Ethanol and food deprivation induced enhancement of hepatotoxicity in rats given carbon tetrachloride at low concentration

H Ikatsu, T Okino, T Nakajima

Abstract

Effects of chronic ethanol consumption and one day food deprivation on the hepatotoxicity of low dose carbon tetrachloride (CCl_4 ; 0 to 100 ppm inhalation for eight hours) in rats were investigated by using biochemical and histopathological methods. Liver malondialdehyde (MDA) contents were significantly increased by exposure to 5 ppm to 50 ppm CCl_4 in ethanol treated rats or by exposure to 25 ppm to 50 ppm CCl_4 in food deprived rats but not in rats without ethanol or food deprivation. The MDA concentrations reached a maximum at 10 ppm and 50 ppm CCl_4 in ethanol treated and food deprived rats, respectively, and decreased to the non-exposed concentration at 100 ppm CCl_4 . At ≥ 50 ppm CCl_4 plasma MDA contents increased significantly only in ethanol treated rats. None of the exposure concentrations influenced plasma glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities in rats that were only exposed to CCl_4 whereas exposure to 10 ppm or higher concentrations combined with ethanol increased both activities. To a lesser extent food deprivation combined with exposure to ≥ 25 ppm CCl_4 had the same effect. No histopathological changes were found in the liver of rats exposed to ≤ 10 ppm CCl_4 , and only a few ballooned hepatocytes were seen in centrilobular areas when exposure was 25 ppm or higher. The presence of ballooned hepatocytes became a regular feature of mid-zonal areas in ethanol treated rats and in the centrilobular areas of food deprived rats after exposure to ≤ 10 and ≤ 25 ppm CCl_4 , respectively. Necrotic hepatocytes were seen in centrilobular areas in liver from ethanol treated and food deprived rats when exposure to CCl_4 was ≥ 25 ppm and

≥ 50 ppm respectively. These results indicate that consumption of ethanol and food deprivation potentiate CCl_4 induced hepatic damage even at low concentrations of CCl_4 by promoting lipid peroxidation. Thus heavy drinking may be a risk factor for CCl_4 induced hepatic damage even though the CCl_4 concentration is as low as the threshold limit value.

Carbon tetrachloride (CCl_4), which was an extensively used industrial solvent has been progressively replaced by solvents such as trichloroethylene and 1,1,1-trichloroethane, which are considered to have lower hepatotoxicity or carcinogenicity.¹⁻³ Thus the number of industrial workers exposed to CCl_4 is decreasing. On the other hand, the exposure of workers to CCl_4 or chloroform is relatively high when they are engaged in the treatment of chemical waste.⁴

It is widely accepted that CCl_4 is metabolically activated by hepatic microsomal cytochrome P-450 mediated reactions to the trichloromethyl radical.⁵⁻⁷ Several different forms of cytochrome P-450 have been isolated and characterised.^{8,9} One of the P-450 isozymes, P-450j (P-450 IIE1) induced by ethanol or food deprivation,^{10,11} contributes to the metabolism of CCl_4 ¹² and other solvents.¹³⁻¹⁵ Therefore, it is of interest to know whether consumption of ethanol and food deprivation potentiate the hepatotoxicity at threshold limit values (TLVs) or lower of CCl_4 .

Threshold limit values of CCl_4 provided by the American Conference of Governmental Industrial Hygienists (ACGIH) and by the Japan Association of Industrial Health are 5 ppm and 10 ppm respectively.^{16,17} It is difficult to extrapolate the dose-response relation in CCl_4 induced hepatotoxicity from the high concentrations used in animal studies to the low concentrations generally encountered by man in the workplace.¹⁸ Therefore, the dose-response relation of inhalation exposure at low concentration must be investigated when trying to determine a TLV of exposure concentration for man. In the present study, we investigated the dose-

response relations of CCl_4 induced hepatic damage in chronic ethanol treated and one day food deprived rats with biochemical and histopathological methods.

Materials and methods

ANIMALS AND DIETS

Male Wistar rats six weeks old were purchased from Nippon SLC Co, Shizuoka, Japan. The animals were housed individually in stainless steel cages. The environmental temperature was kept at $20 \pm 2^\circ\text{C}$ with an alternating 12 hour light dark cycle. After being placed on a commercial solid diet (Clea CE-2, Nippon Clea, Tokyo) and water for two weeks, rats were switched to liquid diet.

