

HCG
ASA
-1

OCT 31 1977

10/1/77

STIMULATION OF HEPATIC DRUG HYDROXYLATION AND
CONJUGATION BY A CUTANEOUSLY APPLIED PCB-MIXTURE
(CLOPHEN A 50)

JUKKA MARNIEMI^a, MARKKU NOKKALA^b, HARRI VAINIO^c, and KAARLO J.W.
HARTIALA^b.

^aThe Rehabilitation Examinations Centre of The Social Insurance Institution, 20720
Turku 72, ^bDepartment of Physiology, University of Turku, 20520 Turku 52 and
^cDepartment of Industrial Hygiene and Toxicology, Institute of Occupational Health,
00290 Helsinki 29 (Finland)

(Received January 31st, 1977)

(Revision received May 18th, 1977)

(Accepted May 26th, 1977)

Introduction

The polychlorinated biphenyls (PCBs) are widely used compounds [1,2]. Because of their widespread use and long half-life, the environment has become contaminated with PCBs [3]. They have been found in tissues of many birds and fish [4,5], human milk [6] and fat [7].

PCBs are known to be strong inducers of both the hepatic microsomal monooxygenase [8-10] and UDP glucuronosyltransferase [11]. PCB-compounds have been reported to differ catalytically as inducers of drug hydroxylation from the two earlier known inducer types, namely those of phenobarbital and 3-methylcholanthrene [12] exhibiting properties of both of them.

It is also known that components of PCB-mixtures like those of Aroclor 1254 are rapidly absorbed through the skin, leading to enhancement of hepatic cytochrome P-450 and drug hydroxylation reactions after cutaneous administration [13].

The present study was carried out to investigate the effects of small amounts of a commercial PCB-mixture (Clophen A 50) administered via the skin, on epoxide hydrase and glucuronide and glutathione conjugation of the liver, compared to effects of monooxygenase.

Materials and methods

Adult male random-bred Wistar rats (weight about 250 g) were used. Clophen A 50 (50% chlorinated, Oy Suomen Bayer, Helsinki, obtained as a

Abbreviations: PCB, polychlorinated biphenyls.

DSW 031564

gift from the State College of Veterinary Medicine, Finland) in acetone (200 or 400 μ l/kg) was applied on the shaved nuchal region of the rat skin (4 or 8 mg/kg) once daily for 3 or 6 consecutive days. Corn oil and dimethyl sulfoxide were also tested as solvents but the results were similar to those obtained with acetone. The rats were killed 24 h after the last administration and the microsomes of the liver were prepared by the CaCl_2 -aggregation method as earlier described [14]. The microsomes were treated in vitro with digitonin and trypsin as described by Hänninen [15] and Marniemi [16], respectively.

Enzyme assays. The aryl hydrocarbon hydroxylase activity in hepatic microsomes was determined fluorimetrically was 3,4-benzpyrene as substrate [17,18] and *p*-nitroanisole *o*-demethylase was measured spectrophotometrically [19] by quantitating the formation of *p*-nitrophenol [18,20].

NADPH cytochrome c reductase and the cytochrome P-450 content were determined according to Phillips and Langdon [21] and Omura and Sato [22], respectively.

UDP Glucuronosyltransferase activity was assayed with *p*-nitrophenol as the aglycone by following spectrophotometrically its disappearance [15,23].

The supernatant enzyme, glutathione S-transferase, measured from CaCl_2 -supernatant [24] and microsomal epoxide hydrase [25,26] were both determined radiochemically using tritium-labeled styrene oxide as substrate.

Results and discussion

Cutaneously applied small doses of Clophen A 50 appeared to enhance drug hydroxylation enzymes of the liver. Both the component enzymes (NADPH cytochrome c reductase and cytochrome P-450) and total hydroxylation reactions (*p*-nitroanisole *o*-demethylase, aryl hydrocarbon hydroxylase) were increased (50–80%) already after 3 days application. Six days administration still increased the enzyme activities, the highest enhancements (dose 8 mg/kg) being obtained in *p*-nitroanisole *o*-demethylase activity and cytochrome P-450 content (220 and 170%, respectively). These results are in accordance with those produced with Aroclor 1254 [13] when a dose of 25 mg/kg was given cutaneously during 6 days. According to the present study as small a dose as 4 mg/kg of Clophen A 50 administered cutaneously for 6 days is enough to cause an observable enhancement of drug hydroxylation in the rat liver.

Also the epoxide transforming enzymes, epoxide hydrase and glutathione S-transferase, were increased with these small doses of the PCB-mixture when administered via the skin (Table I). The highest enhancement (about 170%) was found in epoxide hydrase activity after 6 days application of 8 mg/kg of Clophen A 50. Glutathione S-transferase enhanced (52%) already after three days administration (8 mg/kg) and after 6 days the enhancement was of about the same magnitude. Also the lower PCB-dose (4 mg/kg) increased the activity of both enzymes during six days.

