Inhibition of Ethanol-Induced Liver Disease in the Intragastric Feeding Rat Model by Chlormethiazole (44545)

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Abstract. **The purpose of this investigation was to assess the effect of chlormethiazole treatment on liver damage in the experimental rat intragastric ethanol-feeding model of alcoholic liver disease. Chlormethiazole has been used in the treatment of alcoholic withdrawal and has been shown to inhibit cytochrome P4502E1. Since treatment of experimental alcoholic liver disease with CYP2E1 inhibitors had an ameliorating effect on liver injury in the rat, chlormethiazole was used to see if it had a similar effect. Rats fed ethanol for 2 months had significantly less liver injury when chlormethiazole was added to the diet, fed intragastrically. The CYP2E1 apoprotein levels, which were increased by ethanol feeding, were also increased when chlormethiazole was fed with ethanol. Chlormethiazole inhibited the increase in the ethanol-induced CYP2E1 activity *in vivo*, as measured by chlorzoxazone 6-hydroxylation, but did not affect the level of CYP2E1 apoprotein. Likewise, the reduction in proteasome proteolytic enzyme activity produced by ethanol feeding was blunted in chlormethiazole-fed rats. These results support the conclusion that chlormethiazole treatment partially protects the liver from injury by inhibiting CYP2E1 activity *in vivo*.** [P.S.E.B.M. 2000, Vol 224:302–308]

The aim of this work was to define the effects of chlormethiazole (CMZ) treatment on the liver of rats fed ethanol intragastrically for 2 months. CMZ is currently used to treat alcohol withdrawal symptoms in Europe. Recently, a study on alcohol abusers showed that CMZ treatment inhibited the activity of liver cytochrome P4502E1 (CYP2E1) *in vivo* and *in vitro* in alcoholic patients (1). *In vitro* assays using human liver showed that CMZ was an efficient noncompetitive inhibitor of chlorzoxazone (CHZ) metabolism (1). CHZ hydroxylation by CYP2E1 is increased after ethanol ingestion, and this increase is blocked by CMZ *in vivo* (1). Since ethanol induces CYP2E1, and this induction leads to free radical damage in the liver (2), CMZ is a candidate for alleviating ethanol-induced liver injury caused by free radical generation. In the intragastric, tube-feeding, rat model of ethanol-induced liver injury, inhibition of CYP2E1 prevented free radical damage and ameliorated the liver histopathology (2). However, Hu *et al.* (3) failed to demonstrate CMZ inhibition of liver CYP2E1 activity *in vitro*. They found, rather, that CMZ inhibited transcription of the *CYP2E1* gene, whereas, CMZ inhibited CYP2E1 at a post-translational level in rat hepatoma cells (4). In the present report, the effect of CMZ on CYP2E1 activity *in vivo* was studied using the intragastric, tube-fed rat model.

Some of the data on CMZ and liver injury in the rats studied here have been reported in abstract form (5). In addition, the effect of CMZ on ethanol-induced reduction of liver proteasome peptidase activity has been reported in abstract form (6). Some of the data on cytochrome P450

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derived from the present study have been reported. In these data, CMZ treatment of rats fed ethanol intragastrically for 2 months suppressed autoantibody formation to CYP2E1. This correlated with a reduction in the liver pathology induced by ethanol feeding (7). CYP3A1 studied in the same way as CYP2E1 was not affected by CMZ treatment, indicating specificity for the effect of CMZ on CYP2E1. Thus, CMZ treatment has potential to ameliorate the effects of ethanol on liver disease.

Materials and Methods

Male Wistar rats, weighing about 225 g, were purchased from Charles River Laboratories (Hollister, CA). The intragastric cannula was implanted under pentobarbital sodium (30–50 mg/kg i.p.) anesthesia. The animals were then allowed to recover for 6–10 days before the experiment was started. The rats were maintained according to the guidelines of animal care as described by the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (8). Animals were pair-fed ethanol or isocaloric dextrose with liquid diet by continuous infusion *via* a permanent intragastric cannula for 2 months in the amount necessary to maintain a constant high blood ethanol level as described previously (9). The diet was supplemented with protein, methionine, and choline to ensure adequate methyl donors and with vitamins and minerals (AIN95) to ensure adequate nutrition despite a high percentage of calories derived from ethanol (36%) (10). Water and non-nutrient fiber were given *ad libitum*. Groups of rats fed ethanol or pair-fed dextrose were given chlormethiazole (CMZ) suspended in their diet (80 mg/kg/day). In one subgroup, CMZ was given in a bolus of 80–120 mg/kg/day suspended in the diet without affecting the results. CMZ was supplied as a gift by Astra Arars AB, Södertälie, Sweden. Blood samples were taken from the tail vein before surgery and at 1 and 2 months after treatment.

