MECHANISM OF IN VIVO CARBON TETRACHLORIDE-INDUCED LIVER MICROSOMAL CYTOCHROME P-450 DESTRUCTION

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Received February 24,1975

Summary : Prior administration of aminotriazole (3-amino-1,2,4-triazole) or pyrazole to rats resulted in a significant prevention of the CCl_4-induced decrease in the liver microsomal P-450 content. In A/J mice the CCl_4 activation and P-450 destruction occurred in absolute absence of lipid peroxidation as determined by uv absorption. The data suggest that P-450 destruction is mainly mediated by direct attack of CCl_4 metabolites rather than by CCl_4-induced lipid peroxidation.

In 1967 Smuckler et al. (1) reported that CCl_4 administration to rats decreases the amount of cytochrome P-450 (P-450) in liver microsomal preparations. Studies by Castro et al. (2) showed that 2-diethylaminoethyl-2,2-diphenyl valerate (SKF 525-A) partially prevents the CCl_4-induced P-450 destruction process in contrast to antioxidants like α-tocopherol acetate or diphenyl-p-phenylendiamine (DPPD) suggesting that lipid peroxidation does not mediate the deleterious effect on P-450. Further studies (3) led to the hypothesis that P-450 destruction in vivo is produced by a CCl_4 active metabolite. In 1972 Reiner et al. (4) reported a decrease in microsomal P-450 concentration by in vitro aerobic incubation of isolated liver microsomes with NADPH and further acceleration of this process by CCl_4 addition. Moreover, EDTA or reduced glutathion at concentrations inhibiting lipid peroxidation prevents the CCl_4-induced P-450 destruction (5,6). In spite of this in vitro correlation between P-450 destruction and lipid peroxidation, some in vivo observations from our laboratory suggest that CCl_4 metabolites rather than lipid peroxidation are responsible for P-450 destruction (7-10). Studies attempting to clarify these apparently contradictory observations are reported here.

METHODS

Compounds. Pyrazole was purchased from T. Schuhardt, Germany and 3-amino-1,2,4-triazole was purchased from Aldrich Chemical Co. (^{14}C)CCl_4 (27.5 mCi/mmol) was obtained from the Radiochemical Center, Amersham, England. All

other chemicals employed were reagent grade.

Treatment of animals. Sprague Dawley male rats (220-250 g) or strain A/J male mice (24-28 g) were used throughout the study. Food was withdrawn 12-14 hr before CCl_4 or (^{14}C)CCl_4 administration but water was available ad libitum. Pyrazole was given ip as a saline solution (150 mg/kg) 30 min before CCl_4. A first dose of 3-amino-1,2,4-triazole (3 g/kg po) was given to 24-hr fasted male rats and a second dose was administered 24 hr later, before CCl_4. The latter was given as a 20% v/v solution in olive oil at a dose of 5 ml/kg ip. The (^{14}C)CCl_4 was dissolved in olive oil to give a solution producing 1.4×10^6 dpm/ml, which was administered ip at a dose of 5 ml/kg. Controls received the equivalent amount of water, saline or olive oil. The animals were sacrificed by decapitation at different times after CCl_4 administration.

Isolation of microsomes. The isolation of microsomes for studies on lipid peroxidation, irreversible binding of (^{14}C)CCl_4 to microsomal lipids or P-450 content were previously reported (9-11).

Chemical procedures. The incorporation in vivo of ^{14}C from (^{14}C)CCl_4 into microsomal lipids was estimated according to the procedure described by Castro et al. (9). The results are expressed in dpm/mg of microsomal lipid. Lipid peroxidation in vivo was determined by conjugated diene uv absorption of microsomal extracts as described by Klaassen and Plaa (12). The results are expressed by the absorbance at 243 nm x 1000 for a solution containing 1 mg microsomal lipid/ml. P-450 was determined as described by Schenkman et al. (13). Protein concentrations were estimated according to the method of Lowry et al. (14).

Statistics. The significance of the difference between two mean values was assessed by the Student's t test (15). Differences were considered significant when $p<0.05$.

RESULTS

Effect of 3-amino-1,2,4-triazole treatment to rats on CCl_4-induced liver microsomal P-450 destruction. Liver microsomal P-450 content was lower in aminotriazole or CCl_4-treated rats compared with controls (Table 1).However, the CCl_4-decreasing effect was less striking on aminotriazole pretreated rats than on untreated animals.

Effect of pyrazole treatment to rats on CCl_4-induced liver microsomal P-450 destruction. Pyrazole administration to rats significantly reduced the intensity of CCl_4-induced deleterious action on P-450 compared to that observed in the untreated rats (Table 1). Pyrazole administration by itself did not have any effect on the microsomal P-450 content.

TABLE 1

CCl_4-induced destruction of liver microsomal P-450 in aminotriazole and pyrazole pretreated rats and in mice

Pretreatment	Animals/ group	P-450 n moles/mg protein Control	CCl_4	Decrease %
none	7 rats	0.88	0.33	62
Aminotriazole		0.52	0.41	21
none	7 rats	0.84	0.43	49
Pyrazole		0.95	0.74	22
none	5 mice	0.60	0.21	65

A first dose of aminotriazole (3g/kg po) was given to 24 hr fasted male rats and a second dose was administered 24 hr later, just before CCl_4.

