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EFFECT OF PHENOBARBITAL AND 3-METHYLCHOLANTHRENE PRETREATMENT ON THE HEPATOTOXICITY of 1,1,1-TRICHLOROETHANE AND 1,1,2-TRICHLOROETHANE

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Summary

Pretreatment of rats with phenobarbital potentiated the hepatotoxicity of both 1,1,1- and 1,1,2-trichloroethane given by inhalation. The toxicity of the 1,1,2- isomer was increased to a greater extent than that of the 1,1,1- isomer. 3-Methylcholanthrene pretreatment did not result in increased hepatotoxicity.

Introduction

The two isomers of trichloroethane, 1,1,1- (which is methylchloroform) and 1,1,2-, are important as substitutes for carbon tetrachloride. They are used as solvents, degreasers and intermediates in chemical synthesis. The former is now often encountered in dry cleaning preparations, and the latter is very important industrially (1). The 1,1,1- isomer is markedly less toxic than the 1,1,2- isomer (2).

Garner and McLean (3) demonstrated that pretreatment with the enzyme inducing agent phenobarbital potentiated the hepatotoxicity of CCl_4 when given po. Its toxicity was also potentiated when inhaled (4). 3-Methylcholanthrene, which is also an enzyme inducer but differs in its mechanism and spectrum of enzymes induced, has been shown to protect against CCl_4 hepatotoxicity (5, 6). It was of interest, therefore, to ascertain what effect, if any, pretreatment with these agents would have on the hepatotoxicity of 1,1,1- and 1,1,2-trichloroethane.

Materials and Methods

Adult male albino rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) were injected intraperitoneally with either phenobarbital (PB) at a dose of 50 mg/kg/day for 4 days or 3-methylcholanthrene (MC) at a dose of 40 mg/kg/day for 2 days or saline or corn oil vehicle as respective controls. At 24 hr after the last dose of PB or 48 hr after the last dose of MC, the animals were exposed to either 1,1,1-trichloroethane (1,1,1-TCE) or 1,1,2-trichloroethane (1,1,2-TCE) vapors in a dynamic inhalation chamber previously described (7) for a period of 2 hr. Chamber concentrations were determined using a Packard gas chromatograph equipped with flame ionization detector.

Twenty-two hr after the termination of exposure, the animals were lightly anesthetized with ether and tail vein blood samples were obtained. Liver and body weights were recorded. Hepatotoxicity was assessed by measuring serum glutamic oxalacetic (SGOT) and glutamic pyruvic (SGPT) transaminases by the method of Reitman and Frankel (8) and liver glucose-6-phosphatase according to Harper (9) using maleate buffer, pH 6.25.

The value expressed is the mean ± standard error for a group of 5 animals unless otherwise noted. A two-way analysis of variance technique was used to test the differences between pretreatments, inhalation of air versus TCE, and the interaction between the two. The F values are presented for the interactions. The level of significance was set at 0.05. Where animals received the same pretreatment, means were compared using the least significant difference (lsd) test (10).

Results

The data in Table 1 indicate that there is an effect of phenobarbital pretreatment on both 1,1,2-TCE and 1,1,1-TCE hepatotoxicity. In the case of the 1,1,2-TCE, no hepatotoxicity was noted in the saline pretreated animals under the conditions of exposure employed (890 ppm for 2 hr) using the parameters indicated. There was a decrease in glucose-6-phosphatase and 20-fold elevations in the transaminases in the PB pretreated animals. These values are indicative

of a fairly severe degree of hepatotoxicity.

TABLE 1

Effect of Phenobarbital Pretreatment on 1,1,2-Trichloroethane and 1,1,1-Trichloroethane Hepatotoxicity

Treatment	Liver Wt / 100 g B. Wt.	Glucose-6-Phosphatase[a]	SGPT[b]	SGOT[b]
Saline-Air	4.3 ± 0.09	10.3 ± 0.40	19 ± 1.5	73 ± 3.3
Saline-1,1,2-TCE[c]	4.7 ± 0.17	10.3 ± 0.69	20 ± 1.7	91 ± 7.3
PB-Air	5.1 ± 0.19	9.7 ± 0.36	19 ± 1.8	75 ± 4.9
PB-1,1,2-TCE[c,f]	5.5 ± 0.14[g]	8.2 ± 0.36[h]	465 ± 156.5[h]	1345 ± 187.6[h]
F[d]	1.65	2.42	11.89[i]	65.16[i]
Saline-Air	4.1 ± 0.17	13.8 ± 0.85	13 ± 1.0	44 ± 5.3
Saline-1,1,1-TCE[e]	4.3 ± 0.20	13.2 ± 0.99	17 ± 2.2	59 ± 4.6
PB-Air	4.5 ± 0.08	15.8 ± 0.56	16 ± 1.2	48 ± 2.9
PB-1,1,1-TCE[e]	5.2 ± 0.09[h]	11.3 ± 0.98[h]	95 ± 7.9[h]	178 ± 24.8[h]
F[d]	2.31	5.04[i]	79.91[i]	19.36[i]

[a] µmoles PO_4/g/min

[b] Reitman-Frankel Units

[c] 890 ppm for 2 hr

[d] computed for interaction between pretreatment and inhalation

[e] 11,600 ppm for 2 hr

[f] mean of 4 animals

[g] mean of 3 animals

[h] $P<0.05$ compared to animals with same pretreatment

[i] $P<0.05$

Exposure to 1,1,1-TCE gave results qualitatively similar to the 1,1,2-TCE exposure. The rats were able to tolerate higher concentrations without mortality. The group pretreated with PB and exposed to 1,1,1-TCE had a higher liver to body weight ratio, decreased glucose-6-phosphatase activity, a 6-fold elevation in SGPT and a nearly 4-fold increase in SGOT compared to the air exposed

group.