Blood ethanol (mg/dl) and serum alanine aminotransferase activity (ALT) (IU/l) were measured enzymatically on a clinical analyzer. ALT was measured by a kinetic rate method on a SYNCH RONCX Systems (Beckman Inst, Inc., Brea, CA) BAL was measured using a Radioactive Energy Attenuation Assay on an Abbot AXSYM System Analyzer.

Chlorzoxazone metabolism was measured *in vivo* (6-OH CHZ/CHZ ratio, μg/ml) in some experiments where the ratio of 6-OH-chlorzoxazone (6-OH CHZ) to chlorzoxazone (CHZ) was calculated. Both compounds were measured by HPLC (High Performance Liquid Chromatography) and expressed as μg/ml 1, 2, and 3 hr after intraperitoneal injection of CHZ (150 μmol/kg) into the rats (11, 12).

Euthanasia at the end of the experiments was accomplished using an overdose of intraperitoneal pentobarbital. At the time when the experiments were terminated, livers were removed. A portion was used to prepare microsomes, using a previously described method (13). The relative amounts of CYP2E1, 3A and 4A were assessed by Western blotting using rabbit polyclonal antibodies (7). SDS-Page was performed using a 4% stacking gel and an 8.7% separating gel. Liver microsomes were solubilized in SDS gel loading buffer, [3% SDS (w/v), 0.2 *M* Tris-HCl, pH 6.8, 26% glycerol (w/v) with 2 *M* 2-mercaptoethanol] and heated for 2 min at 100°C.

Electrophoresis was run for 40 min × 200 V in the Mini-Protean apparatus (Bio-Rad, Hercules, CA) (7). The proteins were electro-transferred at 50 mA × 15 hr, to H-Bond Extra (Amersham, Piscataway, NJ) nitrocellulose in transfer buffer (20 m*M* Tris, 154 m*M* glycine, and 20% v/v methanol). Blots were washed in 0.1 *M* phosphate buffer (pH 7.4) and air-dried on Whatman filter paper. They were then placed in 50 m*M* Tris-HCl (pH 7.4), 200 m*M* NaCl, and 0.05% Tween 20 in a 5% solution of baker's powdered milk, for 4 hr to block the free binding sites. Blots were washed three times for 5 min in 10-ml volumes of TBS-Tween 20 buffer and then probed with 1:5000 diluted rabbit anti-CYP2E1, or rabbit anti-CYP3A1, or rabbit anti-CYP2E1–4A sera in blocking buffer for 4 hr, with shaking. After repeated washes, immunoblots were performed for 2 hr with shaking, using peroxidase linked goat antirabbit IgG heavy and light chains, diluted 1:2000. The blots were developed on photographic film (Kodak x-Omat; Kodak, Rochester, NY) with luminol substrate and hydrogen peroxide of enhanced chemiluminescence (ECL) reagent (Amersham). The CYP4A antibody was a generous gift from Dr. Gordon Gibson, University of Surrey. The results were calculated as arbitrary units, which are densitometric units (UA/mg protein). CYP2E1 was also stained in liver slices by immunohistochemistry using the same polyclonal antibody as was used for western blot (7).

The immunohistochemical stain for CYP2E1 used the same rabbit polyclonal antibody that was used for Western blotting except that it was diluted 1:600. Paraffin sections of formalin-fixed liver were used. Biotin-labeled second antibody was applied, to visualize the location of CYP2E1 using Vector ABC kit (Burlingame, CA) (2).

The activities of the proteasome peptidases (chymotrypsin-like (Cht-L), trypsin-like (T-L), and peptidylglutamyl-peptide hydrolase (PGPH)) were measured in the liver cytosol fraction after the method of Donohue *et al.* (14) Liver cytosolic fractions were assayed for the chymotrypsin-like (Cht-L) [hydrolysis on succinyl-Leu-Leu-Val-Tyr-Amido-4-methylcoumarin (LLVY-AMC)], Tyrosine-like (T-L) I hydrolysis of N-t-Boc-Leu-Ser-Thr-Arg-777-Amido-4-Methylcoumarin (LSTR-AMC)], and peptidylglutamyl-peptide hydrolyzing (PGPH) [hydrolysis of N-CBZ-Leu-Leu-Glu-β-Nathylamide (LLE-NA)] activity (Sigma Chemical Co., St. Louis, MO). Assays were performed at 30°C with continuous shaking for 60 min in 0.1 *M* Tris-HCl (pH 7.5) using 20–200 μg of sample protein and a peptide substrate concentration of 40 μ*M*. Fluorescence of AMC for LLVY-AMC and LSTR-AMC was measured at excitation and emission wavelengths of 390 and 440 nm, respectively.

