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KETONE POTENTIATION AND CHEMICAL TOXICITY

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INTRODUCTION

The toxicity of a large number of chemicals can be markedly influenced by preexposure or simultaneous exposure to other agents. In the occupational environment, this phenomenon is of particular interest because of the potential presence of chemical mixtures. Industrialized societies are now quite concerned about this situation and research in this area deserves a high priority.

A number of questions can be raised and they are not easily answered by treating the subject in its entirety. There are, however, several interesting model forms of toxicity that can be useful to elucidate the problems that can arise. The interaction between certain aliphatic ketones and some aliphatic halogenated hydrocarbons, leading to an exaggerated hepatotoxic response, is such a model system and is the subject of this presentation.

POTENTIATION OF CARBON TETRACHLORIDE LIVER INJURY BY KETONES

The discovery that certain aliphatic ketones could potentiate hepatotoxic effects of other agents occurred quite by accident. It arose from a series of studies designed to explain why the aliphatic alcohol isopropanol appeared to be a more potent potentiator of carbon tetrachloride hepatotoxicity than ethanol (1). In a series of studies (1-5) we demonstrated that isopropanol itself was not the potentiating agent, but that its major metabolite, acetone, was most likely responsible for the enhanced hepatotoxicity when given in combination with CCl_4; in these experiments we used biochemical indices of liver injury as well as a morphological assessment of the histological changes occurring with the various treatment schedules. We found that the biochemical indices, which are more easily quantified, reliably portrayed the histological observations.

Table 1 illustrates the potentiation of CCl_4 liver injury in the rat as measured by two biochemical indices, elevation of plasma alanine aminotransferase (ALT) activity and accumulation of hepatic triglycerides. Figure 1 is a summary of experiments designed to show that acetone and not isopropanol is mainly responsible for the marked potentiation. In this study

TABLE 1
POTENTIATION OF CCl_4 HEPATOTOXICITY BY ISOPROPANOL AND ACETONE

Treatment	Plasma ALT Activity (units/mL)	Hepatic Triglycerides (mg/g liver)
Control	71	10.3
CCl_4	84	13.1
Isopropanol + CCl_4	3598	19.7
Control	54	8.8
CCl_4	52	8.9
Acetone + CCl_4	1410	21.1

Isopropanol (2.5 mL/kg, po) or acetone (1.0 mL/kg, po) was administered 18 hr before CCl_4 (0.1 mL/kg, ip); rats were killed 24 hr after CCl_4. Data obtained from (5).

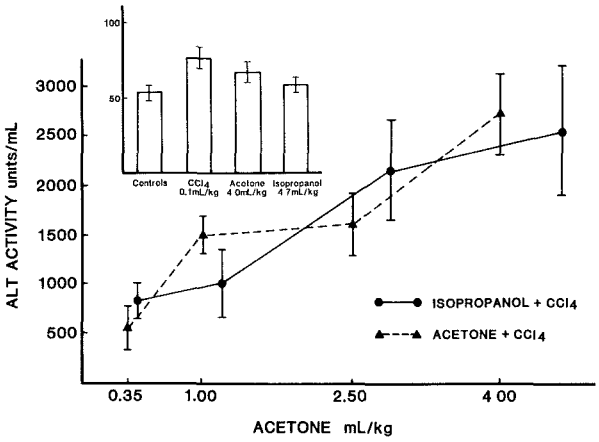

Fig. 1. Dose-effect curves for acetone and isopropanol potentiation of CCl_4-induced hepatotoxicity (elevation of plasma ALT activity). The agents were administered po 18 hr before the CCl_4 challenge (0.1 mL/kg, ip); the rats were killed 24 hr after the haloalkane. The dosages of acetone administered correspond to the acetone equivalent derived in vivo following the administration of varying dosages of isopropanol (~ 85% of isopropanol is biotransformed to acetone). Data redrawn from (4).

the amount of acetone formed from isopropanol in the rat was first calculated. Groups of animals were then pretreated with either isopropanol or the calculated amount of acetone derived from the isopropanol; the rats then received CCl_4 and liver injury assessed 24 hr later. Dose-effect curves were calculated for the two combinations -- isopropanol/CCl_4 or acetone/CCl_4. The two dose-effect curves were virtually superimposable indicating that acetone was most likely responsible for the potentiating properties of isopropanol. Other work (2,4) showed that if one diminishes the formation of acetone from isopropanol, isopropanol potentiation is also decreased.

