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CYTOCHROME P-450 LOWERING EFFECT OF ALKYL HALIDES, CORRELATION WITH DECREASE IN ARACHIDONIC ACID

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SUMMARY

Treatment of male rats with carbon tetrachloride, bromotrichloromethane, chloroform, 1,2-dibromoethane, 1-bromo-2-chloroethane, and 1,2-dibromo-3-chloropropane results in a decrease in cytochrome P-450 content and alterations in the relative content of fatty acids in hepatic microsomes. A high correlation was found between the loss of cytochrome P-450, the decrease in arachidonic acid (r=0.93), and the increases in linoleic (r=-0.91) and oleic acids (r=-0.89).

INTRODUCTION

Exposure of male rats to a single dose of the alkyl halides, carbon tetrachloride, bromotrichloromethane, halothane, and 1,2-dibromo-3-chloropropane is associated with a decrease in the content of hepatic microsomal cytochrome P-450 (1-4). Following carbon tetrachloride and bromotrichloromethane the decrease in cytochrome P-450 reaches 50% of maximum loss within four hours. This rapid loss of the microsomal cytochrome is accompanied by a pulse of peroxidation of the microsomal lipids and a decrease in the microsomal content of the polyunsaturated arachidonic acid (2,5). The loss of cytochrome P-450 is not detectable until 12-18 hours after treatment with 1,2-dibromo-3-chloropropane or halothane, and no increase in the peroxidation of microsomal lipids has been detected (4,6). This data is consistent with the notion that the early loss of cytochrome P-450 in animals treated with alkyl halides with low bond-dissociation energies (i.e., CCl_4 and $CBrCl_3$) is associated with peroxidation of the microsomal lipids. The relationship of the lowering effect of alkyl halides with higher bond dissociation energies to changes in the microsomal lipids has not been fully assessed.

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We have examined the microsomal fatty acid composition and cytochrome P-450 content of animals treated with a number of alkyl halides. Treatment with all of these compounds caused a decrease in the percentage of arachidonic acid. The extent of this loss correlated significantly with the extent of cytochrome P-450 loss.

METHODS

Male Sprague-Dawley rats (C.D., Charles Rivers, Wilmington, Mass) weighing 200-250 grams were housed in steel-screened bottom cages and provided with food and water ad libitum. Twelve hours prior to treatment, the food was removed. The alkyl halides were administered in mineral oil by gastric intubation. The doses were: carbon tetrachloride (2.5 ml/kg), bromotrichloromethane (0.26 ml/kg), chloroform (1.0 ml/kg), 1,2-dibromoethane (0.10 ml/kg), 1-bromo-2-chloroethane (0.15 ml/kg) and 1,2,-dibromo-3-chloropropane (0.30 ml/kg). Control animals received mineral oil (5.0 ml/kg) alone. The animals were sacrificed eighteen hours after treatment under light ether anesthesia, the livers perfused with 0.9% saline, homogenized in 0.2 M potassium phosphate (pH 7.4) and the microsomes isolated as previously described (4). Cytochrome P-450 content was measured in suspensions in phosphate buffer using the method of Omura and Sato (7). Lipids were extracted after the method of Folch et al. (8), transesterified, and the fatty acid composition determined by gas chromatography as previously described (9), using a Hewlett-Packard 5830 A gas chromatograph equipped with a Supelco SP-2330 column and a flame ionization detector.

Results and Discussion

The fatty acid composition of microsomal lipids from control and treated animals is presented in Table 1. Eighteen hours after exposure, the relative abundance of the fatty acids was significantly altered by all the compounds tested. The relative content of palmitic (16:0), oleic (18:1), and linoleic acid (18:2) was increased by all treatments, except for a decrease in oleic acid of animals treated with 1,2-dibromo-3-chloropropane. The relative content of stearic (18:0), arachidonic (20:4) and docosahexanoic acid (22:6) were decreased except for an increased percentage of docosahexanoic acid in the animals treated with chloroform. The consistency of the alterations suggest that the response of the microsomal fatty acids arose from a mechanism common to intoxication by all the alkyl halides tested.

Treatment with the different alkyl halides resulted in a range of losses in microsomal cytochrome P-450 (Fig. 1). Carbon tetrachloride and bromotri-

Table I. Fatty Acid Composition of Hepatic Microsomes from Rats Treated with Alkyl Halides.[a]

Treatment	16:0	18:0	18:1	18:2	20:4	22:6
Control	15.44±0.48	25.76±0.92	7.02±0.52	0.87±0.52	30.25±0.76	6.14±0.71
Carbon tetrachloride	20.46±1.56[b]	23.40±0.91[b]	11.54±1.39[b]	14.02±1.01[b]	18.64±1.65[b]	6.09±1.21
Bromotrichloromethane	21.84±0.28[b]	23.67±1.21[b]	12.40±0.80[b]	13.87±0.89[b]	17.65±0.41[b]	4.16±0.88[b]
Chloroform	19.58±0.51[b]	21.57±0.42[b]	8.04±0.29[b]	11.60±0.45[b]	25.19±0.49[b]	7.11±0.48
1,2-Dibromoethane	17.47±0.91[b]	24.29±0.54	9.07±0.59[b]	12.81±0.69[b]	25.80±0.58[b]	4.80±0.48
1-bromo-2-chloroethane	15.50±0.50	23.29±1.06[b]	9.68±0.33[b]	13.41±0.94[b]	24.36±1.57[b]	4.30±0.17[b]
1,2-Dibromo-3-chloropropane	18.68±0.58[b]	24.00±1.69[b]	6.64±0.60	10.66±1.21	28.09±1.35[b]	5.89±0.97

a-Microsomes were isolated from animals eighteen hours after receiving a single dose of the compound listed and fatty acid composition determined on lipid extracts by gas chromatography as described in the methods.

