CARBON TETRACHLORIDE EFFECT ON RAT LIVER AND ADRENALS RELATED TO THEIR MIXED-FUNCTION OXYGENASE CONTENT

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Summary

In liver: CCl_4 interacts with microsomal cytochrome P-450 (P-450) giving type I spectral changes; $^{14}CCl_4$ irreversibly binds to microsomal lipids and less to those of mitochondria; CCl_4-induced lipid peroxidation occurs in microsomes and not in mitochondria; P-450 destruction is intense; "in vitro" CCl_4 increases lysosomal permeability. In adrenals: CCl_4 gives either type I or type II spectral changes by acting on either mitochondria or microsomal P-450 respectively; $^{14}CCl_4$ binds irreversibly to a similar extent to either mitochondrial or microsomal lipids; lipid peroxidation occurs in microsomes and less in mitochondria; P-450 destruction occurs in microsomes and not in mitochondria; "in vitro" CCl_4 increases lysosomal permeability.

It is currently accepted that CCl_4 hepatotoxicity depends on its own metabolism (1,2,3,4). It is also known that CCl_4 is metabolized in liver by the NADPH and O_2 requiring hydroxylating systems from microsomes (5). Considerably knowledge has been recently gained about the role of cytochrome P-450 and cytochrome P-450 reductase in these hydroxylating reactions (6). This hemoprotein as well as its reductase are also present in the microsomal fraction of a number of tissues (6). The adrenal cortex is particularly rich in P-450 content and also presents the unusual property of having considerably amounts of this hemoprotein not only in microsomes but also in mitochondria (6). If present theories on CCl_4 hepatotoxicity are correct, one should expect the occurrence of very important alterations not only in the adrenal endoplasmic reticulum but also in adrenal mitochondria. The following report describes the occurrence or not in adrenal microsomes, mitochondria and lysosomes of some of the most characteristic alterations found in the corresponding liver fraction after CCl_4 action.

Materials and Methods

All the chemicals employed were reagent grade. Sprague-Dawley male rats (170-260 g) were used in these experiments. Food was withdrawn 12-14 hr before CCl_4 administration. CCl_4 was given intraperitoneally as a 20 % (v/v) solution in olive oil at a dose of 5 ml of solution/kg. The animals were sacrificed by

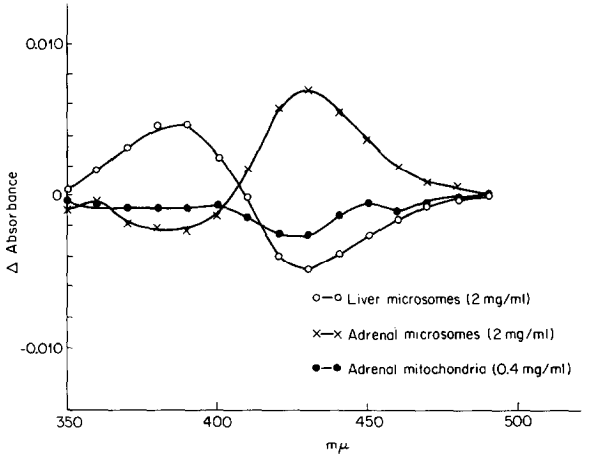

Figure 1. Spectral changes caused by CCl_4. No spectral changes were observed with adrenal mitochondria at higher (2 mg/ml) or at lower concentrations (0.1 mg/ml) than (0.4 mg/ml). CCl_4 final concentration was 27 mM.

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decapitation in a Harvard guillotine and bled. Their livers and adrenals were rapidly removed, carefully defatted, weighed and processed. Liver or adrenal mitochondria and microsomes were separated according to the method of Schneider and Hogeboom (7) but adding 3 mM EDTA to the sucrose of the homogenization media for the studies on lipid peroxidation. The lysosome-rich fraction from either liver or adrenals was isolated according to the quantitative fractionation procedure described by Appelmans, Wattiaux and De Duve (8). The production of spectral changes by interaction of CCl_4 with liver microsomes or with adrenal microsomes and mitochondria was performed according to the procedure described by Schenkman et al (9). The irreversible binding of ^{14}C from $^{14}CCl_4$ to microsomal and mitochondrial lipids was measured according to the method described by Castro et al (10). Lipid peroxidation in microsomal or mitochondrial lipids was measured by the diene-hyperconjugation technique as described by Klaassen and Plaa (11). P-450 content in liver microsomes or in adrenal microsomes and mitochondria was determined as described by Schenkman et al (9). Phosphatase activity was measured by a procedure similar to that employed by Linhardt and Walter (12). For the optical observation of adrenals, they were fixed in Bouin's solution, dehydrated, embedded in paraffin and stained with hematoxylin-eosin.

