

Effects of Aliphatic Chlorohydrocarbons on Drug-metabolizing Enzymes in Rat Liver *in vivo*

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1. Various polychlorinated hydrocarbons were administered intragastrically to rats to examine their effects on the biotransformation capacity of the liver. Due to high toxicity, 1,1,2,2-tetrachloroethane and pentachloroethane were given at a dose level equivalent to one quarter of that of CCl_4 and the other chlorohydrocarbons (i.e. 2.6 mmol/kg).

2. Carbon tetrachloride at 10.3 mmol/kg was the most active in decreasing cytochrome P-450 content and the overall drug hydroxylation activities in rat liver. 1,1,2,2-Tetrachloroethane was the next most active in decreasing the hepatic drug oxidizing enzymic activities.

3. Epoxide hydratase activity in rat liver declined significantly after CCl_4 , 1,1,2,2-tetrachloroethane and pentachloroethane administrations.

4. UDP-Glucuronosyltransferase was affected to a lesser extent than the microsomal mono-oxygenase or epoxide hydratase by chlorohydrocarbon treatment.

111 Tetrachloroethane

Introduction

Aliphatic chlorinated hydrocarbons have a widespread and essential role in the chemical industry and in a variety of manufacturing operations. As a group, the chlorohydrocarbons share several biological properties. Some properties, such as toxicity are associated with only certain members of the series, and these manifestations may present a wide spectrum according to which compound is responsible for the poisoning. Plaa, Evans & Hine (1958) evaluated hepatotoxicity in mice by judging the impairment of detoxication of barbiturates after exposure of the mice to chlorinated hydrocarbons. More recently hepatic damage has been evaluated in mice by determination of the serum transaminase levels after exposure (Gehring, 1968).

The binding of substrates to P-450 haemoprotein is supposed to be required for the compound to be metabolized by the mono-oxygenase system. The metabolism may result in either a decrease or an increase of biological activity; e.g. carbon tetrachloride is activated to its toxic form by microsomal mono-oxygenase system (cf. Recknagel, 1967). In a previous study we found that the addition of various chlorinated hydrocarbons to liver microsomal suspension resulted in a type I spectral interaction (Pelkonen & Vainio, 1975). The increasing number of chlorine atoms resulted in an increased magnitude of the type I spectral change and an increased affinity to cytochrome P-450. To evaluate the relationship between the binding of chlorohydrocarbons to cytochrome P-450 and their toxicity, the various chlorinated hydrocarbons were

administered intragastrically to rats and the activities of hepatic microsomal-bound mono-oxygenase, epoxide hydratase and UDP-glucuronosyltransferase measured.

Materials and methods

Male Wistar rats weighing about 250 g fed *ad libitum* on commercial pellets (Hankkija Ltd., Turku, Finland) were used. Carbon tetrachloride (analytical grade, purity 99.8% (g.l.c.), Merck AG, Darmstadt, Germany) 10.3 mmol/kg, 1,1,1-trichloroethane (practical grade, purity at least 95% (g.l.c.) Fluka AG, Buchs, Switzerland) 10.3 mmol/kg, 1,1,2,2-tetrachloroethane (reagent grade, purity 98% (g.l.c.) Merck-Schuchardt AG, Munchen, Germany) 2.6 mmol/kg, tetrachloroethylene (technical grade, Tamro Ltd., Finland) 10.3 mmol/kg, pentachloroethane (practical grade, purity at least 95% (g.l.c.) Fluka AG, Buchs, Switzerland) 2.6 mmol/kg, and hexachloroethane (practical grade, purity at least 95% (g.l.c.) Fluka AG, Buchs, Switzerland) 10.3 mmol/kg were administered intragastrically in olive oil 24 h before the rats were killed. Control animals were treated with an equivalent volume (5 ml/kg) of olive oil.

CaCl₂-precipitated microsomes were prepared as described earlier (Vainio & Aitio, 1974). Digitonin (E. Merck AG, Darmstadt, Germany) and trypsin (type XII, from bovine pancreas, Sigma Chemical Company, St. Louis, Mo, U.S.A.) treatments used in connection with UDP-glucuronosyltransferase activity determination were performed as previously described (Hänninen & Puukka, 1970; Vainio, 1973).

