual eosinophils were also occasionally observed.

Western blotting for CYP2E1. P-450 reductase expression was increased significantly by enteral ethanol in both CYP2E1+/+ and CYP2E1−/− mice (Fig. 6). CYP2E1 expression was minimal in the liver from CYP2E1+/+ mice given high-fat control diet without ethanol after 4 wk (Fig. 6). In contrast, enteral ethanol increased CYP2E1 expression significantly. However, CYP2E1 expression was not detected in livers from CYP2E1−/− mice fed high-fat liquid diets with or without ethanol as expected (20).

**Fig. 2.** Representative plots of daily urine alcohol concentrations of ethanol-fed CYP2E1+/+ (A) and CYP2E1−/− (B) mice. Urine alcohol concentrations were measured daily as described in MATERIALS AND METHODS. Typical urine alcohol concentrations are shown.

**Fig. 3.** Effect of chronic ethanol on serum aspartate aminotransferase (AST) levels in CYP2E1+/+ and −/− mice. Blood samples were collected via the aorta at 4 wk, and AST was measured as described in MATERIALS AND METHODS. Data represent means ± SE (n = 6). *P < 0.05 compared with CYP2E1+/+ mice fed control diet; #P < 0.05 compared with CYP2E1−/− mice fed control diet by two-way ANOVA using Bonferroni’s post hoc test.
The effect of ethanol treatment on several P-450 subfamilies was assessed by monitoring the hydroxylation of testosterone and the dealkylation of 7-ethoxyresorufin and 7-pentoxyresorufin (Table 1). For all of activities measured, there were no significant differences between the CYP2E1 control 1/1 and control CYP2E1 2/2 mice, suggesting that the constitutive loss of CYP2E1 did not significantly alter the expression of other isoforms such as the CYP3A (testosterone 6β hydroxylation activity), CYP1A1 (ethoxyresorufin dealkylation), or the CYP2B family (pentoxyresorufin dealkylation) (14a). There was a great deal of variation in the expression levels of the enzymes responsible for these activities, which was confirmed for the CYP3A family by immunoblot analysis. In both strains, ethanol treatment resulted in an induction of the CYP3A family. Testosterone 7α hydroxylation was also induced, as was the dealkylation of pentoxyresorufin. In contrast, there was no induction of CYP1A in either CYP2E1 1/+ or 2/2 animals with ethanol. Although the results of the

![Fig. 4. Representative photomicrographs of livers from CYP2E1 1/+ and 2/2 mice after 4 wk of enteral ethanol treatment.](http://apgi.physiology.org/)

Effects of enteral ethanol on induction of P-450 subfamilies. The effect of ethanol treatment on several P-450 subfamilies was assessed by monitoring the hydroxylation of testosterone and the dealkylation of 7-ethoxyresorufin and 7-pentoxyresorufin (Table 1). For all of activities measured, there were no significant differences between the CYP2E1 control 1/+ and control CYP2E1 2/2 mice, suggesting that the constitutive loss of CYP2E1 did not significantly alter the expression of other isoforms such as the CYP3A (testosterone 6β activity), CYP2A12 (testosterone 7α hydroxylation activity), CYP1A1 (ethoxyresorufin dealkylation), or the CYP2B family (pentoxyresorufin dealkylation) (14a). There was a great deal of variation in the expression levels of the enzymes responsible for these activities, which was confirmed for the CYP3A family by immunoblot analysis. In both strains, ethanol treatment resulted in an induction of the CYP3A family. Testosterone 7α hydroxylation was also induced, as was the dealkylation of pentoxyresorufin. In contrast, there was no induction of CYP1A in either CYP2E1 1/+ or 2/2 animals with ethanol. Although the results of the
current study clearly demonstrate that CYP2E1 is not present or induced in the knockout mice, there is normal constitutive expression of other isoforms and ethanol treatment causes a variable induction of the CYP3A and CYP2B families.