Eighty rats were divided into three groups: two groups were fed 80 ml of basal diet and the third group 80 ml of ethanol diet daily at 1600 for three weeks. The basal diet, prepared according to the recipe of DeCarli and Liever,¹⁹ contained 3.47 g casein, 12.0 g sucrose, 41 mg L-cystine, 25 mg DL-methionine, 2 g oil (olive:corn = 4:1), 212 mg ethyl linoleate, 407 mg vitamin mixture, 2 mg DL- α -tocopherol, 813 mg mineral mixture and 212 mg carrageenin per 80 ml. The ethanol diet was made by adding 2.0 g of ethanol to 80 ml of basal diet and reducing sucrose content to 4.0 g. Half of the rats on the basal diet, which formed the food deprived group, received only water on the day before exposure to CCl_4 .

DOSE-RESPONSE EXPERIMENT WITH CCl_4

Rats of all three groups were exposed to 0 ppm, 5 ppm, 10 ppm, 25 ppm, 50 ppm, or 100 ppm CCl_4 for

eight hours (800–1600) in a dynamic air flow chamber (volume 360 l). Carbon tetrachloride was introduced quantitatively to the chamber with a microfeeder (KN model, Natsume Seisakusho, Tokyo). Concentration in the chamber was monitored every 15–30 minutes by gas chromatography with a flame ionisation detector (163 type, Hitachi, Tokyo). From the end of CCl_4 exposure to killing none of the animals were given food, but water was available.

THE EFFECT OF AMOUNT OF ETHANOL ON CCl_4 HEPATOTOXICITY

Liquid diets containing differing amounts of ethanol were freshly made by adding 0, 1.0, 1.5 or 2 g ethanol to 80 ml basal diet. Sixteen male rats were divided into four equal groups, each group being fed a different ethanol base (80 ml dosed diet for each rat) for three weeks. After three weeks rats were exposed to 10 ppm CCl_4 for eight hours (800–1600) according to the method described above.

BIOCHEMICAL DETERMINATIONS

All rats were killed by decapitation 22 hours after ending exposure to CCl_4 . Blood was collected and liver, kidneys, testicles, and lungs were taken. A part of the liver was cut off for histopathological examination. The remaining liver, perfused with cold 1.15% KCl via the portal vein, and other organs without perfusion were homogenised in ice cold 1.15% KCl solution with a Potter-Elvehjem homogeniser. Lipid peroxide concentration in each tissue was measured indirectly by determining thiobarbituric acid (TBA)-positive reactants according to the method of

Table 1 Effect of exposure to CCl_4 on plasma transaminase activity and MDA concentration

Treatment	CCl_4 (ppm)	Treatment before killing	GOT (IU/l)	GPT (IU/l)	Liver MDA (nmol/g liver)	Plasma MDA (nmol/ml)
None	0	A	88 (12)*	44 (6)	105 (9)	4.3 (0.9)
	0	B	61 (5)	46 (6)	209 (43)	4.0 (0.3)
	5	B	67 (13)	45 (10)	205 (28)	4.2 (0.3)
	10	B	89 (20)	48 (4)	241 (9)	3.5 (0.3)
	25	B	92 (10)	48 (4)	265 (71)	3.5 (0.5)
	50	B	99 (14)	47 (3)	242 (55)	4.3 (0.7)
	100	B	85 (23)	43 (8)	207 (87)	4.5 (1.3)
Three weeks ethanol treatment	0	A	95 (13)	59 (8)	293 (20)	4.0 (0.4)
	0	B	99 (24)	41 (5)	302 (29)	2.8 (0.4)
	5	B	99 (16)	52 (5)	391 (12)†	3.1 (0.3)
	10	B	253 (135)	418 (289)†	519 (98)†	2.9 (0.2)
	25	B	3520 (2060)†	4680 (2180)†	438 (35)†	3.8 (0.5)
	50	B	13500 (4970)†	16100 (5150)†	356 (19)†	8.2 (1.2)†
	100	B	8750 (1060)†	12400 (1420)†	222 (17)‡	6.3 (0.6)†
One day food deprivation	0	B	70 (12)	41 (7)	361 (34)	3.4 (0.2)
	5	B	69 (10)	37 (5)	346 (42)	3.4 (0.3)
	10	B	90 (15)	44 (8)	379 (44)	3.5 (0.7)
	25	B	203 (112)	220 (217)	484 (41)†	2.3 (0.1)
	50	B	1040 (841)	1610 (1370)	592 (81)†	3.3 (0.3)
	100	B	1550 (1150)†	2780 (2030)†	387 (20)‡	4.1 (1.3)

A—Rats were given daily diet until death. B—Rats were given only water in place of daily diet on the day of exposure to CCl_4 .