When the inducing agent was MC (Table 2), there were few indications of either potentiation or protection. Only very minor changes were seen in the parameters measured as a result of the 1,1,2-TCE pretreatment. In the case of the liver to body weight ratio, there was a significant interaction resulting in enlarged livers in the MC pretreated animals inhaling 1,1,2-TCE. This group

TABLE 2

Effect of 3-Methylcholanthrene Pretreatment on 1,1,2-Trichloroethane and 1,1,1-Trichloroethane Hepatotoxicity

Treatment	Liver Wt. / 100 g B. Wt.	Glucose-6-Phosphatase[a]	SGPT[b]	SGOT[b]
Corn oil-Air	3.5 ± 0.11	12.6 ± 0.49	14 ± 1.0	50 ± 2.9
Corn oil-1,1,2-TCE[c,f]	3.4 ± 0.12	12.3 ± 1.25	33 ± 12.0[h]	93 ± 24.5
MC-Air	4.5 ± 0.08	12.7 ± 0.57	9 ± 0.9	54 ± 3.7
MC-1,1,2-TCE[c]	5.5 ± 0.16[h]	9.8 ± 0.71[h]	24 ± 5.6[h]	107 ± 34.5
F[d]	18.89[i]	2.98	0.39	0.06
Corn oil-Air	4.4 ± 0.19	17.2 ± 1.36	17 ± 1.2	54 ± 3.9
Corn oil-1,1,1-TCE[e]	4.4 ± 0.15	14.8 ± 0.46	19 ± 3.1	56 ± 4.8
MC-Air	6.0 ± 0.32	13.1 ± 1.18	18 ± 1.9[g]	73 ± 3.1[g]
MC-1,1,1-TCE[e]	5.5 ± 0.31	15.8 ± 0.66	15 ± 0.9	60 ± 5.5
F[d]	1.28	6.86[i]	2.00	2.70

[a] μmoles PO_4/g/min

[b] Reitman-Frankel Units

[c] 2080 ppm for 2 hr

[d] computed for interaction between pretreatment and inhalation

[e] 13,070 ppm for 2 hr

[f] one animal died overnight; blood samples were unobtainable from 2 others

[g] mean of 4 animals

[h] $P<0.05$ compared to animals with same pretreatment

[i] $P<0.05$

also showed a slight decrease in glucose-6-phosphatase activity although the F value indicates that this was not a significant interaction. The changes in the serum transaminases were minimal and were similar with both pretreatments. Higher concentrations of 1,1,2-TCE than the 2080 ppm used could not be employed because of a low survival rate among the animals with increased concentration. Even at the concentration used, one corn oil animal died overnight and two others were so moribund that tail vein blood samples were unobtainable.

Following 1,1,1-TCE exposure, there were no changes in either group which could be associated with the inhalation despite the high concentration (13,070 ppm) used. Although the changes in glucose-6-phosphatase activity due to inhalation were not statistically significant, the F value is significant since the changes occurred in opposite directions. In the case of 1,1,1-TCE exposure, therefore, it is not possible to show protection because of the lack of ability of the 1,1,1-TCE to cause significant hepatotoxicity.

Discussion

The lack of ability of 1,1,1- and 1,1,2-TCE inhalation to cause hepatotoxicity in the rats is not surprising. Platt and Cockrill (11) administered the compounds po for 7 days and found no increase in liver to body weight ratios or decrease in glucose-6-phosphatase. Klaassen and Plaa (12) administered the compounds ip and observed no decrease in glucose-6-phosphatase and no increase in lipid peroxidation. Because of the lack of liver damage in these present experiments, it could not be determined if MC had a protective effect. Mortality prohibited the use of higher concentrations. It can be concluded, however, that MC did not potentiate the hepatotoxicity of the trichloroethanes.

PB pretreatment did result in an enhancement of the hepatotoxicity of both compounds. In actuality, it might better be expressed that hepatotoxicity was manifested that otherwise would not have appeared. A much greater enhancement was seen with 1,1,2-TCE than with 1,1,1-TCE.

It is difficult to speculate how the potentiation occurs since the exact mechanism by which the chlorinated hydrocarbon solvents produce their hepato-

toxicity is unknown. Recknagel (13) suggests that the metabolism of CCl_4 results in trichloromethane free radicals which cause peroxidation of structural lipids and thus necrosis of hepatocytes. Garner and McLean (3) demonstrated that PB pretreatment results in an increased metabolism of CCl_4. Following the above hypothesis, this would be expected to result in the generation of more free radicals and consequently more lipid peroxidation and cell destruction. Van Dyke and Wineman (14) found that 1,1,1-TCE was dechlorinated in only trace amounts but that 1,1,2-TCE was a good substrate for dechlorination and the reaction could be induced by pretreatment with PB but not with MC. However, Klaassen and Plaa (11) were unable to detect any increase in lipid peroxidation in rats treated with 1,1,1-TCE or 1,1,2-TCE. Thus, it is important from both a practical and a theoretical viewpoint that PB will potentiate the toxicity of the trichloroethanes, but the molecular basis of this potentiation, and indeed the mechanism of hepatotoxicity of the agents themselves, remains unknown.

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