Table I. Effect of Chlormethiazole Treatment on Cytochrome CYP2E1 Isoforms and Pathology Score in Rats Fed Ethanol

	Dextrose	Ethanol	ETOH + CMZ	Dex + CMZ
Body wt (g)	347 ± 24 (6)*	372 ± 38 (7)[a]	301 ± 61 (14)[a]	340 ± 31 (8)
Liver wt (g)	11.9 ± 1.8 (6)[b]	18.8 ± 2.9 (7)[b,c]	16.6 ± 5 (14)	13.3 ± 1.4 (8)[c]
% Body wt	3.4 ± 0.4 (6)[d]	5.0 ± 0.3 (7)[d]	5.4 ± 0.9 (14)[e]	3.9 ± 0.3 (8)[e]
BAL† mg/dl	—	197 ± 49 (8)	239 ± 172 (8)	—
ALT IU/L	90 ± 80 (6)	102 ± 73 (8)	66 ± 38 (6)	33 ± 13 (6)
CYP2E1 (UA/mg)	37 ± 15 (6)[f]	233 ± 17 (7)[f]	215 ± 79	22 ± 17 (8)
CYP4A (UA/mg)	65 ± 27 (6)	126 ± 57 (7)	158 ± 91 (8)	111 ± 57 (8)
CYP3A (UA/mg)	153 ± 141 (6)	239 ± 265 (7)	349 ± 218 (8)	121 ± 85 (8)

* Mean ± SD, number in parentheses = number measured.
† BAL = blood alcohol levels, taken at the time of sacrifice.
$P < 0.05$ when *a* is compared with *a*, *b* with *b*, *c* with *c*, *d* with *d*, *e* with *e*, *f* with *f*.
Statistics: ANOVA, one-way analysis of variance, all pair-wise comparisons (Bonferroni *t* test method).

β-Naphthylamine (LLE-NA) was measured at excitation and emission wavelengths of 335 and 410 nm, respectively.

The liver pathology score was determined morphologically using the scoring method of Tsukamoto *et al.* (9) where fat is scored 0–4 +, inflammation 0–2 +, necrosis 0–2 +, and scarring 0–2 + for a possible total score of 10. The sections were stained with hematoxylin and eosin. For assessment of scarring, the sections were stained with Sirius red.

Lipid tissue malondialdehyde MDA was measured as an indication of lipid peroxidation (pmol MDA/mg protein) (13). MDA was measured using the thiobarbituric acid reagent.

Results

The purpose of the first set of experiments was to assess the effect of CMZ on ethanol-induced pathology and cytochrome P450-induction. Rats fed ethanol without CMZ and their pair-fed controls grew at similar rates, but the CMZ-fed rats that were fed ethanol gained less weight than their corresponding controls over the experimental period of 2 months (Table I). Mean weight gain for the ethanol group was 3 g/day; dextrose control group was 2.2 g/day; CMZ + ethanol group was 1.5 g/day, and the CMZ dextrose group was 2.1 g/day. The blood ethanol levels between the rats fed ethanol compared with rats fed ethanol + CMZ were not significantly different (Table I). CMZ treatment did not inhibit the ethanol-dependent induction of CYP2E1 apoprotein as was assessed at the end of the experiment (Table I). CMZ did not affect the increased liver weights induced by ethanol (Table I). ALT levels were not different when the ethanol- and CMZ-treated rats were compared with controls (Table I). Likewise, there was no significant difference in the CYP4A and 3A found in the ethanol- and CMZ-treated rats (Table I). Malondialdehyde was increased ($P \leq 0.013$) in the ethanol-fed rats (58.7 ± 21.8 pmol MDA/mg homogenate protein, $n = 7$, SD) compared with the rats fed ethanol + CMZ (37.5 ± 9.1, $n = 9$), pair-fed controls (34.9 ± 7.1, $n = 8$), and CMZ pair-fed controls (31.1 ± 9.3, $n = 8$).

