DIFFERENCES IN THE CARBON TETRACHLORIDE-INDUCED DAMAGE TO COMPONENTS OF THE SMOOTH AND ROUGH ENDOPLASMIC RETICULUM FROM RAT LIVER

J. A. Castro, M. I. Diaz Gomez, E. C. de Ferreyra, C. R. de Castro, N. D'Acosta and C. M. de Fenos

Laboratorio de Quimica Bio-Toxicologica, CITEFA, Zufriategui y Varela, Villa Martelli, Pcia. de Buenos Aires, Argentina

Received November 27,1972

Summary

There is a higher activity of ethyl morphine N-demethylase (EM-ase) and cytochrome P-450 (P-450) reductase as well as higher P-450 content in the smooth endoplasmic reticulum (SER) than in the rough endoplasmic reticulum (RER). The extent of the irreversible binding of the ^{14}C from $^{14}CCl_4$ to lipids and proteins, as well as the CCl_4-induced destruction of P-450 is more intense in SER than in RER while the opposite was found for glucose 6-phosphatase (G6P-ase) destruction. CCl_4-induced lipid peroxidation is as intense in SER as is in RER. ^{14}C from $^{14}CCl_4$ gets irreversibly bound to ribosomal proteins.

It has been postulated that CCl_4 hepatotoxicity is related to an activation process occurring in liver during an intermediate step of its own metabolism (1,2,3,4). This metabolic transformation would be mediated by the mixed-function oxygenase system from the endoplasmic reticulum (4). It is also known that the endoplasmic reticulum presents two morphologically different forms, the rough (RER) and the smooth (SER) endoplasmic reticulum, distinguished respectively by the presence or the absence of bound polysomes. Several investigators have established the heterogeneous nature of enzyme distribution between both forms. For example, many of the microsomal enzymes that require NADPH and oxygen, utilize cytochrome P-450 (P-450) and catalyze the metabolism of foreign compounds, are concentrated in smooth microsomal membranes (5,6,7,8,9) while glucose 6-phosphatase (G6P-ase) activity is higher in rough membranes than in smooth ones (10,11,12).

If present theories about the mechanism of CCl_4 hepatotoxicity were corect,

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one should expect an heterogeneous effect of CCl_4 on the two forms of the endoplasmic reticulum, to give a more intense damage on the smooth component than on the rough one. In this work we have analyzed this possibility.

Materials and Methods

All the chemicals employed were reagent grade. Sprague-Dawley male rats (190-250 g) were used in this study. Food was withdrawn 12-14 hr before administration of either $^{14}CCl_4$ or CCl_4, but water was available "ad libitum". The $^{14}CCl_4$ (27.5 mCi/mM) was dissolved in olive oil to give a solution producing 1,400,000 dpm/ml. This solution was given ip at a dose of 5 ml of solution/kg. CCl_4 was given ip as a 20% solution in olive oil at a dose of 5 ml solution/kg. Controls received olive oil. The animals were sacrificed by decapitation 3 hr after administration of either $^{14}CCl_4$ or CCl_4. After bleeding, the livers were rapidly excised and processed. Fractions of hepatic smooth and rough microsomes were prepared by the method of Dallner as described by Holtzman et al. (8). Free and membrane-bound polysomes were isolated according to Ragnotti et al. (13). The methods for the measurement of the extent of the irreversible binding of $^{14}CCl_4$ to lipids, lipid peroxidation, G6P-ase, ethylmorphine N-demethylase (EM-ase), P-450 content, as well as the statistical treatment of the data were previously described (14,15). The extent of the irreversible binding of $^{14}CCl_4$ to proteins was measured according to Rao and Recknagel (16). P-450 reductase activity was determined according to Gigon et al. (17).

Results

As shown in Table 1, the EM-ase and the P-450 reductase activities as well as the P-450 content in SER are higher than in RER, the ratios SER/RER being 2.13; 1.83 and 2.34 respectively. The irreversible binding of ^{14}C from $^{14}CCl_4$ to microsomal lipids and proteins is more intense in SER than in RER (Table 2). The ratio SER/RER is 1.3 for the case of the irreversible binding to lipids and 1.2 for the irreversible binding to proteins.

Table 1

EM-ase ACTIVITY, P-450 CONTENT AND NADPH P-450 REDUCTASE ACTIVITY IN SMOOTH AND ROUGH MICROSOMES FROM RAT LIVER

	SER	RER
EM-ase	51 ± 6	24 ± 3
P-450	0.57 ± 0.05	0.31 ± 0.10
P-450 reductase	0.89 ± 0.30	0.38 ± 0.18

Preparations and assays are given in Methods. EM-ase activity is given in mμmoles of formaldehyde formed in 15 min. per mg of protein at 37°; P-450 content is expressed in mμmoles per mg of protein and P-450 reductase activity is given in mμmoles of P-450 reduced at 25° per min. per mg of protein.
Values are given as mean ± S.D. of the results obtained with ten animals per group. Values from SER are significantly higher than those for RER for the three parameters ($p < 0.001$).