Furthermore the enzyme catalyzing glucuronide conjugation, microsomal UDPglucuronosyltransferase was shown to be elevated (Table I). After a

TABLE I

EFFECT OF CUTANEOUSLY APPLIED CLOPHEN A 50 ON HEPATIC EPOXIDE HYDRASE, GLUTATHIONE S TRANSFERASE AND UDPGLUCURONOSYLTRANSFERASE ACTIVITIES

Activities were measured after 3 or 6 days administration. Results are means from 4-5 animals. The statistical significance of the differences (Student's *t*-test) has been denoted as follows: (X), $2P < 0.05$; (XX), $2P < 0.01$.

	Glutathione S-transferase (μ mole/h $\cdot$ mg prot.)	Epoxide hydrase (nmole/h $\cdot$ mg prot.)	UDP Glucuronosyltransferase Non-activated microsomes	(nmole/h/mg prot.) Digitonin-treated microsomes
3 days				
Control	3.91 $\pm$ 0.58	280 $\pm$ 24	16.5 $\pm$ 2.2	263 $\pm$ 44
Clophen A 50	5.95 $\pm$ 0.53 (X)	370 $\pm$ 51	26.4 $\pm$ 3.3 (X)	343 $\pm$ 34
6 days				
Control	4.61 $\pm$ 0.35	214 $\pm$ 27	12.2 $\pm$ 1.8	207 $\pm$ 33
Clophen A 50 (4 mg/kg)	5.80 $\pm$ 0.51	559 $\pm$ 56 (XX)	23.0 $\pm$ 2.4 (X)	340 $\pm$ 74
Clophen A 50 (8 mg/kg)	6.56 $\pm$ 0.64 (X)	573 $\pm$ 85 (XX)	25.9 $\pm$ 6.6	402 $\pm$ 70 (X)

DSW 031566

three-day application the increment was highest when the enzyme activity was measured from the native, non-activated microsomes (60%) but also both in digitonin and trypsin treated microsomes slightly increased activities were observed. After six days two- (native and digitonin treated microsomes) to threefold (trypsin treated microsomes, not shown in the table) activities were to be seen when compared to the control level. The enhanced activities could be seen also with the smaller dose (4 mg/kg).

The data obtained indicate that even cutaneously applied small doses of PCB-mixtures easily reach the liver at concentrations high enough to induce besides the monooxygenation step also the subsequent epoxide hydration and glucuronide and glutathione conjugation steps of drug metabolism. In a recent study we have shown that the epoxide inactivating enzymes, glutathione S-transferase and epoxide hydratase of the rat liver could be doubled after a single intraperitoneal dose (100 mg/kg) of Clophen A-50 [27].

The present and earlier [13,28] results on the effects of cutaneously applied PCBs and those on the percutaneous penetration [29] of some pesticides and herbicides stress the importance of the skin as a route to the organism of many harmful pollutants. Fortunately, this tissue has shown also a high degree of adaptation in its ability to metabolize these compounds to less harmful and more water soluble derivatives [13,28].

Supported by Grant No. NIH AM 06018 and Juho Vainio Foundation, Finland.

- 1 J. Pichirallo, Leaks of toxic substances raises issues of effects, regulation, *Science*, 173 (1971) 899.
- 2 Y. Masuda, R. Kagawa and M. Kuratsune, Polychlorinated biphenyls in carbonless carbon paper, *Nature (London)*, 237 (1972) 41.
- 3 S. Jensen, Report of a new chemical hazard, *New Sci.*, 32 (1966) 612.
- 4 R.W. Risebrough, P. Reiche, D.B. Peakall, S.G. Herman and M.N. Kirven, Polychlorinated biphenyls in the global ecosystem, *Nature (London)*, 220 (1968) 1098.
- 5 J.H. Koeman, M.C.T.N. de Brauw and T.H. de Vos, Chlorinated biphenyls in fish, mussels and birds from the river Rhine and the Netherlands coastal area, *Nature (London)*, 221 (1969) 1126.
- 6 L. Acker and E. Schulte, Über das Vorkommen von chlorierten Biphenylen und Hexachlorbenzol neben chlorierten Insektiziden in Humanmilch und menschlichem Fettgewebe, *Naturwissenschaften*, 57 (1970) 497.
- 7 E.J. Biros, A.C. Walker and A. Medbery, Polychlorinated biphenyls in human adipose tissue, *Bull. Environ. Contam. Toxicol.*, 5 (1970) 317.
- 8 D.C. Villeneuve, D.L. Grant, W.E.J. Phillips, M.L. Clark and D.J. Clegg, Effects of PCB administration on microsomal activity in pregnant rabbits, *Bull. Environ. Contam. Toxicol.*, 6 (1971) 120.
- 9 C.L. Litterst, T.M. Farber, A.M. Baker and E.J. Van Loon, Effect of polychlorinated biphenyls on hepatic microsomal enzymes in the rat, *Toxicol. Appl. Pharmacol.*, 23 (1972) 112.
- 10 D.R. Bickers, L.C. Harber, A. Kappas and A.P. Alvares, Polychlorinated biphenyls. Comparative effects of high and low chlorine containing Aroclors on hepatic mixed function oxidase, *Res. Commun. Chem. Pathol. Pharmacol.*, 3 (1972) 505.
- 11 H. Vainio, Enhancement of microsomal drug oxidation and glucuronidation in rat liver by an environmental chemical, polychlorinated biphenyl, *Chem.-Biol. Interact.*, 9 (1974) 379.