*Values are mean (SD) for four rats.

†Significantly different from corresponding non-exposed rats ($p < 0.05$).

‡Significantly different from corresponding rats exposed to 50 ppm CCl_4 ($p < 0.05$).

Uchiyama and Mihara²⁰ and determined in plasma by the method of Jose and Slater.²¹ The content of TBA reactants was expressed in terms of malondialdehyde (MDA) concentration using 1,1,3,3-tetraethoxypropane as a standard.²² Activities of plasma glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were determined enzymatically by measuring the rate of NADH disappearance using Boehringer Mannheim kits. Triglyceride was determined by the acetylacetone method using Wako Pure Chemical kits (Wako Pure Chemicals, Osaka, Japan).

HISTOPATHOLOGY

For histopathological examinations, liver was fixed in 10% formalin buffer solution (pH 7.4), and sections were stained with haematoxylin-eosin.

Quantitative analysis of hepatic damage (necrosis and ballooning degeneration of hepatocytes) induced by CCl₄ was performed by the method of Mitchell *et al.*²³ and Hewitt *et al.*²⁴ Ten hexagonal lobules were chosen randomly for each liver section. The distance from the central vein to the periportal area was divided into three parts per lobule to define arbitrarily the centrilobular, mid-zonal, and periportal areas. Scanning was performed in each area per lobule. In each field, the tissue element immediately beneath each of the 16 points of reference was termed a "hit"; thus 16 hits were examined per field. A total of 480 hits (160 hits in each area of the lobule) was examined in each section. The hits were categorised as (1) normal parenchymal hepatocyte; (2) ballooned parenchymal hepatocyte; (3) necrotic parenchymal hepatocyte; and (4) other cellular structures. Hits on

each of the first three categories were recorded and expressed as a percentage of the total number of hepatocytes examined in that area.

STATISTICAL ANALYSIS

Statistical analysis was by analysis of variance. When significant differences were found among groups, means were tested by Student's *t* test. The 0.05 level of probability was used as the criterion of significance. A logarithmic transformation was applied to plasma GOT and GPT activities before statistical analysis.

Results

PLASMA TRANSAMINASE ACTIVITY

Carbon tetrachloride did not influence the plasma GOT and GPT activities in non-treated rats at any concentration, but increased both the activities at 10 ppm or higher in ethanol treated rats, and at 25 ppm or higher in one day food deprived rats (table 1).

LIPID PEROXIDE IN LIVER AND PLASMA

Chronic ethanol treatment and one day food deprivation increased liver MDA content by threefold and twofold respectively compared with non-treated rats. One-day food deprivation of ethanol treated rats, however, did not cause further increases in concentrations of liver MDA (table 1).

Carbon tetrachloride did not affect MDA concentrations in kidneys, lungs, and testicles of any rat (data not shown), or in the liver of rats without additional treatment. In ethanol treated rats, the liver MDA concentration was significantly increased at CCl₄ concentrations of 5 to 50 ppm but not at a

Table 2 Effect of exposure to CCl₄ on liver triglyceride concentration and ratio of liver to body weight

Treatment	CCl ₄ (ppm)	Treatment before killing	Triglyceride (mg/g liver)	Liver:body weight (w/w (%))	Body weight (g)
None	0	A	22.8 (3.4)*	4.05 (0.13)	291 (10)
	0	B	9.3 (1.0)	3.14 (0.13)	280 (7)
	5	B	10.6 (1.6)	3.36 (0.08)	300 (16)
	10	B	12.2 (0.9)†	3.22 (0.09)	274 (7)
	25	B	18.5 (4.4)†	3.24 (0.08)	277 (9)
	50	B	22.7 (3.5)†	3.25 (0.07)	267 (5)
	100	B	44.5 (6.6)†	3.29 (0.10)	266 (13)
Three weeks ethanol treatment	0	A	11.5 (1.1)	3.58 (0.17)	247 (14)
	0	B	6.4 (1.1)	2.93 (0.03)	236 (7)
	5	B	6.8 (0.4)	3.01 (0.09)	282 (8)
	10	B	12.9 (3.4)†	3.15 (0.17)	233 (7)
	25	B	14.6 (2.5)†	3.52 (0.08)†	229 (11)
	50	B	20.3 (3.2)†	3.83 (0.13)†	219 (15)
	100	B	24.8 (1.5)†	3.80 (0.13)†	217 (11)
One day food deprivation	0	B	18.1 (1.4)	2.51 (0.04)	274 (13)
	5	B	19.6 (1.5)	2.79 (0.09)†	259 (10)
	10	B	25.7 (2.2)†	2.78 (0.06)†	271 (6)
	25	B	31.1 (1.7)†	3.01 (0.11)†	257 (7)
	50	B	17.9 (4.3)	3.12 (0.22)†	256 (18)
	100	B	24.6 (1.8)†	3.14 (0.30)†	235 (13)