It is interesting to note that when the initial studies were published, we predicted (3) that such a potentiation might occur in humans in the occupational setting provided the exposure conditions were analogous to those employed in the animal studies. In 1976, Folland et al. (6) described such an industrial accident in an isopropanol packaging plant, where CCl_4 was temporally used as a degreasing agent; they attributed the acute hepatotoxicity and nephrotoxicity observed in the workers to the phenomenon we had previously described with rodents.

Acetone potentiation of CCl_4 is observed when the ketone is administered by inhalation or by gavage (7). One can establish minimally effective doses (MED) for both the oral and inhalation routes. Exposures below the MED exert no effect, nor are they cumulative (7,8). There is an excellent correlation between the amount of acetone present in the blood and the severity of the CCl_4 potentiation (7,8).

Acetone is not the only ketone that can potentiate haloalkane hepatotoxicity. Table II lists the various aliphatic ketones studied and found to possess potentiating properties. In addition, n-hexane and 1,3-butanediol, substances that are biotransformed to ketones in the rat, also enhance haloalkane hepatotoxicity (9-13). Chlordecone, a cyclic chlorinated pesticide containing a ketonic group, potentiates the hepatotoxicity induced by $CHCl_3$ (14) or CCl_4 (15).

HALOALKANES AFFECTED

CCl_4 and $CHCl_3$ are not the only haloalkanes that can be potentiated by ketone pretreatment. Table III summarizes the substances that have been investigated. It is striking that not all haloalkanes are affected similarly. With the chlorinated methane, ethane and ethylene derivatives, it appears that those agents exhibiting weak hepatotoxic properties (in terms of severity of the lesion) are not potentiated by ketone pretreatment (3, 16, 17). However, the correspondence is not sufficiently exact to permit a

TABLE II

KETONES OR KETOGENIC SUBSTANCES KNOWN TO POTENTIATE HALOALKANE HEPATOTOXICITY

Ketones	Ketogenic Substances
Acetone	Isopropanol
2-Butanone (MEK)	1,3-Butanediol
2-Pentanone (MPK)	n-Hexane
2-Hexanone (MnBK)	4-Methyl-2-pentanol
2,5-Hexanedione	Alloxan (diabetic state)
4-Methyl-2-pentanone (MIBK)	Streptozotocin (diabetic state)
4-Hydroxy-4-methyl-2-pentanone	
2-Heptanone (MAK)	
Chlordecone	

TABLE III

HALOALKANES TESTED FOR POTENTIATION BY KETONES OR KETOGENIC SUBSTANCES

Haloalkanes Potentiated	Haloalkanes Not Potentiated
Carbon tetrachloride	1,1,1-Trichlorethane
Chloroform	1,1,2,2-Tetrachloroethane
1,1,2-Trichloroethane	Trichloroethylene (?)
1,1-Dichloroethylene	Tetrachloroethylene
Bromoform	
Bromodichloromethane	
Dibromochloromethane	

generalization that weak hepatotoxicants are not affected by ketones. The results obtained with brominated derivatives (bromoform, dibromochloromethane, and dichlorbromomethane) (16, 18, 19) are particularly interesting, since these haloalkanes are not potent hepatotoxicants (in terms of severity of the lesion); at near-lethal doses, hepatotoxic manifestations are very mild in intensity. Yet, acetone and chlordecone greatly enhance the hepatotoxic properties of these brominated methane derivatives. An adequate explanation for these findings is not yet available. However, it is known that brominated alkanes can form reactive metabolites (20); perhaps pretreatment with the ketones somehow favors the formation of these highly reactive moieties.

BIOACTIVITATION OF CARBON TETRACHLORIDE AND THE POTENTIATION PHENOMENON

It is well know that CCl_4 is bioactivated by the cytochrome P-450 system (20), resulting in the formation of reactive metabolites that appear responsible for initiation of the hepatic lesion. We demonstrated indirectly (4) that enhanced microsomal enzymes were probably responsible for the acetone-isopropanol potentiation phenomenon. Others (21) showed that the covalent binding of ractive metabolites of CCl_4 to microsomal protein was enhanced following isopropanol or acetone administration, a finding consistent with the bioactivation hypothesis as the mechanism involved in the potentiation phenomenon. Chlordecone, 2-hexanone, 2,5-hexanedione, and 2-butanone exhibit similar properties (22-25). Several ketones (acetone, 2-butanone, 2-hexanone, chlordecone, 2,5-hexanedione) increase hepatic cytochrome P-450 content within 24 hr in the rat. Thus, the evidence indicates that induction of cytochrome P-450 is involved in the process.