Results and Discussion

Present theories on CCl_4 hepatotoxicity involve the interaction of CCl_4 with the liver microsomal electron-transport chain. It has been postulated that as result of this interaction $\cdot CCl_3$ and $\cdot Cl$ ($R\cdot$) are produced (1,2,3,4)

Table 1

IRREVERSIBLE BINDING OF ^{14}C FROM $^{14}CCl_4$ TO DIFFERENT SUBCELLULAR FRACTIONS

Subcellular fraction	dpm/mg lipid ± SD	P value	dpm/mg protein ± SD	P value
Liver microsomes	56 ± 4	0.001	17 ± 3	0.001
Liver mitochondria	36 ± 3		9 ± 1	
Kidney microsomes	8 ± 2	0.05	2 ± 1	0.001
Kidney mitochondria	7 ± 1		1 ± 0	
Adrenal microsomes	18 ± 12	0.05	24 ± 11	0.05
Adrenal mitochondria	20 ± 12		15 ± 10	

12-14 hr starved male rats were injected i.p. with a solution of $^{14}CCl_4$ (27.5 mCi/mM) in olive oil (1,400,000 dpm/ml of solution) at a dose of 5 ml of solution/kg. The animals were sacrificed 3 hr after administration of $^{14}CCl_4$. The results are expressed either in dpm/mg of lipid or as the dpm associated with the lipid present in the amount of sample containing one mg of protein. Free levels of $^{14}CCl_4$ in liver, kidney and adrenals were 368±39, 856±103 and 462±105 respectively. (in dpm/g organ).
Five animals were used in the experiments on liver and kidney. The results from adrenals are the mean of three different experiments using the pooled adrenals from 12, 21 and 41 animals respectively. The significance for the differences between results from liver microsomes and those from adrenal microsomes or mitochondria is $P > 0.1$ when results are in dpm/mg protein.

and that those R· initiate a lipid peroxidation process of the membrane components of the endoplasmic reticulum and mitochondria (1,2). This last alteration is usually considered to be a fundamental reason for the liver cell death (1). Since a similar electron-transport system to that occurring in liver microsomes is also present in adrenal mitochondria and adrenal microsomes, the presently accepted theories may imply that similar or equivalent alterations to those observed in liver should also occur in adrenals. Here we found that CCl_4 interacts with adrenal microsomal and mitochondrial P-450 to give type II spectral changes in the case of microsomes and a very small but observable type I spectral change in the case of mitochondria. Both results were unexpected, since in the case of liver microsomes the interaction with CCl_4 results in a type I spectral change, while in that with adrenal microsomes a type II one was obtained; in the case of adrenal mitochondria, the usual type I spectral

Table 2

CCl_4-INDUCED LIPID PEROXIDATION IN SUBCELLULAR FRACTIONS *

Subcellular fractions		Time after CCl_4 (hr)	abs. at 243 mμ/mg lipid Mitochondria	Microsomes
Adrenal	Control	3	271	303
	Treated	3	291	341
	Control	6	359	318
	Treated	6	372	393
Liver	Control	3	336	353
	Treated	3	348	463**
	Control	6	312	252
	Treated	6	332	401**

CCl_4 was given i.p. as a 20 %(V/V) solution in olive oil at a dose of 5 ml of solution/kg. Control rats received olive oil i.p.
*The lipid peroxidation value is expressed as Δ absorbance at 243 mu x 1,000 for a solution having 1 mg of lipid/ml. Five animals/group were used in the liver experiments and the standard deviation of the values was of ± 10 % as a maximum. Results from adrenals were obtained by pooling the glands from 36 rats for each group.
** $P < 0.01$.

change was observed, but having a magnitude not in correspondence with its high P-450 content. In the case of adrenal mitochondrial P-450, the interaction is usually not observed when protein concentration in the suspension is about 2 mg/ml, but becomes evident when it is diluted to 0.4 mg/ml. This fact may mean that adrenal mitochondrial P-450 is already bound to an endogenous spectral change-forming compound, e.g. an steroid (which are known to give spectral changes) and that this endogenous compound competes either with the binding of CCl_4 itself or with the expression as spectral change of that binding. We also found that CCl_4 not only interacts with the P-450 containing electron-transport chain from adrenal mitochondria and microsomes as it should, but also that, as occurs for the case of liver microsomes, ^{14}C from $^{14}CCl_4$ irreversibly bounds to their lipids even to those of mitochondria, while binding to lipids from mitochondria in liver or kidney is much less. P-450 destruction in adrenals only occurs in microsomes and to a smaller extent than the one observed for the case of liver microsomes. Since we found that activation of CCl_4 in adrenal mitochondria occurs (as it is shown by the high irreversibly bound ^{14}C