Protein determination was carried out by the biuret method using bovine serum albumin (Armour Pharmaceuticals, Eastbourne, England) as reference (Layne, 1957).

Aryl hydrocarbon hydroxylase (EC 1.14.14.2) was measured fluorometrically with an Aminco-Bowman spectrofluorometer using 3,4-benzpyrene as substrate (Nebert & Gelboin, 1968; Vainio, 1973). In determination of *p*-nitroanisole *O*-demethylase activity the formation of *p*-nitrophenol from *p*-nitroanisole was recorded with a Unicam SP-800 spectrophotometer (Netter & Seidel, 1964). The cytochrome P-450 content was measured according to the method of Omura and Sato (1964). NADPH-cytochrome *c* reductase activity was determined by monitoring the reduction of cytochrome *c* at 550 nm (Phillips & Langdon, 1962; Vainio & Hänninen, 1972).

Epoxide hydratase activity was determined by the method of Oesch, Jerina & Daly (1971) as described earlier (Vainio & Parkki, 1974). The water phase containing styrene glycol formed was counted in Monophase 40 (Packard Instrument Co. Inc., Illinois, U.S.A.) without any further extraction with ethyl acetate. The counting efficiency achieved was 29% as determined by internal tritium standardization. (³H₂O was a gift from Dr. Niilo Kaartinen, Department of Physiology, University of Turku). The tritiated styrene oxide (NEN Chemicals Ltd, Dreieichenhain, Germany) was treated as described earlier (Marniemi & Parkki, 1975) to remove soluble product formed during storage.

UDP-Glucuronosyltransferase (EC 2.4.1.17) activity was determined with *p*-nitrophenol (0.5 mmol/l, Merck AG, Darmstadt, Germany) as aglycone. The UDP-glucuronic acid (Sigma Chemical Company) concentration used was 2.2 mmol/l (Hänninen, 1968).

Table 1. The effect of peroral administration of several aliphatic chlorohydrocarbons on drug biotransformation enzymes in rat liver

Compound	NADPH-Cyt. <i>c</i> reductase ($\mu\text{mol/g liver/min}$)		Cyt. P-450 (nmol/g liver)		Epoxide hydratase (nmol/g liver/min)		3,4-Benzpyrene hydroxylase (nmol/g liver/min)		<i>p</i> -Nitroanisole <i>O</i> -demethylase (nmol/g liver/min)	
Oil	0.55 $\pm$ 0.06	(6)	6.95 $\pm$ 0.54	(6)	187 $\pm$ 14	(6)	2.20 $\pm$ 0.20	(7)	10.6 $\pm$ 0.89	(6)
Carbon tetra- chloride	0.41 $\pm$ 0.06	(5)	1.47 $\pm$ 0.39***	(5)	113 $\pm$ 7***	(5)	0.76 $\pm$ 0.13***	(6)	3.1 $\pm$ 1.5**	(5)
1,1,1-Trichloro- ethane	0.57 $\pm$ 0.05	(5)	3.93 $\pm$ 0.50**	(5)	138 $\pm$ 14*	(5)	1.96 $\pm$ 0.21	(6)	7.5 $\pm$ 1.7	(5)
1,1,2,2-Tetrachloro- ethane	0.43 $\pm$ 0.11	(4)	3.70 $\pm$ 0.74**	(4)	93.0 $\pm$ 12**	(4)	0.65 $\pm$ 0.24**	(4)	4.6 $\pm$ 1.5**	(5)
Tetrachloro- ethylene	0.63 $\pm$ 0.09	(5)	5.81 $\pm$ 0.61	(5)	156 $\pm$ 19	(5)	1.25 $\pm$ 0.12**	(5)	7.2 $\pm$ 1.1*	(4)
Pentachloro- ethane	0.61 $\pm$ 0.05	(5)	4.54 $\pm$ 0.56*	(5)	124 $\pm$ 8**	(5)	1.56 $\pm$ 0.27	(5)	7.7 $\pm$ 0.6	(5)
Hexachloro- ethane	0.73 $\pm$ 0.05	(5)	3.38 $\pm$ 0.73**	(5)	184 $\pm$ 12	(5)	0.75 $\pm$ 0.07***	(5)	5.9 $\pm$ 0.5**	(5)