There are some interesting temporal relationships that also indicate enzyme induction is a major mechanism involved in the potentiation phenomenon. In the rat, maximal potentiation of $CHCl_3$ is observed 18-48 hr after the administration of the ketone (16). When the time interval between ketone administration and the haloalkane challenge is increased, the potentiation diminishes and is eventually lost. However, the duration of the critical ketone-haloalkane time interval during which potentiation is elicited when the $CHCl_3$ challenge is administered depends on the ketone employed. With acetone, the interval is short (24 hr), whereas with 2,5-hexanedione it is longer (> 72 hr). With chlordecone, the critical time interval for potentiation of $CHCl_3$ lasts for at least 20 days (26). We attempted to correlate enhanced covalent binding of $CHCl_3$-derived ^{14}C to hepatic constituents, following ketone administration, and presence or absence of haloalkane potentiation. We demonstrated (25, 26) that there is a very good correlation between these two events. With acetone, 2-butanone, 2-hexanone

and 2,5 hexanedione, the critical time interval increased and covalent binding of $CHCl_3$-derived material was demonstrable during this time interval; potentiation did not occur when enhanced covalent binding was absent. With chlordecone, a qualitatively similar pattern was observed (Fig. 2); during the time that enhanced covalent binding was present, potentiation occurred.

POTENTIATION OF A CHRONIC LESION

Acetone accelerates and exaggerates the appearance of cirrhosis produced in the rat by the chronic administration of CCl_4 (27). In this experiment, the acetone and the CCl_4 were administered twice a week for 12 weeks; the acetone was administered 18 hr before the CCl_4. The data summarized in Figure 3 demonstrate that after 10 weeks, the amount of collagen present in the group treated with acetone plus CCl_4 was greater than that observed in the group treated only with the haloalkane; acetone by itself produced no abnormalities. The enhanced cirrhotic response was confirmed by morphologic analysis (Table IV). Thus, some chronic effects of haloalkanes can be potentiated by ketone administration.

ENHANCED CHOLESTASIS AFTER KETONE ADMINISTRATION

Another form of liver injury, cholestasis, can be enhanced by ketone pretreatment. In rats, experimentally induced cholestasis can be produced by the injection of taurolithocholate or the injection of a combination of manganese and bilirubin (28). Both forms of cholestasis can be aggravated by pretreating the animals with 1,3-butanediol (28). Furthermore, pretreatments with 2-hexanone or 4-methyl-2-pentanone, before administration of the cholestatic agent, result in an exaggerated cholestatic response (13, 29, 30).

Curtis et al. (15) demonstrated that CCl_4 induces hepatobiliary dysfunction, which is independent of its necrogenic action. This property of CCl_4 is potentiated when one pretreats rats with the ketogenic agent, isopropanol (28). More recently, we demonstrated that $CHCl_3$ also possesses cholestatic properties and that it can be enhanced in animals pretreated with acetone, 2-butanone, 2-hexanone, or chlordecone (31). In these studies it appears that the ketones enhance the effect of chloroform on canalicular membrane permeability, as determined by segmented retrograde injection of markers of canalicular membrane function (31). The mechanisms involved in the potentiation of cholestatic responses by ketones are not well understood. However, temporal and dose-effect relationships suggest that the mechanism underlying this phenomenon is different from that causing the potentiation of haloalkane-induced necrogenic responses, although enzyme induction is still a possible explanation (29).