For explanations, see footnote to table 1.

concentration of 100 ppm. The increase reached a maximum at 10 ppm CCl_4 . In one day food deprived rats, no significant increase in concentration of liver MDA was found up to 25 ppm CCl_4 , whereas it increased liver MDA concentration at 25 and 50 ppm but not at 100 ppm. These results indicate that ethanol treatment and one day food deprivation enhanced the hepatic accumulation of lipid peroxides at low concentrations of CCl_4 .

Plasma MDA concentrations were raised only in ethanol treated rats exposed to 50 and 100 ppm CCl_4 (table 1).

RATIO OF LIVER TO BODY WEIGHT AND LIVER TRIGLYCERIDE

The ratio of liver to body weight increased with increasing exposure concentration of CCl_4 in ethanol treated and one day food deprived rats, and was also increased, but to a lesser degree, in non-treated rats (table 2).

Liver triglyceride concentrations were significantly increased at 10 ppm or higher concentration of CCl_4 in all rats (table 2).

HISTOPATHOLOGICAL FINDINGS

No alterations were found in the livers of non-treated rats exposed to 5 and 10 ppm CCl_4 , whereas fat deposits (data not shown) were found after exposure to 25, 50, and 100 ppm CCl_4 . Negligible ballooned hepatocytes and necrotic hepatocytes were seen in the livers of non-treated rats exposed to ≥ 25 ppm CCl_4 and of ethanol treated, and food deprived rats exposed to 5 ppm CCl_4 (table 3). Ballooned hepatocytes were mainly seen in mid-zonal areas when ethanol treatment was combined with exposure to 10 ppm CCl_4 and in centrilobular areas when food deprivation was combined with exposures to 10 and 25 ppm CCl_4 . Necrotic hepatocytes were found in centrilobular areas of ethanol treated rats exposed to more than 25 ppm CCl_4 and of food deprived rats exposed to more than 50 ppm CCl_4 . Only a few ballooned and necrotic hepatocytes were seen in periportal areas of both ethanol treated and food deprived rats after 10 ppm or higher exposure to CCl_4 (table 3).

The figure shows photomicrographs of liver sections from ethanol treated rats (figures A and B) and one day food deprived rats (figures C and D). Livers from ethanol treated rats showed scattered ballooning degeneration in mid-zonal areas at exposure to 10 ppm CCl_4 (figure A) whereas necrotic hepatocytes surrounded with ballooned hepatocytes in centrilobular areas were seen on exposure to 25 ppm CCl_4 (figure B). Liver from one day food deprived rats showed ballooning degeneration mainly in centrilobular areas on exposure to 25 ppm CCl_4 (figure C) and necrotic hepatocytes were also found in centrilobular areas with ballooned

hepatocytes on exposure to 50 ppm CCl_4 (figure D).

ETHANOL CONSUMPTION AND CCl_4 INDUCED HEPATOXICITY

Table 4 presents the results. Plasma GOT and GPT activities were unchanged in rats treated with 0, 0.5, and 1.0 g ethanol, but increased in rats treated with 2.0 g ethanol.

Ethanol treatment itself increased liver MDA concentration at the 1.0 or 2.0 g dose. Carbon tetrachloride exposure raised liver MDA concentration only in rats given 2.0 g ethanol.

Discussion

A considerable amount of research on CCl_4 induced hepatic damage has been conducted.¹⁻³ Adams *et al* investigated species differences in the hepatotoxicity of CCl_4 and reported that no detectable injury was noticed in rat liver exposed to 50 ppm CCl_4 for seven hours.²⁵ Uemitsu *et al* proposed that the no effect level of CCl_4 on GOT activity is 500 ppm CCl_4 .²⁶ In the present study, plasma GOT and GPT activities, and liver MDA contents were not changed by exposure to 100 ppm CCl_4 for eight hours in non-treated rats. The accumulation of triglycerides and fat deposits were seen, however, after exposure to 25 ppm CCl_4 .

