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EFFECTS OF DICHLOROMETHANE ON CARBON TETRACHLORIDE HEPATOTOXICITY IN RATS

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ABSTRACT: *A non-hepatotoxic dose of dichloromethane (DCM) was examined for potential effects on the hepatotoxicity of carbon tetrachloride (CT) in adult male rats. A concomitant treatment of DCM (0.45 ml / kg, po) significantly potentiated the hepatotoxicity of CT at varing doses (0.06 to 0.63 ml / kg, po) as determined by increases in SGOT and SGPT activities 24 hr following the treatments. The carboxyhemoglobin (COHb) saturation induced by DCM was significantly decreased by CT treatments. The potentiation of CT hepatotoxicity by DCM does not appear to be associated with increased metabolism of CT.*

Key words: *Dichloromethane, Carbon tetrachloride, Combination, Carboxyhemoglobin, Hepatotoxicity, Mixed-function oxidase*

INTRODUCTION

Carbon tetrachloride (CT) is a potent hepatotoxin. Its hepatotoxicity is mediated by trichloromethyl free radicals ($\bullet CCl_3$) generated during the metabolism of this solvent (Recknagel, 1967; Recknagel and Glende, 1973). Also mixed-function oxidase (MFO) activities and cytochrome P-450 contents are reduced by CT (Smuckler *et al.*, 1967; Glende, 1972; Head *et al.*, 1981).

Dichloromethane (DCM) is widely used in industry as a solvent, degreaser and extraction medium. Dichloromethane has physicochemical properties similar to CT but is not associated with liver and kidney injuries except at extremely high exposure levels (Moskowitz and Shapiro, 1944; Hanke *et al.*, 1974). The major toxicological hazard of DCM lies in the formation of carbon monoxide (CO) during its biotransformation (Stewart *et al.*, 1972; Rateney *et al.*, 1974; Peterson, 1978; Divincenzo and Kaplan, 1981). Kubic and Anders (1975) demonstrated that DCM was metabolized to CO and inorganic chloride by a rat liver microsomal fraction requiring NADPH and molecular

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oxygen.

Carbon tetrachloride and dichloromethane share some common uses in industry. A combined exposure of workers to CT and DCM may be a frequent occurrence. The present study was undertaken to evaluate the effect of DCM administration on the CT hepatotoxicity.

MATERIALS AND METHODS

Adult male Wistar rats (150-250g) were acclimated in environmentally controlled rooms (light: 0700-1900, dark: 1900-0700) at least two weeks prior to experimentation. Lab chow and tap water were allowed *ad libitum*.

Rats were treated with DCM at a dose of 0.23, 0.45 or 0.90 ml/kg and/or with CT at a dose of 0.06, 0.32 or 0.63 ml/kg orally. The vehicle used was corn oil. Control animals were treated with corn oil only.

The COHb level was measured using the spectrophotometric method of Rodkey *et al.* (1979). Activities of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruric transaminase (SGPT) were measured 24 hrs following the treatments using a clinical chemistry analyzer (Impact 400E, Gilford).

The results were analyzed by Student's *t*-test or two way analysis of variance.

RESULTS AND DISCUSSION

At the doses used in the present study DCM alone did not alter SGPT or SGOT activities (Table 1). However, combinations of DCM and CT treatments significantly elevated the parameters of hepatotoxicity compared to CT treatments alone.

The COHb levels plateaued 3 to 6 hr after the DCM treatment depending on the dose of DCM administered (Fig. 1). Increasing DCM doses failed to show correspon-

Table 1. Effect of dichloromethane and/or carbon tetrachloride on SGPT and SGOT activities in male rats[a]

Group	DCM	CT	SGOT (IU/L)	SGPT (IU/L)
I	—	—	35.4 ± 2.8	113.7 ± 12.1
II	0.45 ml/kg	—	37.0 ± 2.7	126.2 ± 29.6
III	—	0.06 ml/kg	156.1 ± 48.4[b]	896.7 ± 358.6[b]
IV	—	0.32 ml/kg	223.8 ± 51.7[b]	989.7 ± 285.0[b]
V	—	0.63 ml/kg	453.9 ± 86.4[b]	3264.8 ± 1163.3[b]
VI	0.45 ml/kg	0.06 ml/kg	609.4 ± 191.4[c]	4562.5 ± 1862.7[c]
VII	0.45 ml/kg	0.32 ml/kg	757.7 ± 129.9[c]	2916.0 ± 831.5[c]
VIII	0.45 ml/kg	0.63 ml/kg	2255.1 ± 550.8[c]	8786.3 ± 543.0[c]

[a]Dichloromethane and/or carbon tetrachloride were administered to rats orally. Control rats (Group I) were given corn oil only. Twenty four hr following the treatment SGPT and SGOT activities were determined. Each value represents the mean ± S.E. for 6 rats.

[b] The effect of CT treatment is significant (Students *t*-test, $p<0.01$).