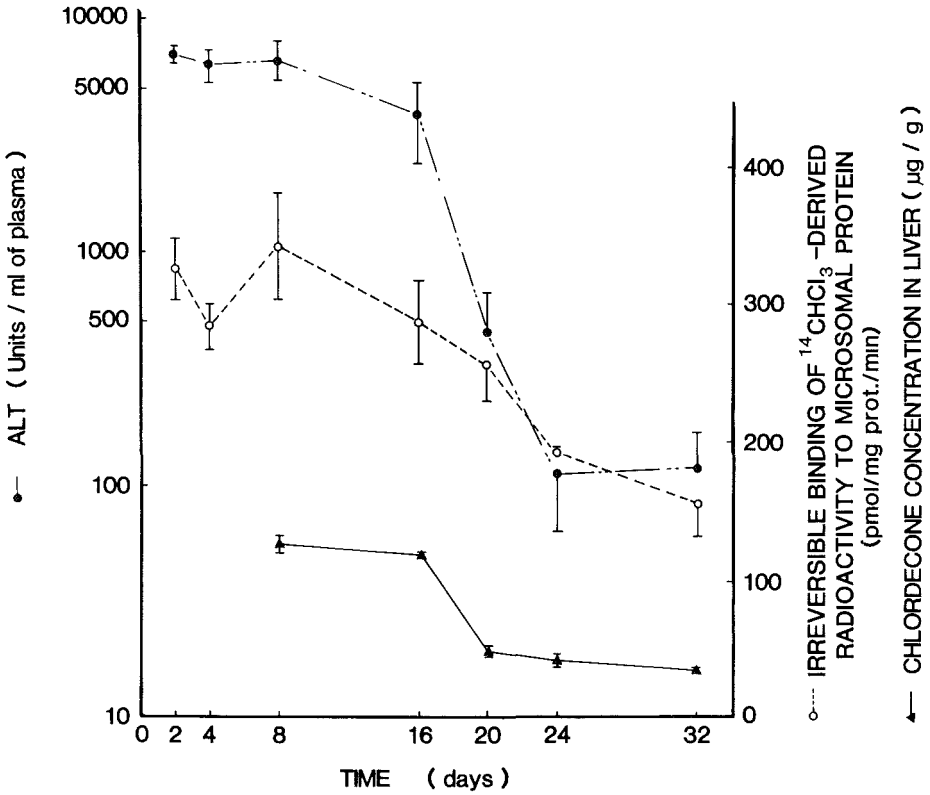

Fig. 2. Temporal relationships of chlordecone potentiation of $CHCl_3$-induced (0.5 mL/kg, po) hepatotoxicity (elevation of plasma ALT activity with hepatic chlordecone content (µg/g) and in vitro $^{14}CHCl_3$-derived covalent binding to microsomal protein (pmol/mg/min). Time is expressed in days after a single administration of chlordecone (50 mg/kg, po). Potentiation of $CHCl_3$ toxicity is still observed 20 days after chlordecone treatment. Correlation between severity of injury and covalent binding, $r = 0.88$ ($p < 0.05$); correlation between severity of injury and hepatic chlordecone content, $r = 0.96$, ($p < 0.05$); correlation between in vitro covalent binding and hepatic chlordecone content, $r = 0.90$, ($p < 0.05$). Reproduced from (26).

