CARBON TETRACHLORIDE ACTIVATION IN LIVER MICROSOMES FROM RATS INDUCED WITH 3-METHYLCHOLANTRENE

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Summary

The irreversible binding of ^{14}C from $^{14}CCl_4$ to microsomal lipids is decreased in animals treated with 3-methylcholantrene (3-MC), while it is increased in animals induced with phenobarbital (PB). CCl_4-induced lipid peroxidation in 3-MC treated rats is as intense as in controls. Destruction of glucose 6-phosphatase (G6P-ase) by CCl_4 is smaller in 3-MC treated rats than in controls. Destruction of total cytochrome P-450 (P-450 + P_1-450) by CCl_4 is smaller in 3-MC treated than in PB treated rats but similar to that obtained in controls. Results would indicate that P-450 would participate in CCl_4 activation much more effectively than P_1-450.

It is believed that CCl_4 hepatotoxicity depends on an activation step occurring during its biotransformation (1-8). The liver microsomal electron-transport system was found to be involved in the metabolism of CCl_4 to CO_2(3). CCl_4 activation would lead to the formation of $\cdot CCl_3$ and $\cdot Cl$ free radicals (7,8) which either may bind irreversibly to the unsaturated fatty acid moieties of the liver microsomal lipids (9) or they may set into motion a lipid peroxidation process (2). Previous studies showed that CCl_4 interacts with the liver microsomal electron-transport chain by binding either to oxydized P-450 to give a type I spectral change (10,11) or to reduced P-450 to give a distinctive spectral change (12). More recently, we found further evidence for the possibility that CCl_4 activation were related to its interaction with the mixed-function oxygenase system, since we found activation of CCl_4 not only in liver microsomes but also in adrenal microsomes and mitochondria where similar electron-transport systems are also present (13). Furthermore, the study of that activation step in liver microsomes under different experimental conditions led us to anticipate the possibility for an activation of CCl_4 occurring during

the reduction of the CCl_4/P-450 complex mediated by P-450 reductase (14). The present study is devoted to verify if the different type of P-450 known to be induced by 3-MC and usually named either P_1-450 or P-448 (15) is able to participate in CCl_4 activation. The results here described would suggest that P_1-450 is either less able than P-450 to activate CCl_4 or that it does not activate CCl_4 at all.

Experimental

Male Sprague Dawley rats weighing 80-120 g were used. 3-MC was dissolved in olive oil with gentle heating (7 mg/ml) and administered ip (35 mg/kg) once daily for 3 days. Control animals received olive oil. PB (80 mg/kg) was given as a saline solution once daily for 3 days. Control animals received saline. The animals were deprived of food 12-14 hr before CCl_4 administration. At the morning of the fourth day CCl_4 was given ip as a 20 % (v/v) solution in olive oil at a dose of 5 ml of solution/kg. In the experiment using labelled $^{14}CCl_4$ the animals were injected ip with a solution of $^{14}CCl_4$ (27.5 mCi/mM) in olive oil (1,400,000 dpm/ml) at a dose of 5 ml of solution/kg. The animals were sacrificed by decapitation 3 hr after CCl_4 administration. The livers were excised and processed. The methods for isolation of microsomes, the measurement of the extent of the irreversible binding of $^{14}CCl_4$ to lipids, lipid peroxidation, G6P-ase activity, cytochrome P-450 content, CCl_4 levels in liver as well as the statistical treatment of the data (5,16) were previously described.

Results and Discussion

The obtained results on the effect of CCl_4 on the lipid peroxidation, G6P-ase and P-450 content from 3-MC-treated rats can not be merely attributted to a lowering effect of the 3-MC treatment on the CCl_4 levels in liver, since CCl_4 levels under that experimental condition were not significantly different from those occurring in control rats (Table 4).

Similarly, it is not possible to explain the decrease in the extent of the irreversible binding of $^{14}CCl_4$ to microsomal lipids found in 3-MC-treated

Table 1

IRREVERSIBLE BINDING OF ^{14}C FROM $^{14}CCl_4$ TO LIVER MICROSOMAL LIPIDS FROM RATS PREVIOUSLY TREATED WITH EITHER 3-MC OR PB

Treatment * (10 rats/group)	dpm/mg lipid	% of control
Control	43	
3-MC	31 **	71
Control	58	
PB	81 **	140

* 12-14 hr starved male rats were injected ip with a solution of $^{14}CCl_4$ (27.5 mCi/mM) in olive oil (1,400,000 dpm/ml) at a dose of 5 ml of solution/kg. Controls and treated animals were sacrificed 3 hr after administration of $^{14}CCl_4$. Microsomes were isolated and analyzed for irreversible bound ^{14}C to lipids as described in Methods.
3-MC (35 mg/kg) and PB (80 mg/kg) were given ip once daily for 3 days. $^{14}CCl_4$ was given in the morning of the fourth day. Controls received either olive oil or saline.

** Significantly different from controls, $p < 0.001$. The mean standard deviation was 15 %.

rats in terms of differences in levels of free $^{14}CCl_4$ under these circumstances, since we found in livers of 3-MC-treated rats significantly higher levels of free $^{14}CCl_4$ than in livers from control rats. PB administration does not modify CCl_4 levels in liver (24).

As shown in Table 1, the extent of the irreversible binding of ^{14}C from $^{14}CCl_4$ to microsomal lipids from livers of 3-MC treated rats is less intense than in controls and the one occurring in those from PB treated rats is 1.4 fold greater than that obtained from controls. Since that irreversible binding to microsomal lipids would arise from the addition of $\cdot CCl_3$ and $\cdot Cl$ to polyunsaturated fatty acids (9), its extent should be proportional to the extent of CCl_4 activation to $\cdot CCl_3$ and $\cdot Cl$. In this case, our results

would indicate that CCl_4 activation is greater in PB treated rats than in controls and in controls than in 3-MC treated animals.