The pathology score was significantly higher in the rats fed ethanol compared with the rats fed ethanol with CMZ (Table II). All of the parameters measured (i.e., fat, necrosis, inflammation, and fibrosis) were significantly worse in the rats fed ethanol compared with the rats fed ethanol with CMZ. Focal fibrosis was prominent in some of the livers from rats fed ethanol (Figs. 1A–C). This fibrosis was centrilobular (Figs. 1A–C) with central-central bridging fibrosis (Fig. 1A) or pericellular fibrosis (Fig. 1C). Fatty change, necrosis, and inflammation were prominent in the livers of rats fed ethanol (Fig. 1D) compared with the livers from rats fed ethanol and CMZ.

The staining for CYP2E1 in the centrilobular zone was assessed morphologically using immunohistochemical staining. In rats fed ethanol alone, the increased staining was the same as that seen in the rats fed ethanol with CMZ (Fig. 2). Controls not fed ethanol showed less staining in hepatocytes located in the central zones (Fig. 3). This cor-

Table II. The Effect of Chlormethiazole (CMZ) Treatment on Ethanol-Induced Liver Pathology

Regimen	*n*	Pathology Score (P.S.) Mean ± SD				
		Fat[a]	Necrosis[b]	Inflammation[c]	Fibrosis[d]	Total P.S.[e]
Dextrose	6	0.17 ± 0.41*	0.83 ± 0.75	0.83 ± 0.75	0	1.83 ± 1.5
ROH†	8	3.0 ± 1.07	1.1 ± 0.99	1.6 ± 0.52	0.75 ± 1.04	6.45 ± 2.4
ROH† + CMZ	14	1.8 ± 1.05	0.14 ± 0.36	0.6 ± 0.8	0	2.54 ± 1.8
Dex‡ + CMZ	10	0.25 ± 0.6	0	0.6 ± 0.84	0	0.85 ± 0.88

Note. Compare ROH group with ROH + CMZ group: [a]$P = < 0.019$, [b]$P = < 0.007$, [c]$P = < 0.003$, [d]$P = < 0.01$, [e]$P = < 0.05$.
* Mean ± SD
† ROH = Alcohol
‡ Dex = Dextrose