b-Significantly different from controls, $p<0.01$

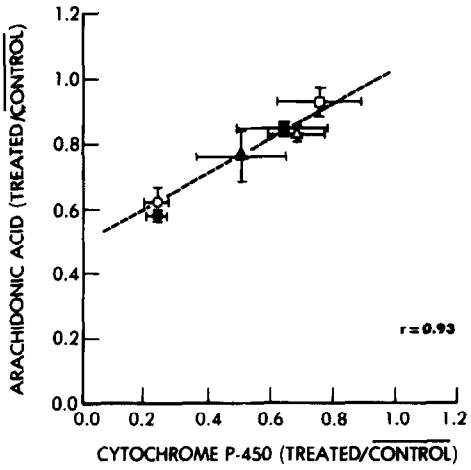

Figure 1. Scattergram of the changes in hepatic microsomal cytochrome P-450 and arachidonic acid (20:4). The contents of microsomal cytochrome P-450 and arachidonic acid were determined from the livers of animals eighteen hours following treatment with carbon tetrachloride (○), bromotrichloromethane (●), chloroform (△), 1,2-dibromoethane (▲), 1-bromo-2-chloroethane (■), and 1,2-dibromo-3-chloropropane (□), as described in the methods section. Values were expressed as a ratio to their mean control values, with the vertical and horizontal bars representing one S.D. (control value for cytochrome P-450 was 69.4 ± 5.9 nmole/liver/ 100 g body weight).

chloroethane treatment reduced the cytochrome P-450 levels the most to 24% of control levels. Successively lesser effects resulted from exposure to 1-bromo-2-chloroethane (51%), 1,2,-dibromoethane (64%), chloroform (69%) and 1,2-dibromo-3-chloropropane (76%). All of the changes are significant after eighteen hours, and progress to lower values within two days after treatment (Moody and Smuckler, unpublished data).

If a relationship exists between the changes in the microsomal lipids and changes in the content of cytochrome P-450, the extent of the alterations would be expected to be consistent in individual animals. Therefore, the fatty acid and cytochrome P-450 values were expressed as ratios to their mean control values. The changes in cytochrome P-450 were plotted against the changes in the different fatty acids (as depicted in Fig 1) and correlation coefficients determined.

Table II. Correlation of Changes in Microsomal Cytochrome P-450 with the Changes in Microsomal Fatty Acids.[a]

Fatty Acid	Correlation Coefficient
16:0	-0.37 -(0.00-0.76)[b]
18:0	0.14 (0.00-0.63)
18:1	-0.89[c] -(0.68-0.96)
18:2	-0.91[c] -(0.73-0.97)
20:4	0.93[c] (0.79-0.98)
22:6	0.26 (0.00-0.70)

a-correlation coefficients were determined from scattergrams as depicted in Figure 1, N=18

b-values in parenthesis are 95% confidence intervals

c-r is significantly different from zero, $p<0.01$

An extremely high correlation (r=0.93) was found between the loss of cytochrome P-450 and the decrease in arachidonic acid. This was associated with high negative correlations with the increases in linoleic, r=-0.91, and oleic acid, r=-0.89 (Table 2). No significant correlation was noted with the changes in palmitic, stearic, and docosahexanoic acid.

These results have demonstrated that intoxication with alkyl halides, irrespective of their ability to cause detectable peroxidation of microsomal fatty acids, does cause significant alterations in the cytochrome P-450 and fatty acid composition of the isolated microsomes. The acute effect of chloroform and 1,2-dibromoethane on cytochrome P-450 may now be added to those reported previously (1-4). The close correlation found between the

decrease in cytochrome P-450 and changes in arachidonic, oleic, and linoleic acids suggest a common mechanism of response. This may result from a common susceptibility of the factors which regulate the biological content of these microsomal components. In particular, the shift in the composition of the fatty acids is consistent with a defect in the synthesis of arachidonic acid from its precursor, linoleic acid, after treatment with alkyl halides.

At this time, however, a more direct relationship between the decrease in cytochrome P-450 and the alteration in fatty acid content cannot be excluded. The interdependnece of the microsomal cytochrome with the lipid within its immediate membrane environment is not yet established. The level of cytochrome P-450 is known to depend upon the polyunsaturated fatty acid content of the diet (10,11). Also, diacylphospholipids are required for the reconstitution of the cytochrome P-450 dependent mixed function oxidase activities (12), and the substrate binding spectrum of cytochrome P-450 is effected by the presence of polyunsaturated fatty acids (13,14). Studies on the mechanism of decrease in microsomal cytocrome P-450 and arachidonic acid following exposure to alkyl halides may provide information on the inter-relationship of cytochrome P-450 with the fatty acids in its membrane environment.

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