Effects of chronic enteral ethanol on free radical formation in bile. After 4 wk of high-fat control diet, free radical formation in bile was minimal in both CYP2E1+/+ and −/− mice (data not shown). In contrast, enteral ethanol for 4 wk increased free radical formation about threefold but to the same extent in both groups (Fig. 7). There were no differences in radical intensities between the groups. Thus CYP2E1 deficiency did not affect radical formation caused by alcohol feeding in mice.

DISCUSSION

Advantages of knockout technology for the study of alcohol-induced liver disease. Knockout technology, developed by Smithies (28), has distinct advantages. The advantage of knockout technology is that deletions or disruptions in DNA result in alterations that are specific. Specific genetic manipulation allows deletion of key enzymes or receptors so that potentially nonspecific agents can be avoided. Application of chronic enteral ethanol feeding to knockout mice provides a powerful tool for mechanistic studies in alcohol research in which interpretations in vivo may be complicated.

Application of a new enteral feeding model to alcohol-induced liver injury studies in mice. The new model described in this study was designed to provide enteral nutrition for long-term studies in mice. Surgery in the mouse is more difficult than the rat due to the smaller body size; however, postoperative survival rates of over 90% were achieved with care. Administration of antibiotics postsurgically and fasting for 24 h before surgery were important factors for success. Furthermore, it was also important that diets were initiated 1 wk after surgery to allow for full recovery from surgery for optimal survival.
The continuous intragastric infusion of a high-fat liquid diet with or without ethanol has been successfully achieved for periods of 28 days and could be easily extended. During continuous intragastric feeding, careful observations for intoxication and nursing care were required at least every 12 h for maximal success. Accordingly, animals given ethanol were observed frequently for signs of severe alcohol intoxication, i.e., sluggish movement, relaxation of legs, and loss of consciousness, using the 0–3 scoring system detailed in MATERIALS AND METHODS. With this system, it was possible to detect tolerance to alcohol during the first 1–2 wk, thus allowing alcohol delivery to be increased gradually. Because histopathological findings were similar in livers from wild-type mice (Fig. 4) and rats, it is concluded that long-term enteral feeding in the mouse is practical.

Nutrition and dietary factors are important in the pathogenesis of alcoholic liver injury (11). Therefore, it is important that weight gain be demonstrated to minimize possible nutritional complications. In the present study, adult mice achieved steady weight gains, indicating that nutrition was adequate (Fig. 1). Furthermore, early alcohol-induced liver injury was prevented in tumor necrosis factor receptor-1 knockout mice given enteral ethanol using the same protocol (34). Both controls and knockout mice received the same diet, but only wild-type mice exhibited pathology. Thus it is concluded that nutritional complications cannot explain these results.

**Table 1. Effects of enteral ethanol on induction of P-450 subfamilies**

<table>
<thead>
<tr>
<th>Catalytic Activity</th>
<th>CYP2E1+/+ Control</th>
<th>CYP2E1+/+ Ethanol</th>
<th>CYP2E1−/− Control</th>
<th>CYP2E1−/− Ethanol</th>
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<tr>
<td>Testosterone 6β hydroxylation</td>
<td>531 ± 259</td>
<td>1,296 ± 400</td>
<td>540 ± 234</td>
<td>789 ± 541</td>
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<td>Testosterone 7α hydroxylation</td>
<td>42 ± 14</td>
<td>182 ± 67</td>
<td>32 ± 10</td>
<td>73 ± 23</td>
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<td>Ethoxyresorufin dealkylation</td>
<td>1,612 ± 1288</td>
<td>2,327 ± 537</td>
<td>1,138 ± 489</td>
<td>1,817 ± 813</td>
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<tr>
<td>Pentoxyresorufin dealkylation</td>
<td>918 ± 728</td>
<td>2,102 ± 813</td>
<td>980 ± 706</td>
<td>1,941 ± 679</td>
</tr>
</tbody>
</table>