Table 2

IRREVERSIBLE BINDING OF ^{14}C FROM $^{14}CCl_4$ TO LIPIDS AND PROTEINS OF SMOOTH AND ROUGH MICROSOMES FROM RAT LIVER

Irreversible binding of ^{14}C from $^{14}CCl_4$ dpm/mg lipid or protein	SER	RER
to lipids	342 ± 25	265 ± 29
to proteins	140 ± 15	114 ± 14

Preparations and assays are given in Methods. Values are given as mean ± S.D. of the results obtained with ten animals per group. Values from SER are significantly higher than those for RER for both parameters ($p < 0.01$).

In Table 3 we can see that the destruction of P-450 by CCl_4 is more intense in SER than in RER, since a 52.6% decrease was observed in SER while only

Table 3

CCl_4-INDUCED LIPID PEROXIDATION AND DESTRUCTION OF P-450 AND G6P-ase IN SER AND RER FROM RAT LIVER

	SER	RER
P-450 *		
Control	0.57 ± 0.05	0.31 ± 0.10
CCl_4	0.27 ± 0.04	0.23 ± 0.03
G6P-ase *		
Control	45 ± 7	53 ± 5
CCl_4	27 ± 5	27 ± 5
Lipid peroxidation		
Control	173 ± 22	170 ± 23
CCl_4	231 ± 17	263 ± 18

Preparations and assays are given in Methods. Values are given as mean ± S.D. of the results obtained with 8 animals (P-450); 10 animals (G6P-ase) and 5 animals (Lipid peroxidation) per group. The effect of CCl_4 on all the parameters in a given fraction was statistically significant ($p < 0.001$; except for the effect on P-450 in RER when $p < 0.05$).

* The effect of CCl_4 in SER was significantly different to that in RER ($p < 0.05$ for G6P-ase and $P < 0.001$ for P-450).

25.8% in RER. Conversely, in the case of the G6P-ase activity suppression by CCl_4, we found that it is slightly more pronounced in RER than in SER since 49% destruction was obtained in the former and 40% in the later. CCl_4-induced lipid peroxidation is as intense in SER as in RER. As shown in Table 4, the ^{14}C from $^{14}CCl_4$ is able to bind in an irreversible manner to the protein moieties of both, the membranous portion and the polysomal component of the RER.

Discussion

As expected if present views about the mechanism of CCl_4 hepatotoxicity were correct, most of the CCl_4-induced damage to components of the endoplas-

Table 4

IRREVERSIBLE BINDING OF ^{14}C FROM $^{14}CCl_4$ TO LIPIDS AND/OR PROTEINS OF FREE AND MEMBRANE-BOUND POLYSOMES

Polysomes	Irreversible binding of ^{14}C from $^{14}CCl_4$ to lipids and proteins	
	dpm/mg lipid	dpm/mg protein
membrane-bound	174 ± 17	160 ± 77
free	----	59 ± 9

Preparations and assays are given in Methods. Values are mean ± SD of the results obtained with 8 animals per group.
The effect of CCl_4 on proteins was significant ($p < 0.01$).

mic reticulum is more intense in the SER than in the RER since the P-450 content, P-450 reductase activity and EM-ase activity are higher in SER than in RER. In agreement with our previous assumptions (14,15,18,19) that P-450 is destroyed by the $\cdot CCl_3$ and $\cdot Cl$ arised from CCl_4 activation and not by a lipid peroxidation process, we found more destruction of P-450 in SER than in RER and a more intense activation of CCl_4 to $\cdot CCl_3$ and $\cdot Cl$ in the SER than in the RER (as indicated by the relative extents of the irreversible binding of $^{14}CCl_4$ to microsomal lipids, which arises from the addition of the $\cdot CCl_3$ and $\cdot Cl$ to the double bonds of their unsaturated fatty acids (20)) while lipid peroxidation is as intense in SER as in RER. It is interesting to point out that part of the irreversible binding of the ^{14}C from $^{14}CCl_4$ to the proteins of the RER is due to the contribution of the binding to the polysomal component, since we also found labeled the proteins from free polysomes. The irreversible binding of $^{14}CCl_4$ to ribosomes might be involved in the CCl_4-induced breakdown of polysomes to ribosomal monomers and sub-

units (21), since several antibiotics which are also able to get bound to ribosomal proteins cause a similar effect (22).

In contrast to our expectations, G6P-ase was slightly more damaged by CCl_4 in RER than in SER. This apparently contradictory result does not necessarily mean that our previous assumptions (14,19) were not correct, since it is known that G6P-ase activity in the rough subfraction is appreciably more labile to several treatments than in its smooth counterpart (23) and consequently the observed different behavior to CCl_4 damage, may merely reflect a different sensitivity to CCl_4 rather than a different exposure to a deleterious agent.

Acknowledgements

This research was supported by grant AM 13195-04 from the National Institutes of Health (USA), and by grants from the Consejo Nacional de Investigaciones Cientificas y Tecnicas and from the Instituto Nacional de Farmacologia y Bromatologia, Argentina.

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