Pyrazole was given ip to 12-14 hr fasted rats as a saline solution at a dose of 150 mg/kg, 30 min before CCl_4.

CCl_4 was given ip as a 20% v/v solution in olive oil at a dose of 5 ml/kg. Controls received the equivalent amount of olive oil and saline. The animals were sacrificed 3 hr after CCl_4 administration.

All $p<0.01$ and $SD<0.1$

Microsomal P-450 destruction in livers of CCl_4-treated mice, its relation to CCl_4 activation and to lipid peroxidation. CCl_4 administration to mice (Table 1) led to a P-450 destruction as intense as that produced in livers from CCl_4-treated rats. This effect was obtained even though there was no observable microsomal lipid peroxidation, resulting in uv spectral change, within 24 hr after CCl_4 administration (Table 2). The intense activation to reactive metabolites, as measured by the irreversible binding of CCl_4 to microsomal lipids from mice livers, was comparable to that previously reported for rat liver preparations (9,10) (Table 3).

DISCUSSION

Several authors (4-6) have provided convincing evidence for an in vitro CCl_4-induced decrease in P-450 content mediated by lipid peroxidation.Howev-

TABLE 2

Effect of CCl_4 at different times after administration on the *in vivo* mouse liver microsomal lipid peroxidation

Treatment 10 mice/group	In vivo lipid peroxidation ± SD		
	3 hr	6 hr	24 hr
Control	324 ± 56	185 ± 12	118 ± 52
CCl_4	323 ± 30	195 ± 37	103 ± 24

CCl_4 was given as in Table 1. Animals were sacrificed at either 3; 6 or 24 hr and their livers processed for measuring microsomal lipid peroxidation as described in Methods. Two similar experiments were carried out at each time.

The lipid peroxidation value is expressed as absorbance at 243 nm x 1000 for a solution containing 1 mg microsomal lipid/ml.

$p>0.1$

er, an equivalent correlation between loss of P-450 and intensity of lipid peroxidation was not observed under different experimental conditions *in vivo* (7-10). In those cases a better correlation was found between loss of P-450 and intensity of CCl_4 activation to $\cdot CCl_3$, estimated by the irreversible binding of (^{14}C)CCl_4 to microsomal lipids. In our present experiments *in vivo* P-450 destruction correlates better with CCl_4 activation than with microsomal lipid peroxidation. Aminotriazole, which is capable of decreasing both the intensity of CCl_4 activation and the CCl_4-induced peroxidation (16), also decreases the destruction of microsomal P-450 in CCl_4-poisoned rats.This suggests that either one or both processes may be involved in P-450 destruction, excluding any direct solvent effect of CCl_4 on the P-450 molecule. Pyrazole, which is capable of decreasing CCl_4 activation without modifying the occurrence of lipid peroxidation (17), partially prevents the CCl_4-induced loss of microsomal P-450. This result suggests that lipid peroxidation is not the key factor in P-450 destruction. Studies on A/J mice provide more evidence to support our view. In this species liver microsomal P-450 destruction occurs in the absence of lipid peroxidation although CCl_4 activation and necrosis are observed (these results and other on different species will be reported).

These interesting findings not only reinforce our hypothesis that P-450 destruction *in vivo* by CCl_4 is mediated by the attack of reactive intermedi-

TABLE 3

Irreversible binding of ^{14}C from $(^{14}C)CCl_4$ to liver microsomal lipids and $(^{14}C)CCl_4$ concentrations in liver 3 hours after administration to mice

Irreversible bound ^{14}C to liver microsomal lipids dpm/g lipid±SD	$(^{14}C)CCl_4$ concentration in liver dpm/g liver±SD	R
81,000 ± 11,000	91 ± 22	849 ± 53

Fasted (12-14 hr) A/J male mice were injected ip with a solution of $(^{14}C)CCl_4$ (27.5 mCi/mmol) in olive oil (1.4×10^6 dpm/ml) at a dose of 5 ml/kg. The animals were sacrificed 3 hr after CCl4 administration. Microsomal fractions were isolated and analyzed for irreversible bound ^{14}C to lipids as described in Methods. Sixteen animals, divided in four groups of four pooled mouse livers each, were used in the experiment.

The CCl4 concentrations were estimated by collecting the $(^{14}C)CCl_4$ in toluene in the center well of a microdiffusion cell followed by scintillation counting.

R is the ratio between the values of irreversible binding and its respective $(^{14}C)CCl_4$ concentration.

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ates on its molecule and not by lipid peroxidation, but also raise serious doubts about the importance of lipid peroxidation in CCl_4 liver injury. Apparently, lipid peroxidation is an important mechanism in vitro, while irreversible binding to cellular components may play an important role under in vivo experimental conditions.

Acknowledgments : This work was supported by grant 5R01-AM 13195-06 from the National Institutes of Health, USA, and by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

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