Consumption of ethanol and food deprivation potentiated CCl_4 induced hepatotoxicity.²⁷⁻³⁰ Our study shows that transaminase activities increased at CCl_4 concentrations of 10 ppm or higher in ethanol treated rats and of 25 ppm or higher in one day food deprived rats. Liver MDA concentrations had increased already at 5 ppm CCl_4 in ethanol treated rats and at 25 ppm in one day food deprived rats. In agreement with these biochemical findings, significant histopathological changes were seen in both ethanol treated and one day food deprived rats at exposures of 10 ppm and 25 ppm CCl_4 , respectively. These results indicate that both consumption of ethanol and deprivation of food potentiate CCl_4 induced hepatotoxicity at low CCl_4 concentrations: ethanol potentiates the hepatotoxicity at CCl_4 concentrations as low as 5 ppm and 10 ppm. These are the proposed TLV concentrations in the United States and Japan.^{16,17} This indicates that heavy drinkers are at risk when exposed to CCl_4 at TLV concentrations.

Workers engaged in treatment of waste water were found to be exposed to comparatively high concentration of CCl_4 (up to 39.3 ppm) or chloroform (up to 45.2 ppm) when handling organic solvents.⁴ Thus these workers should be no more than temperate drinkers to avoid becoming intoxicated by CCl_4 .

Lipid peroxide concentrations were estimated by MDA formation.^{31,32} A poor correlation between liver MDA concentrations and plasma transaminase activities in CCl_4 exposed rats was found. Liver MDA concentration increased linearly with plasma

Table 3 Histological evaluation of the effects of exposure to CCl_4 on liver

Treatment	CCl_4 (ppm)	Centrilobular areas		
		No*	BH† (%)	NH‡ (%)
None	5	153 (1)	0	0
	10	152 (2)	0	0
	25	154 (2)	1.0 (1.0)	0
	50	152 (3)	0.7 (0.5)	0
	100	149 (2)	0.2 (0.3)	0
Three weeks ethanol treatment	5	152 (2)	0.5 (0.5)	0
	10	153 (2)	11.2 (7.0)	0.7 (1.1)
	25	155 (1)	15.8 (7.0)	75.0 (7.2)
	50	153 (2)	5.6 (3.5)	83.9 (10.8)
	100	154 (2)	3.0 (0.6)	94.1 (3.5)
One day food deprivation	5	153 (1)	1.1 (1.0)	0
	10	148 (6)	14.9 (4.7)	0
	25	150 (5)	35.0 (10.1)	8.0 (7.0)
	50	152 (3)	21.5 (7.9)	45.7 (31.5)
	100	151 (2)	18.6 (11.8)	72.8 (14.0)

*Values are Nos of normal, ballooned, and necrotic hepatocytes among 160 hits of areas and shown as mean (SD) for four rats.

†Ballooned hepatocytes (BH); values are % of Nos of hepatocytes.

‡Necrotic hepatocytes (NH); values are % of Nos of hepatocytes.

GPT activity up to 1000 IU/l, but not further. The MDA concentration ceased to increase when exposure reached concentrations of 10 and 25 ppm CCl_4 in ethanol treated rats, and 25 and 50 ppm in one day food deprived rats—that is, when hepatic cells changed from ballooning to necrosis. The basis for this discrepancy is not clear. Plasma MDA concentration tended to rise after liver MDA concentration reached a maximum or began to decrease (table 1), suggesting that liver MDA may leak into the blood from necrotic hepatic cells, in agreement with the view of Harris *et al.*³³

As ethanol consumption and food deprivation induce P-450 IIE1, which contributes to CCl_4 bioactivation,¹⁰⁻¹⁵ these treatments seem to increase the rate of lipid peroxidation. On the other hand, both consumption of ethanol and food deprivation decreased liver GSH concentration, and therefore impaired the scavenging of toxic free radicals.³⁴⁻³⁶ Thus the mechanism whereby consumption of ethanol and food deprivation potentiate CCl_4 hepatotoxicity can be an increase in the generation of radical metabolites or a decrease in the GSH concentration of liver, or both. Although the concentration of GSH in liver in one day food deprived rats (1.05 (SD 0.11) mg/g liver) was less than that in ethanol treated rats (1.56 (SD 0.16) mg/g liver) in the present study, the effect of ethanol on CCl_4 hepatotoxicity was more prominent than that of one day food deprivation. This indicates that lipid peroxidation and the metabolic activation of CCl_4 were more important than detoxication by GSH.