Previous observations from other laboratories and our own observations (10-13) suggest that P-450 may be involved in the activation of CCl_4; moreover, our studies seem to indicate that CCl_4 activation may occur during the reduction of the CCl_4/P-450 complex by P-450 reductase (14). If that mechanism for CCl_4 activation were correct, the present observations could be interpreted as due either to a lack of ability or to a very decreased ability of P_1-450 to participate in CCl_4 activation when compared to the one of P-450. This is particularly likely if one considers that 3-MC treatment does not modify P-450 reductase activity (17) but fundamentally alters drug metabolism by inducing the synthesis of an unusual cytochrome called by some authors P_1-450 (15) which constitutes the half of the total microsomal P-450 in 3-MC treated rats (15).

In contrast to the previously reported increases in CCl_4-induced lipid peroxidation observed in PB induced rats (18), results in Table 2 indicate that CCl_4-induced lipid peroxidation is as intense in 3-MC treated rats as in control rats despite the fact that less activation to $\cdot CCl_3$ and $\cdot Cl$ was found. These results may be interpreted as showing that only a small part of the $\cdot CCl_3$ and $\cdot Cl$ free radicals is enough to spark lipid peroxidation to maximum levels or alternatively that both processes are independent.

The prevention of the CCl_4-induced liver necrosis by 3-MC (19) appears to follow more closely the early decreases in the extent of the irreversible binding of $^{14}CCl_4$ to liver microsomal lipids than the early changes in CCl_4 induced lipid peroxidation observed in 3-MC treated rats, suggesting that the effect of the initial $\cdot CCl_3$ and $\cdot Cl$ free radicals could be more pertinent than lipid peroxidation to the development of necrosis.

Similarly, G6P-ase activity destruction by CCl_4 in 3-MC treated rats is smaller than in controls (Table 3). These results on 3-MC induced rats are in agreement with our previous assumption (16,20) that G6P-ase is destroyed by the $\cdot CCl_3$

Table 2

CCl_4-INDUCED LIPID PEROXIDATION IN 3-MC TREATED RATS

Treatment * (4 rats/group)	absorbance at 243 mu/mg lipid
Control	40
CCl_4	111 **
3-MC	95
3-MC + CCl_4	128 **

* 3-MC was given as indicated in Table 1. CCl_4 was given ip as a 20 % (v/v) solution in olive oil at a dose of 5 ml of solution/kg. Controls rats received olive oil ip. The animals were sacrificed 3 hr after CCl_4 administration. Microsomes were isolated and analyzed for lipid peroxidation as described in Methods.

** Significantly different from controls, $P < 0.01$. The overall effect of 3-MC on CCl_4-induced lipid peroxidation was not significant, $p > 0.05$.
The mean standard deviation was 19 %.

and •Cl free radicals and not by lipid peroxidation as postulated by other authors (21). In contrast to previous observations on PB treated rats (5), 3-MC induction does not increase the destruction of P-450 caused by CCl_4 (Table 3). These results are in agreement with the hypothesis that P-450 is destroyed by $•CCl_3$ and •Cl free radicals (5), since in the case of PB induction both, the extent of the irreversible binding to lipids which is proportional to the amount of $•CCl_3$ and •Cl formed and the destruction of P-450 are increased while in the case of 3-MC induction the irreversible binding of $^{14}CCl_4$ to lipids is slightly decreased and the P-450 destruction is either not altered as we found in these studies or it is decreased as previously described by other authors (22,23).

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Table 3

EFFECT OF 3-MC PRETREATMENT ON THE DESTRUCTION OF MICROSOMAL G6P-ase ACTIVITY AND P-450 BY CCl_4 ADMINISTRATION

Treatment * (5 rats/group)	P-450 (muMol/mg protein)	G6P-ase (ug inorganic phosphorus/ 15 min/mg protein)
Control	0.50	34.1
CCl_4	0.26 **	22.5 **
3-MC	0.91 **	25.2
3-MC + CCl_4	0.51	24.4

* 3-MC and CCl_4 were given as indicated in Table 1 and 2. The animals were sacrificed 3 hr after CCl_4 administration. Microsomes were isolated and analyzed for P-450 content and G6P-ase activity as described in Methods.

** $p < 0.05$ when compared to its respective control. The significance of the overall effect of the prior treatment with 3-MC obtained by analysis of variance was $p > 0.1$ for the results on P-450 and $p < 0.05$ for G6P-ase activity. The mean standard deviation was 24 %.

Table 4

$^{14}CCl_4$ AND CCl_4 LEVELS IN LIVER AFTER THEIR ADMINISTRATION TO RATS PREVIOUSLY TREATED WITH 3-MC

Treatment * (8 rats/group)	$^{14}CCl_4$ levels (dpm/gr liver)	CCl_4 levels (ug/gr liver)	
	3 hr	1 hr	3 hr
Control	585	772	605
3-MC	826 **	952	483

* 3-MC, $^{14}CCl_4$ and CCl_4 were given as indicated in Tables 1 and 2. Levels of $^{14}CCl_4$ and CCl_4 were measured by separation by microdiffusion followed by either counting or colorimetric analysis respectively as described in Methods.

** Significantly different from controls, $p < 0.01$. The mean standard deviation was 30 %.

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