[c] The synergistic interaction between DCM and CT is significant (two way ANOVA, $p<0.02$)

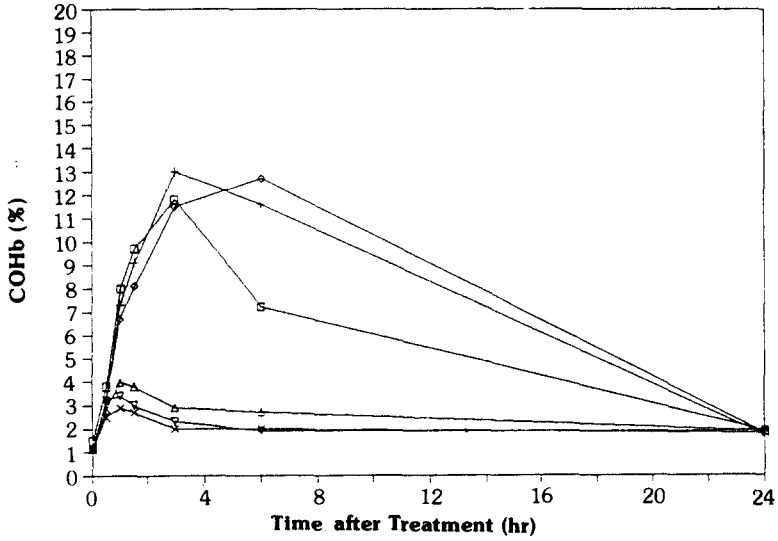

Fig. 1. Effect of CT on the DCM-induced COHb generation. Rats were treated orally with DCM at a dose of 0.23 ml/kg (□), 0.45 ml/kg (+), 0.90 ml/kg (◇), or with a combination of DCM (0.5 ml/kg) and CT at 0.06 ml/kg (△), 0.32 ml/kg (×) or 0.63 ml/kg (▽). Each value is the mean for 6 rats. Regardless of the dose used, COHb levels of the rats treated with CT only ranged 1 to 2% at all time points. These levels are not different from the COHb level in normal rats and omitted from the figure.

ding increases in the peak COHb level suggesting that the metabolism of DCM to CO was saturated. However, the COHb decline appeared to be dependent on the dose of DCM. These results are in good agreement with those observed by Kim and Carlson (1986) who used inhalation as the route of administration of DCM.

The hepatotoxicity of CT is known to be potentiated by many chemicals. Among these potentiators, CO is of special interest. It has been well-known that *in vitro* system CO binds to cytochrome P-450 and inhibits cytochrome P-450-dependent MFO activities. However, the effect of CO inhalation on the drug metabolizing activity *in vivo* is not well-understood. Suarez *et al.* (1972) reported that CO inhalation exposure significantly enhanced the increases in SGPT and SGOT activities induced by 1000 ppm CT exposure in rats. The authors hypothesized that an imbalance in the microsomal electron transport system resulted in a shunt of electrons into lipid peroxidation and subsquent celluar damage. On the other hand Pankow and Ponsold (1976) suggested that the enhanced drug metabolizing activity caused by hypoxic stress might be responsible for the increase in CT toxicity. However, CO exposure at lower concentrations (500 ppm or less) did not affect the drug metabolizing activity (Staland *et al.*, 1973; Kim and Carlson, 1983) and as the concentration increased the drug metabolizing activity was rather inhibited (Montgomery and Rubin, 1973; Roth and Rubin, 1976). Recently Trela *et al.* (1988) demonstrated that CO could inhibit aminopyrine metabolism in the isolated perfused rabbit lung, but the threshold of CO concentration in the ventilated air for this inhibition was 7.5% which is much higher than the level one could tolerate.

Shen *et al.* (1982) reported that exposure of rats to 5000 ppm CT in the presence of 12% oxygen resulted in a significant increase in SGPT activities compared to rats exposed to an identical concentration of CT in the presence of 21% oxygen. An increase

in conjugated dienes in microsomal lipids was minimal, but the covalent binding of CT metabolites to microsomal lipids/proteins was remarkably increased. The authors concluded that the increased covalent binding was responsible for the potentiation of liver toxicity.

The mechanism of potentiation of CT-induced hepatotoxicity by DCM is unknown. Decreases in COHb level in rats treated with both DCM and CT (Figure 1) appear to be caused by less metabolic degradation of DCM due to competition between the two solvents for the drug metabolizing enzymes and/or decreases in drug metabolizing activities resulting from CT-induced hepatotoxicity. Likewise, the metabolism of CT would be also inhibited due to the competition and/or the effect of CO, generated from DCM, on the cytochrome P-450. Therefore, increased metabolism of CT does not appear to be involved in the potentiation of its hepatotoxicity by DCM. An indirect effect of DCM, that is, generation of CO *in vivo* leading to tissue hypoxia may be responsible for the potentiation of CT hepatotoxicity.

The present study indicates that in order to protect the workers from chemical hazards in their workplaces properly as is suggested by ACGIH (1988), an additional safety factor should be considered for the combination effects of a chemical otherwise assumed to be relatively innocuous.

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