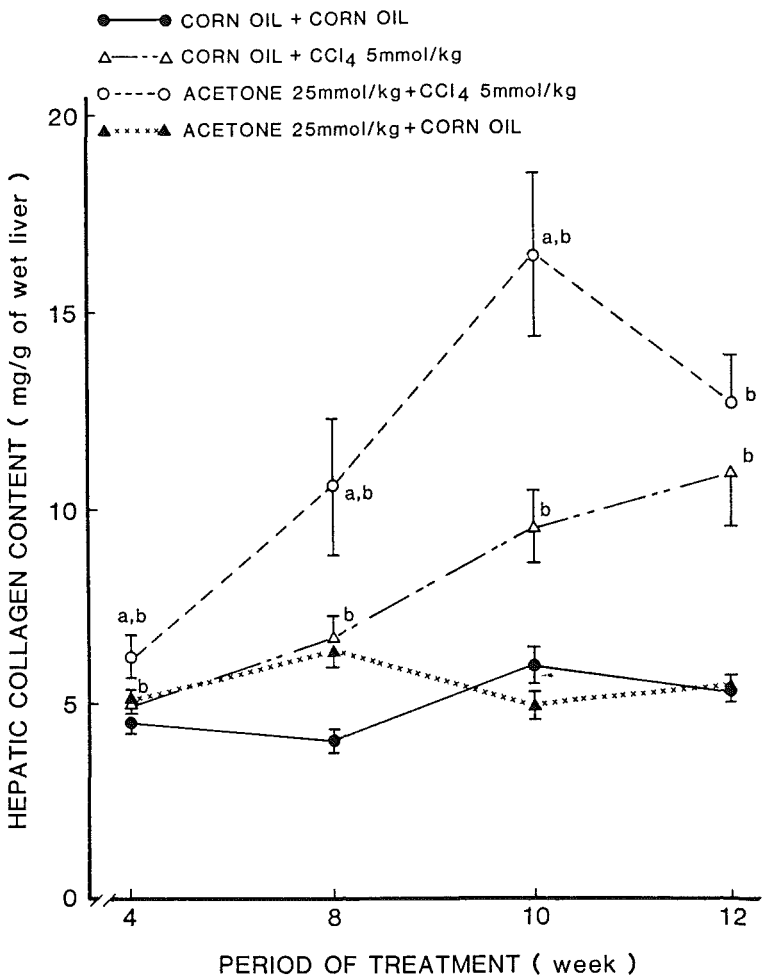

Fig. 3. Temporal progression of hepatic collagen content in rats treated orally with acetone (1.8 mL/kg, po) twice weekly (Tuesday and Thursday) and challenged with CCl_4 (0.5 mL/kg, po) 18 hr later (Wednesday and Friday) for 4, 8, 10 or 12 weeks. a = [acetone + CCl_4] group is significantly different ($p < 0.05$) from [corn oil + CCl_4] group; b = CCl_4-challenged groups differ significantly ($p < 0.05$) from corn oil-challenged groups. Data redrawn from (27).

TABLE IV

MORPHOLOGIC EVALUATION OF HEPATIC CHANGES IN RATS AFTER CHRONIC CCl_4 ADMINISTRATION FOR 10 WEEKS

	Percentage of Liver Occupied by:		
Treatment + Challenge	Bile Ducts	Fibrous Tissue	Parenchymal Tissue
Oil + Oil	0.03	0.53	99.44
Oil + Acetone	0.01	0.76	99.24
Oil + CCl_4	0.31	16.01	83.68
Acetone + CCl_4	1.23[a]	36.75[a]	62.12[a]

[a] Significantly different ($p < 0.05$) from [Oil + CCl_4] group Student's t-test. Data obtained from (27).

CONCLUSION

In addition to haloalkane-induced hepatotoxicity, some forms of haloalkane-induced nephrotoxicity can be potentiated by ketones or ketogenic agents (32). It is clear that other potential interactions may also exist. Thus, interactions by this class of chemicals merits extensive investigation.

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Discussion - Ketone potentiation and chemical toxicity.

D. Mansuy

Can you comment on the possible lipid peroxidation induced by these potentiating agents?

G.L. Plaa

It is related to the degree of liver damage observed. With chloroform we do not see signs of enhanced lipid peroxidation but with carbon tetrachloride we definitely detect lipid peroxidation.

H. Vainio

Liver damage in workers exposed to the concentrations allowed nowadays, at least in the industrialized Western world, is a very rare phenomenon and if one detects an increase in serum transaminases it is more likely due to alcohol intake than to occupational exposure. In this setting, I think that it would be most interesting if you could produce data showing that the agents you discussed also potentiate kidney or central nervous system toxicity, in which an alcoholic basis would be less likely.

G.L. Plaa

MnBK is neurotoxic but at doses that are much higher than the TLV. However, with acetone, particularly if trichloroethylene is also present, potentiation of toxicity occurs in the rat at dosages that are below the human TLV. That is why our interest lies in the lesser neurotoxic ketones.

O. Pelkonen

You mentioned chlordecone among the ketones known to potentiate haloalkane toxicity. What about mirex?

G.L. Plaa

We have not studied it, but mirex has been shown to enhance carbon tetrachloride and chloroform liver toxicity. Mirex is, to some extent, a methylcholanthrene type of enzyme inducer, whereas chlordecone is more like phenobarbital.

M.M. Reidenberg

I would like to comment again on the possible potentiation of renal toxicity. There are three epidemiological studies of patients with chronic glomerulonephritis that show a greater degree of exposure to volatile organic solvents than appropriately selected controls. (Beirne GJ, Brennan JT, Arch Environ Health 25 : 365, 1972; Zimmerman SW, Groehler K, Beirne GJ, Lancet 2 : 199, 1975; Lagrue G, Lancet 1 : 1191, 1976; Ravnskov U, Forsberg B, Skerfving S, Acta Med Scand 205 : 585, 1979.

J.V. Castell

Can you comment on the ketone potentiation of chemical hepatotoxicity in circumstances in which these ketones are produced in the body, either physiologically or in a pathological situation?

G.L. Plaa

Experimentally, fasting will potentiate haloalkane hepatotoxicity, but the potentiation can still be increased by the administration of exogenous ketones. Haloalkane hepatotoxicity can also be enhanced by experimental diabetes either induced by alloxan or streptozocin, and administration of insulin blocks this potentiation. There is evidence that in ketosis induced by fasting, diabetes and, certainly, by acetone, a subspecies of cytochrome P 450 that has a particularly high affinity for carbon tetrachloride is induced.