Values are means ± SD for 4 different animals in each group and are expressed as pmol product·min⁻¹·mg protein⁻¹. Catalytic activities were determined using S9 fraction as described in MATERIALS AND METHODS. +/+, Wild-type cytochrome P-450 (CYP)2E1 mice; −/−, CYP2E1 knockout mice; both groups were fed either control diets or ethanol-containing diets.
The main pathological change in early alcohol-induced liver injury in the mouse was steatosis; however, inflammation and necrosis were also observed. Fat accumulation in livers from CYP2E1+/+ mice given ethanol was observed mainly in pericentral to midzonal regions, except for one to three layers of hepatocytes around central veins, which may be hypoxic. Enteral ethanol causes hypoxia in pericentral regions of the liver in rats (4). Because ATP levels decline in anoxia, decreased fat accumulation in the last cell layers may be due to a lack of energy for triglyceride synthesis.

Is CYP2E1 involved in early alcohol-induced liver injury? Reactive oxygen species are involved in mechanisms of alcohol-induced liver injury in the rat (17). CYP2E1 induced predominantly in the hepatocyte after alcohol could be a source of reactive oxygen species (15, 26). Indeed, French et al. (12) correlated the level of CYP2E1 with liver pathology, lipid peroxidation, and formation of the α-hydroxethyl radical adduct produced in vivo and in vitro. Furthermore, inhibitors of CYP2E1 partially decreased alcohol-induced liver injury (6). Moreover, Wong et al. (33) reported that CYP2E1 was the major factor in carbon tetrachloride-induced hepatotoxicity in mice. These data are consistent with the hypothesis that mechanisms of alcohol-induced liver injury involve oxidant stress from CYP2E1. On the other hand, reactive oxygen species are also produced by various white blood cells such as monocytes and macrophages. Kupffer cells, the resident macrophage in the liver, could be a rich source of reactive oxygen species via NADPH oxidase. Indeed, destruction of Kupffer cells by GdCl3 blunted free radical formation in the Tsukamoto-French model and prevented early alcohol-induced liver injury (17, 19). Thus whether oxidant stress is from CYP2E1 predominantly in hepatocytes or NADPH oxidase in Kupffer cells has remained unclear.

Recently, it was reported that CYP2E1 was induced to the same extent in ethanol- and ethanol + GdCl3-treated rats in the Tsukamoto-French model; however, liver pathology was reduced by GdCl3 (19). Furthermore, Lytton et al. (22) reported that autoantibodies against CYP2E1 were detected in sera from alcoholics; however, only 10–15% of patients were positive. These data support the idea that CYP2E1 plays only a small role in early alcohol-induced liver injury. Thus evidence exists for and against a role for CYP2E1 in the mechanism of alcohol-induced liver injury. To resolve this controversy, knockout mice were evaluated here. In this study, a similar pattern of increase was observed in serum transaminases in both CYP2E1+/+ and CYP2E1−/− mice (Fig. 3). Furthermore, CYP2E1+/+ and CYP2E1−/− mice given enteral ethanol developed moderate steatosis, mild inflammation, and necrosis to the same degree (Fig. 4). In addition, there were no differences in free radical formation between CYP2E1+/+ and CYP2E1−/− mice fed enteral ethanol (Fig. 7). Thus these data are consistent with the hypothesis that oxidant stress from CYP2E1 plays no role or only a minor role in mechanisms of early alcohol-induced liver injury in mice. The induction of other isoforms of P450 might be a contributing factor in the initiation of early alcohol-induced liver injury. Alternatively, the knockout mice may have adapted to their lifelong CYP2E1 deficiency by expressing or overexpressing other P450 proteins that could replace the CYP2E1 function, since other P450 subfamilies were induced by enteral ethanol similarly in both groups (Table 1).

In conclusion, the data presented here are consistent with the hypothesis that oxidant stress from CYP2E1 plays no role or only a minor role in mechanisms of early alcohol-induced liver injury in mice. Moreover, this mouse model combined with knockout technology will provide a powerful new tool in alcohol research.

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Preliminary accounts of this work have appeared elsewhere (18).
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