Table 3

EFFECT OF CCl_4 ADMINISTRATION ON THE P-450 CONTENT

Subcellular fraction		Time after CCl_4 administration (hr)	Cytochrome P-450 (mμ Mole/mg protein) mitochondria	microsomes
Adrenal	Control	6	0.41 ± 0.06	0.39 ± 0.06
	CCl_4	6	0.46 ± 0.04	0.33 ± 0.01
	Control	10	0.42 ± 0.08	0.31 ± 0.05
	CCl_4	10	0.47 ± 0.01	0.20 ± 0.02 *
	Control	24	0.39 ± 0.12	0.37 ± 0.13
	CCl_4	24	0.44 ± 0.01	0.17 ± 0.06 **
	Control	48	0.46 ± 0.14	0.55 ± 0.01
	CCl_4	48	0.47 ± 0.15	0.52 ± 0.04
	Control	72	0.48 ± 0.15	0.42 ± 0.06
	CCl_4	72	0.35 ± 0.03 +	0.47 ± 0.03
Liver	Control	6	---	0.47 ± 0.10
	CCl_4	6	---	0.23 ± 0.08 *
	Control	10	---	0.56 ± 0.11
	CCl_4	10	---	0.15 ± 0.07 +
	Control	24	---	0.64 ± 0.11
	CCl_4	24	---	0.08 ± 0.08 +

CCl_4 was given as indicated in Table 2. The results for adrenals are the mean of three different experiments using the pooled adrenals from 12 animals in each one. Five animals for each group were used in liver experiments.
+ $P < 0.001$. * $P < 0.01$. ** $P < 0.05$.

from $^{14}CCl_4$ to lipids found in this organelle), our results may show that the endogenous compounds postulated to be bound to P-450 in mitochondria may also stabilize P-450 against damage by R·. We also found that lipid peroxidation, as measured by the UV method described by Klaassen and Plaa (11), occurs in adrenal microsomes but to a lesser extent than in liver, while in adrenal mitochondria lipid peroxidation appears to lack of importance. If as present theories establish, the lipid peroxidation process is initiated by the R· arised from the activation of CCl_4 and the irreversible bound ^{14}C from $^{14}CCl_4$ to lipids is due to the addition of R· to unsaturated lipids (3.4), then, one may expect more lipid peroxidation when more binding of $^{14}CCl_4$ to lipids occurs. In the

Table 4

COMPARATIVE CCl_4-INDUCED IN VITRO LIBERATION OF LYSOSOMAL ENZYMES IN LIVER AND ADRENALS *

		Acid phosphatase activity**	% of control
Liver	Control	32.0	
	CCl_4	66.0	206
Adrenals	Control	8.8	
	CCl_4	22.0	250

* Three ml aliquots of lysosomal suspensions from liver (2.5 mg protein/ml) or adrenals (2.1 mg protein/ml) were incubated with shaking for 30 min at 37° in Warburg flasks with or without 4 ul of pure CCl_4 in the side arm. The content of the flasks was centrifugated 20 min at 20,000 x g and the supernatant was used for enzyme activity measurements.

** Acid phosphatase activity is given in mumoles of p-nitrophenol liberated in 30 min at 37° by 50 ul of a 20,000 x g supernatant of a lysosomal suspension having 1 mg of protein/ml.

case of adrenals, closely similar levels of irreversible binding to those occurring in liver microsomes were not accompanied by similar levels of lipid peroxidation, since less or even almost negligible ones occur in adrenal microsomes and mitochondria. However, these results do not imply that present theories are uncorrect because they also may be due to a different susceptibility of adrenal lipids to act as R· target-sites or to the presence in adrenals of higher levels of endogenous antioxidants than in liver. In order to have a more complete picture about the analogies and differences between liver and adrenals in their response to CCl_4 deletereous action, we also compared the ability of CCl_4 to cause "in vitro" the liberation of enzymes from lysosomes from both organs and we found that they were comparable. In spite of all the similarities between rat liver and adrenal in their response to CCl_4 here described, we were not able to find histologically observable alterations in adrenals after acute CCl_4 administration. Damage to adrenals may depend to an important extent on the species employed to do the test and also on the regime of exposure to CCl_4, since Phelps and Hu (13) reported on necrosis of the adrenals in a patient dying from CCl_4 and this also was observed in guinea pigs poisoned with this solvent, while Gardner (14) saw it only exceptionally in dogs after giving large dosis of CCl_4 and Higgins and Cragg (15) observed hyperplasia of the cortical zone in rats but when they were exposed daily for 6, 8 and 12 weeks to CCl_4 vapours.

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