The dose given was 2.6 mmol/kg body wt. for 1,1,2,2-tetrachloroethane and pentachloroethane, whereas that for other halogenated hydrocarbons was 10.3 mmol/kg body wt. The activities of NADPH-cytochrome *c* reductase (cytochrome *c* reduced, $\mu\text{mol/g liver w.wt./min}$), epoxide hydratase (styrene glycol formed, nmol/g w.wt./min), aryl hydroxylase (activity expressed as nmol hydroxylated benzpyrene formed/g liver w.wt./min), *p*-nitroanisole *O*-demethylase (*p*-nitroanisole formed/nmol/g liver w.wt./min) as well as the amount of cytochrome P-450 (nmol/g liver w.wt.) were measured. The number of experiments is given in parentheses. Statistical analysis as compared to oil controls was performed with Student's *t*-test (***: $2P < 0.001$; **: $2P < 0.01$; *: $2P < 0.05$).

Results

Various chlorinated hydrocarbons were administered intragastrically to rats in equivalent doses (10.3 mmol/kg body wt.) except 1,1,2,2-tetrachloroethane and pentachloroethane, which were given at a dose level of 2.6 mmol/kg body wt. due to the lethality of higher doses to rats. Of the component reactions of the microsomal mono-oxygenase system, NADPH-cytochrome *c* reductase was not significantly affected by the chlorohydrocarbons (Table 1). Cytochrome P-450 was, however, greatly influenced by the treatments. Carbon tetrachloride, especially, was active in decreasing the P-450 haemoprotein in liver microsomes (cf. Recknagel, 1967). The other chlorinated hydrocarbons, at the dosage used, caused a diminution of cytochrome P-450 concentration to about one half of the control level, except for tetrachloroethylene which caused only a slight decrease (18%) (Table 1). The activities of overall drug oxidation reactions; hydroxylation of 3,4-benzpyrene and *O*-demethylation of *p*-nitro-anisole were decreased considerably especially by carbon tetrachloride, 1,1,2,2-tetrachloroethane, and hexachloroethane treatments (Table 1).

Epoxide hydratase activity, which is responsible for conversion of the intermediate epoxides into dihydrodiols (Oesch, 1973), declined greatly after carbon tetrachloride (40%), 1,1,2,2-tetrachloroethane (50%) and pentachloroethane (34%) administration. Statistically a significant decrease was also achieved by 1,1,1-trichloroethane. The other compounds tested had no significant effect on this microsomal-bound enzyme (Table 1).

UDP-Glucuronosyltransferase activity, when measured from intact 'native' microsomes, was increased (53%) by carbon tetrachloride pretreatment of rats (Table 2). If the microsomes had been pre-treated *in vitro* with digitonin, a

Table 2. The effect of peroral administration of several aliphatic chlorohydrocarbons on UDP-glucuronosyltransferase activity.

Compound	Native microsomes		Digitonin-treated microsomes		Trypsin-treated microsomes	
Oil	8.53 ± 1.25	(6)	72.0 ± 2.30	(6)	13.6 ± 2.5	(6)
Carbon tetra- chloride	13.01 ± 1.90	(5)	60.6 ± 3.3*	(5)	13.6 ± 1.3	(5)
1,1,1-Trichloro- ethane	6.02 ± 1.37	(5)	72.8 ± 6.5	(5)	14.1 ± 2.5	(5)
1,1,2,2-Tetrachloro- ethane	6.42 ± 0.73	(4)	60.1 ± 1.8**	(4)	11.0 ± 2.6	(4)
Tetrachloro- ethylene	8.02 ± 1.60	(5)	64.3 ± 4.4	(5)	22.2 ± 8.2	(5)
Pentachloro- ethane	9.11 ± 1.47	(5)	67.9 ± 2.2	(5)	15.5 ± 1.8	(5)
Hexachloro- ethane	4.91 ± 0.54*	(5)	69.9 ± 3.3	(5)	16.5 ± 5.0	(5)

The activity was measured both in native, in digitonin- and in trypsin-activated microsomes and was expressed as nmol *p*-nitrophenol bound/g liver w.wt./min in rat liver. For other details, see Table 1.

decrease in measurable UDP-glucuronosyltransferase activity could be observed in rats treated with carbon tetrachloride or tetrachloroethane (about 16% in both cases). After trypsin digestion of the hepatic microsomes *in vitro*, the transferase activity was at the control level even in carbon tetrachloride pre-treated rats (Table 2). The other chlorohydrocarbons studied had only minor effects on microsomal UDP-glucuronosyltransferase, both when measured in 'native' or in 'activated' microsomes (Table 2).