In recent years physiologically based pharmaco-

kinetic models for inhaled CCl_4 have been developed.^{37,38} Uemitsu reported that CCl_4 metabolism in vivo was perfusion limited at inhaled concentrations below around 100 ppm and was saturated at inhaled concentrations above 100 ppm in naive rats.³⁷ This means that the metabolism of CCl_4 is not rate limited by a Michaelis-Menten mechanism, but by perfusion. In the present study, however, the CCl_4 hepatotoxicity was potentiated at CCl_4 concentrations below 25 ppm by chronic ethanol treatment or one day food deprivation. Moreover, the degree of hepatic injury caused by CCl_4 was more severe in ethanol treated rats than in one day food deprived rats. These results suggest that the metabolism of CCl_4 may be controlled by a metabolic step at a concentration as low as 25 ppm.

It is of practical significance that information is obtained through animal studies of low dose inhalation exposure to CCl_4 . With the help of physiologically based pharmacokinetic models animal data can be scaled to estimate human response.^{39,40}

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Requests for reprints to: Hisayoshi Ikatsu, Department of Hygiene, Shinshu University School of Medicine, Asahi 3-1-1, Matsumoto-shi 390, Japan.

Mid-zonal areas			Periportal areas		
No	BH (%)	NH (%)	No	BH (%)	NH (%)
153 (3)	0	0	152 (2)	0	0
153 (3)	0	0	153 (2)	0	0
152 (2)	0	0	152 (3)	0	0
152 (3)	0	0	152 (2)	0	0
151 (1)	0	0	153 (2)	0	0
152 (3)	0	0	152 (2)	0	0
155 (3)	31.5 (13.0)	0	153 (2)	4.9 (1.9)	0
153 (3)	15.7 (5.9)	29.7 (1.3)	152 (2)	1.3 (0.0)	1.8 (0.5)
154 (1)	6.5 (1.9)	39.2 (16.6)	153 (1)	3.3 (0.7)	2.9 (1.4)
151 (4)	5.6 (1.2)	56.3 (7.7)	150 (2)	2.0 (1.5)	11.7 (5.9)
152 (2)	0	0	153 (2)	0	0
151 (4)	5.4 (3.7)	0	152 (2)	0.7 (0.7)	0
151 (2)	7.9 (2.0)	0.7 (1.1)	153 (3)	0.7 (0.7)	0
153 (2)	8.2 (3.0)	18.8 (16.1)	149 (2)	2.4 (3.3)	1.5 (2.6)
148 (5)	10.5 (6.6)	36.1 (14.8)	149 (3)	3.7 (1.1)	3.4 (1.5)

Figure Hepatic histopathological appearance ($\times 33$) after exposure to CCl_4 . (A) Liver injury in an ethanol treated rat at exposure to 10 ppm CCl_4 (GPT 338 IU/l). Ballooned hepatocytes are seen in mid-zonal areas. (B) Liver injury in an ethanol treated rat at exposure to 25 ppm CCl_4 (GPT 1960 IU/l). Necrotic hepatocytes surrounded with ballooned hepatocytes in centrilobular areas are seen. (C) Liver injury in a one day food deprived rat exposed to 25 ppm CCl_4 (GPT 330 IU/l). Ballooned hepatocytes occur frequently in centrilobular areas. (D) Liver injury in a one day food deprived rat exposed to 50 ppm CCl_4 (GPT 1710 IU/l). Necrotic hepatocytes with ballooned hepatocytes in centrilobular areas are seen. C = Central vein, P = portal triad.

Table 4 Effect of three weeks ethanol consumption on plasma transaminase activity and liver MDA concentration after exposure to CCl_4

Ethanol intake (g/day)	CCl_4 (ppm)	GOT (IU/l)	GPT (IU/l)	Liver MDA (nmol/g liver)
0	0	47 (5)*	21 (1)	170 (6)
	10	76 (15)†	26 (4)	258 (58)†
0.5	0	59 (14)	27 (7)	173 (27)
	10	65 (5)	24 (2)	221 (53)
1.0	0	55 (5)	24 (4)	240 (9)†
	10	72 (9)†	31 (2)	265 (44)
2.0	0	99 (24)†	41 (5)†	253 (30)†
	10	253 (135)†	418 (289)††	519 (98)††

* †See footnotes in table 1.

†Significantly different from no ethanol group ($p < 0.05$).

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