DSW 031567

- 12 A.P. Alvares, D.R. Bickers and A. Kappas, Polychlorinated biphenyls: A new type of inducer of cytochrome P-448 in the liver, *Proc. Natl. Acad. Sci. (U.S.A.)*, 70 (1973) 1321.
- 13 D.R. Bickers, A. Kappas and A.P. Alvares, Differences in inducibility of cutaneous and hepatic drug metabolizing enzymes and cytochrome P-450 by polychlorinated biphenyls and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT) *J. Pharmacol. Exp. Ther.*, 188 (1974) 300.
- 14 H. Vainio and A. Aitio, Enhancement of microsomal drug hydroxylation and glucuronidation in rat liver by phenobarbital and 3-methylcholanthrene in combination, *Acta Pharmacol. Toxicol.*, 34 (1974) 130.
- 15 O. Hänninen, On the metabolic regulation in the glucuronic acid pathway in the rat tissues. *Ann. Acad. Sci. Fenn. Ser. A II*, 142 (1968) 1.
- 16 J. Marniemi, Bilirubin UDPglucosyl- and UDPglucuronosyltransferase of rat liver. A comparative study of the effects of membrane perturbants in vitro and of chrysene administration in vivo., *Chem. Biol. Interact.*, 9 (1974) 135.
- 17 D.W. Nebert and H.V. Gelboin, Substrate inducible microsomal aryl hydroxylase. II Cellular responses during enzyme induction, *J. Biol. Chem.*, 243 (1968) 6250.
- 18 H. Vainio, Drug hydroxylation and glucuronidation in liver microsomes of phenobarbital treated rats, *Xenobiotica*, 3 (1973) 715.
- 19 K.J. Netter and G. Seidel, An adaptively stimulated o-demethylating system in rat liver microsomes and its kinetic properties, *J. Pharmacol. Exp. Ther.*, 146 (1964) 61.
- 20 M.G. Parkki, J. Marniemi and H. Vainio, Action of styrene and its metabolites styrene oxide and styrene glycol on activities of xenobiotic biotransformation enzymes in rat liver in vivo., *Toxicol. Appl. Pharmacol.*, 38 (1976) 59.
- 21 A.H. Phillips and R.G. Langdon, Hepatic triphosphopyridine nucleotidocytochrome c reductase: Isolation, characterization, and kinetic studies, *J. Biol. Chem.*, 237 (1962) 2652.
- 22 T. Omura and R. Sato, The carbon-monoxide binding pigment of liver microsomes. II Solubilization, purification and properties, *J. Biol. Chem.*, 239 (1964) 2379.
- 23 K.J. Isselbacher, Enzymatic mechanisms of hormone metabolism. II. Mechanism of hormonal glucuronide formation, in G. Pincus (Ed.), *Recent Progress in Hormone Research*, Vol. 12, Academic Press, New York 1956, pp. 134-151.
- 24 J. Marniemi and M.G. Parkki, Radiochemical assay of glutathione S-epoxide transferase and its enhancement by phenobarbital in rat liver in vivo., *Biochem. Pharmacol.*, 24 (1975) 1569.
- 25 F. Oesch, D.M. Jerina and J. Daly, A radiometric assay for hepatic epoxide hydrolase activity with [7-³H]styrene oxide, *Biochim. Biophys. Acta* 227 (1971) 685.
- 26 H. Vainio and M.G. Parkki, Protection of microsomal drug biotransformation enzymes against carbon tetrachloride by diethyldithiocarbamate in rat liver, *Res. Commun. Chem. Path. Pharmacol.*, 9 (1974) 511.
- 27 J. Marniemi, M.G. Parkki and H. Vainio, Long term effects of a single dose of DDT and PCB on drug activating-inactivating enzymes in rat liver., *Acta Physiol. Scand.*, Suppl., 440 (1976) 133.
- 28 D.R. Bickers, J. Eiseman, A. Kappas and A.P. Alvares, Microscope immersion oils: Effects of skin application on cutaneous and hepatic drug-metabolizing enzymes, *Biochem. Pharmacol.*, 24 (1975) 779.
- 29 R.J. Feldmann and H.I. Maibach, Percutaneous penetration of some pesticides and herbicides in man, *Toxicol. Appl. Pharmacol.*, 28 (1974) 126.