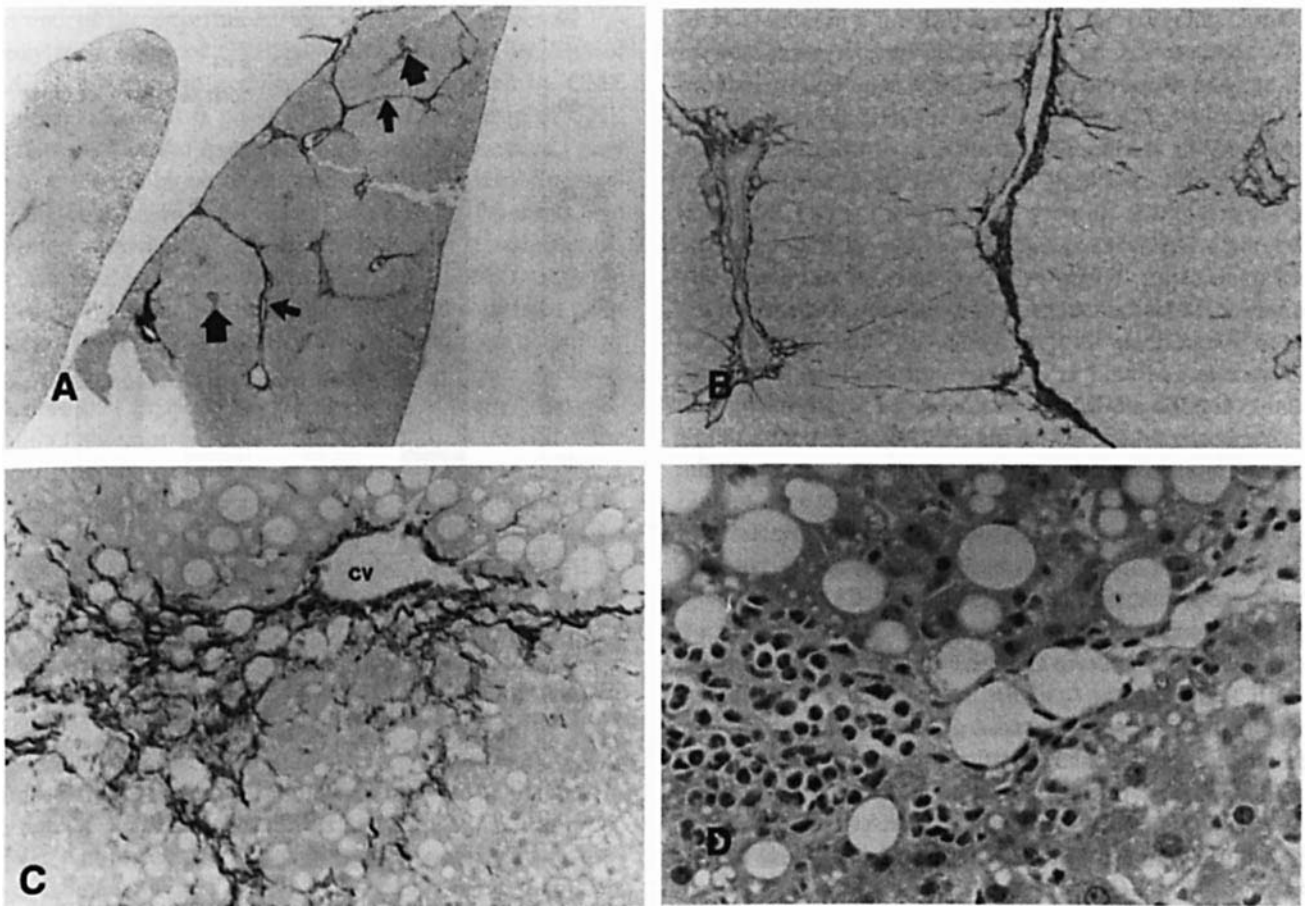

Figure 1. (A) Liver section stained for collagen from a rat fed ethanol without CMZ. Note the focal central bridging fibrosis (small arrow) and intervening portal tracts (large arrow). Sirius red stain. ×31. (B) Liver section from the same rat as Figure 1A showing central scar in the middle and portal tracts at both sides. Note, a few strands of collagen extend toward the portal tracts from the central scar. Sirius red stain. ×156. (C) Liver section from the same rat as in Figures 1A & 1B showing pericellular fibrosis near a central vein (CV). Sirius red stain. ×312. (D) Liver section from the same rat as in Figure 1 showing fatty change, necrosis, and inflammation and fibrosis near a central vein. Most of the inflammatory cells are neutrophils, but mononuclears are also present. Hematoxylin and Eosin stain. ×624.

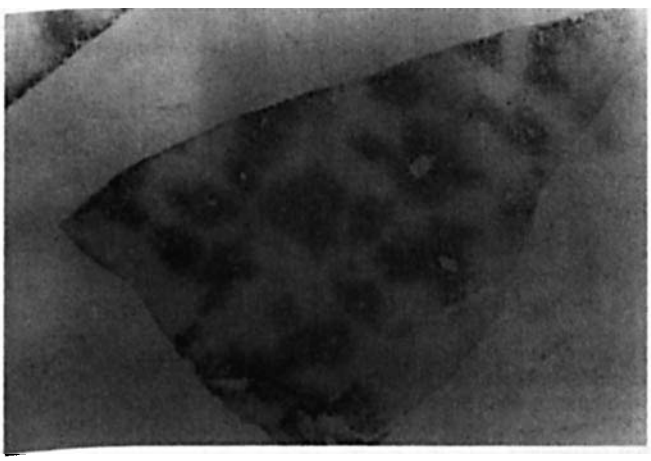

Figure 2. Liver from a rat fed ethanol and CMZ and stained for CYP2E1. Note the absence of fibrosis, inflammation, necrosis, or fatty change. The CYP2E1 positive centrilobular zone was increased compared with controls (Figure 3) and was similar to the livers from rats fed ethanol alone. Immunoperoxidase stain. ×31.

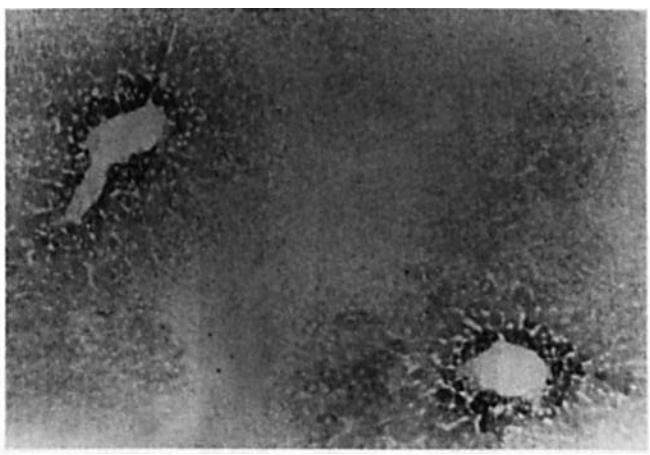

Figure 3. Liver from a control rat fed CMZ but not ethanol showing that the CYP2E1-positive centrilobular zone was much smaller compared with the livers of rats fed ethanol and CMZ (Figure 2). Immunoperoxidase stain. ×156.

related with the quantitative measurements of CYP2E1 apoprotein in the microsomal fractions (Table I).