Discussion

In general, the effects which various chlorinated hydrocarbons have on the activities of different microsomal drug-metabolizing enzymes seem to have no correlation to the previously observed affinity towards cytochrome P-450 (Pelkonen & Vainio, 1975). The effects at the subcellular level are obviously more dependent on specific metabolism of the chlorohydrocarbons than mere binding to P-450 haemoprotein.

Cytochrome P-450, in addition to its catalytic role as terminal oxygen activator in drug metabolism, also functions as an active trapping agent for certain foreign compounds (Grundin *et al.*, 1974). This means that compounds which have a high affinity towards P-450 haemoprotein are effectively extracted from blood into tissues such as liver and kidney. In some instances even more toxic derivatives than the parent compound are formed during oxidation by cytochrome P-450. A good example here is carbon tetrachloride, the active form of which is formed by the microsomal mono-oxygenase system and which later sparks a process of lipid peroxidation (Slater, 1966; Recknagel, 1967). The early destruction of a component of the liver endoplasmic reticulum might be due to either direct attack of free radicals arising from carbon tetrachloride or to the deleterious effect of the lipid peroxidation process (Recknagel & Glende, 1973; Vainio & Parkki, 1974). While destroying the structure of the microsomal membranes, cytochrome P-450 is converted to inactive P-420 form and consequently the drug oxidations are inhibited. However, UDP-glucuronosyltransferase, which resides in a deeper location in the membrane structure (for references, see Vainio, 1975), is only activated *in vivo* by carbon tetrachloride re-treatment of rats (cf. also Aitio, 1974). The other chlorinated hydrocarbons now studied had a distinct decreasing effect on microsomal mono-oxygenation, whereas UDP-glucuronosyltransferase was not much affected by them.

1,1,2,2-Tetrachloroethane and pentachloroethane could not be given at a dosage level equivalent to carbon tetrachloride due to their lethality, but had to be decreased to one fourth of that dose. However, compounds with even still higher chlorine content, e.g. hexachloroethane, which has a high affinity towards cytochrome P-450 (Pelkonen & Vainio, 1975), was well tolerated by rats. Tetrachloroethane, which was administered at a dose level of one fourth of tetrachloroethylene, was surprisingly active in destroying the metabolic functions of microsomes. On the other hand, the metabolism of tetrachloroethylene as well as ethenes generally have been suggested to proceed via epoxides (Leibman & Ortiz, 1970). Epoxides have been implicated in various toxic reactions such as carcinogenesis, mutagenesis and necrogenesis (Jerina & Daly, 1974). It is

possible, however, that the biotransformation of tetrachloroethane proceeds via formation of a radical similarly to carbon tetrachloride (cf. Recknagel & Glende, 1973). The actions of carbon tetrachloride and tetrachloroethane in the present study differed, however, in various aspects, e.g. in behaviour towards UDP-glucuronosyltransferase.

Epoxide hydratase and cytochrome P-450 are possibly intimately linked in the microsomal membrane (Oesch & Daly, 1972). In the present study it was found that epoxide hydratase activity was decreased by administering many chlorinated hydrocarbons to rats, e.g. by carbon tetrachloride, 1,1,2,2-tetrachloroethane and pentachloroethane. It is interesting to note that among the compounds studied tetrachloro- and pentachloroethane were also the most toxic derivatives. This may be suggestive of their possible metabolic conversion to toxic epoxide derivatives. Some differences in the behaviour of cytochrome P-450 and epoxide hydratase could be seen, e.g. hexachloroethane, which decreased the content of cytochrome P-450 to about one half of the original (and highly inhibited the hydroxylation reactions) had no effect on epoxide hydratase activity. This may be due to the high affinity of this compound towards cytochrome P-450 as observed earlier (Pelkonen & Vainio, 1975).

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