The second set of experiments was designed to assess whether or not CMZ treatment would prevent the inhibitory effect of ethanol on the peptidase activity of the proteasome. Rats were fed ethanol at a fixed rate (13 g/kg/day) and CMZ

(80 mg/kg/day) with pair-fed controls for 2 months ($n = 5$) to measure proteasome peptidase activities. The chymotrypsin-like activity of the proteasome was significantly reduced by ethanol feeding for 2 months. This reduction was attenuated by CMZ feeding in that activities in controls were no longer significantly higher than those from CMZ-ethanol-fed rats (E-CMZ) (Fig. 4). The same was true for trypsin-like (T-L) activity (Fig. 5). The activity of the control rat was significantly higher than the ethanol fed rats ($P < 0.05$). However, CMZ alone tended to reduce the T-L activity but not to a significant level, and ethanol + CMZ activity was the same as CMZ alone. In the case of PGPH activity, there was no significant difference among the four groups ($n = 4$) (i.e., control 6.7 ± 4.5; alcohol 14.3 ± 12.5; control CMZ 7.5 ± 6.3; CMZ + ethanol, 9.8 ± 8.5 nmoles/mg protein; mean ± SD)

In a third group of rats, we examined the effects of CMZ treatment on CYP2E1 hydroxylation of CHZ *in vivo*. Rats were fed ethanol (13 g/kg/day) and CMZ (80 mg/kg/day) at a constant rate with pair-fed controls ($n = 5$) for 2 weeks to measure *in vivo* CYP2E1 activity by measuring the 6-OHCHZ/CHZ ratio (μg/ml). The 2-hr post-intraperitoneal injection of CHZ (150 μmoles/kg) serum ratio showed that the ethanol-fed rats had significantly elevated ratios compared with their pair-fed controls (0.18 ± 0.03 vs. 0.11 ± 0.03 SD, respectively) (Fig. 6). Likewise, the ratios were higher in the ethanol-fed rats compared with those treated with ethanol and CMZ ($P \leq 0.05$) and dextrose + CMZ ($P \leq 0.05$, Bonferroni *t* test). CMZ also reduced the ratio compared with the DEX control indicating that CMZ inhibited both the ethanol-induced CYP2E1 and the constitutive CYP2E1 activity *in vivo*.

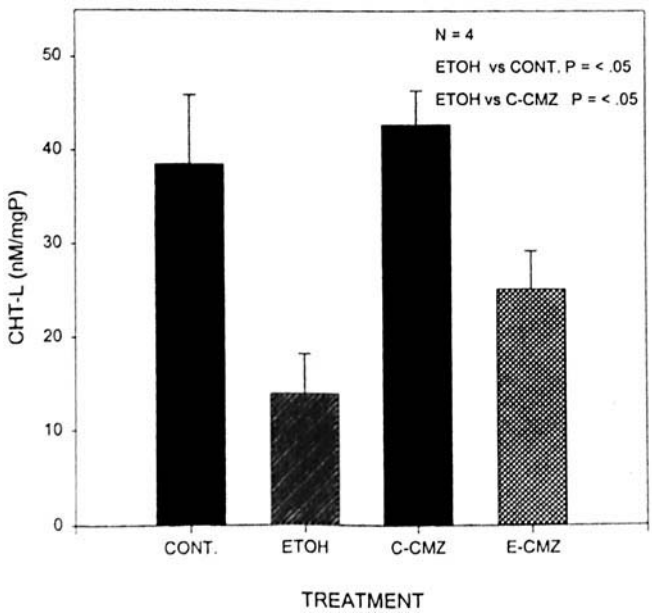

Figure 4. Bar graph of chymotrypsin activity in rats fed ethanol (ETOH) or ethanol with CMZ (E-CMZ) and their pair-fed controls (CONT and C-CMZ) ($n = 4$). ETOH compared with CONT and ETOH compared with C-CMZ = $P < 0.05$ and $P = < 0.05$, respectively (Bonferroni *t* test).

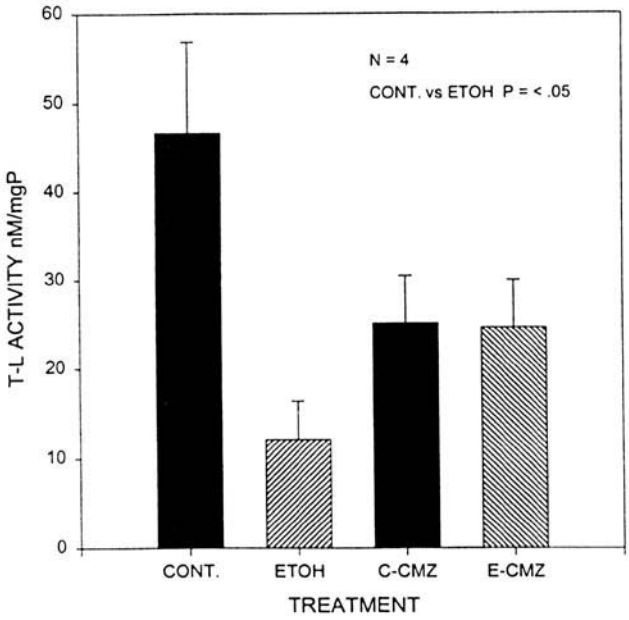

Figure 5. Bar graph of trypsin-like proteasomal enzyme activity of rats fed ethanol (ETOH), ethanol and CMZ (E-CMZ), and their pair-fed controls (CONT and C-CMZ) ($n = 4$). ETOH compared with CONT was significantly decreased ($P < 0.021$, $n = 4$, Bonferroni *t* test). The differences between CONT and E-CMZ were not significant ($P < 0.244$).

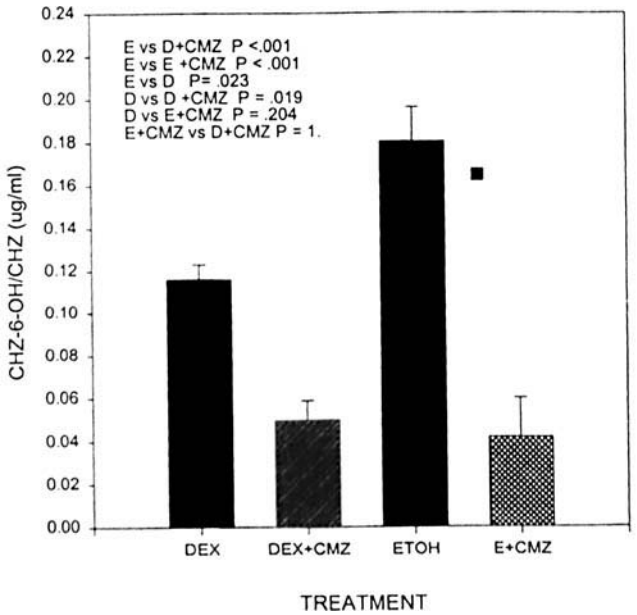

Figure 6. Bar graph of *in vitro* 6-0H CHZ/CHZ ratios 2 hr after intraperitoneal injection of CHZ (150 μM/kg). Note that ethanol (ETOH) feeding increased the ratio compared with DEX ($P < 0.023$) ($n = 5$). CMZ feeding reduced both the ethanol-induced (ETOH + CMZ) and the constituent CYP2E1 activity (DEX + CMZ) *in vivo* ($P < 0.05$, Bonferroni *t* test).

Discussion

The present study documents that CMZ treatment reduces the liver pathology induced by intragastric tube feeding of ethanol. Although the CYP2E1 apoenzyme induction by ethanol feeding was not affected by CMZ treatment, at

the end of the experiment, the *in vivo* chlorzoxazone hydroxylation assay of CYP2E1 activity induced by ethanol and the constitutive activity were both reduced by CMZ treatment. Thus, it is possible that the mechanism of CMZ amelioration of the liver pathology caused by ethanol may involve the inhibition of enzyme catalysis *in vivo*. Supporting this conclusion is the observation made of these rats, and reported elsewhere (7), that CMZ treatment reduced the formation of autoantibodies to CYP2E1 in these rats. In the latter study, the antibody titers to CYP2E1 correlated positively ($P \leq 0.001$) with the severity of the liver pathology and with the CYP2E1 protein levels in the liver assayed by Western blot ($P \leq 0.002$) (7). One cannot exclude the possibility that the apoprotein level of CYP2E1 at the end of the experiment did not reflect the overall inhibition of CYP2E1 obtained throughout the treatment period. The results of the present study might support a clinical trial using CMZ when alcohol abusers with clinical alcoholic liver disease (ALD) cannot stop drinking.

The reason why CMZ reduced CYP2E1 activity *in vivo*, despite the ethanol-induced increase in apoenzyme of CYP2E1, was that CMZ inhibited CYP2E1 by binding at a site on the CYP2E1 molecule other than the enzymatic catalytic site, causing noncompetitive inhibition (1). The mechanism for the enzyme induction was by stabilization of the enzyme. This occurred as a result of ethanol binding, which inhibited the phosphorylation-ubiquitination-proteasome pathway (15, 16). Others have noted that CMZ also inhibits CYP2E1 gene transcription (17), but this was not studied in the present report. It is probably not important to the present study because the apoenzyme levels were the same in the ethanol-fed and the ethanol + CMZ–fed rats. The difference in the pathology scores in the two groups of rats is likely to be due to the inhibition of CYP2E1 by CMZ *in vivo*. This would account for the decreased CYP2E1 antibodies found in the blood of these rats (7). The antibodies are the result of ethanol-derived radical adducts (CYP2E1-hydroxyethyl radical adducts) (18) that are dependent on cytochrome P4502E1 for their formation (7). CMZ inhibition of CYP2E1 would also account for the prevention of the increased malondialdehyde in the livers of ethanol-fed rats.

In the present study, the *in vivo* metabolism of CHZ was studied while the rats were still metabolizing ethanol. This would be expected to reduce the CYP2E1 activity *in vivo* by noncompetitive inhibition (1). Thus the level of CYP2E1 activity measured *in vivo* would not reflect the several-fold induction of CYP2E1 apoprotein measured *in vitro*. Nevertheless, a reduction in the CYP2E1 activity measured *in vivo* was achieved by feeding CMZ. However, CYP2E1 inhibition by CMZ *in vivo* was much more dramatic in humans during alcohol withdrawal because ethanol had been eliminated as a noncompetitive inhibitor (1).

Previously, using the intragastric ethanol feeding rat model of ALD, ethanol-induced inhibition in proteasome peptidase activity was found. However, the mechanism for this observation was not identified, except that phenethyl isocyanate, which inhibits CYP2E1 (2, 10), partially prevented the inhibition of proteasomal proteolytic enzymes by ethanol after 1 month of ethanol feeding (19). In the current studies, CMZ inhibited *in vivo* CYP2E1 activity and ameliorated the liver pathology induced by ethanol. Whether the reduction of the ethanol-induced proteasome inhibition by CMZ is linked to the amelioration of the liver injury by CMZ will require further investigation. Since the liver weight increase caused by ethanol is probably due to the fatty changes and liver cell protein accumulation (14), an inhibition of liver weight gain might be anticipated in the ethanol-fed rats fed CMZ. However, CMZ decreased both the liver fat and the activity of the proteasome in the ethanol-fed rats. Thus, the weights of the livers in the rats fed ethanol were the same as for the rats fed ethanol and CMZ. This happened because the gain in protein and loss of fat balanced each other out. There was no net weight gain or loss. Protein retention in the liver cell cytoplasm, caused by the inhibition of the proteasomal peptidase activity, could cause liver injury by causing cell swelling. Consequently, impediment of sinusoidal blood flow would occur.

In conclusion, CMZ was found to ameliorate the pathologic changes induced by ethanol feeding. Most importantly, CMZ completely prevented ethanol-induced fibrosis. The mechanism by which CMZ reduced the pathology score was most likely through the inhibition of CYP2E1 activity *in vivo*. This conclusion was supported by the fact that lipid peroxidation was prevented by CMZ because the malondialdehyde level was not increased by ethanol when CMZ was fed with the ethanol. The results indicated that CMZ protected the liver from free radical damage by inhibiting CYP2E1 activity. CMZ also reduced the negative impact of ethanol on the function of proteasome peptidases. This suggests that the proteasome is vulnerable to free radical damage caused by CYP2E1, induced by ethanol. CMZ also inhibited anti-CYP2E1 auto antibody formation. In the future, CMZ may prove to be a useful drug in the treatment of liver damage in which CYP2E1 overactivity was involved in the mechanism of the liver injury.

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