Biochemical Pharmacology, Vol. 28, pp. 3667–3668

0006-2952/79/1215-3667 $02.00/0

SHORT COMMUNICATIONS

Effects of inducers on the *in vivo* covalent binding of a vinyl chloride metabolite to liver fractions

(*Received* 31 *May* 1979; *accepted* 4 *July* 1979)

Vinyl chloride is extensively used in the synthesis of plastics [1]. Inhalation of this monomer produces liver fibrosis and angiosarcomas of the liver in rodents [2–5] and in man [1]. Vinyl chloride is transformed by cytochrome P-450 into a chemically reactive metabolite that covalently binds to macromolecules, destroys cytochrome P-450 and depletes hepatic glutathione [6–16]. It is presumed that the chemically reactive metabolite of vinyl chloride may be chloroethylene oxide and/or its chloracetaldehyde derivative [6–10, 13, 15, 16]. Epoxide hydrase converts reactive epoxides into inactive dihydrodiols [17] and might inactivate chloroethylene oxide [9, 15, 16]. Epoxide hydrase is an inducible microsomal enzyme [17]. Recently, we reported *in vitro* data consistent with the hypothesis that pretreatment with DDT* or phenobarbital increases both the formation of the reactive metabolite by cytochrome P-450 and its inactivation by epoxide hydrase [16]. We also reported *in vivo* data consistent with the hypothesis that cytochrome P-450 destruction is related to the formation rate of the metabolite, whereas glutathione depletion seems influenced both by the formation rate and the inactivation rate [16]. It was reasoned that some of the formed epoxide may destroy cytochrome P-450 during or immediately after its formation, and before epoxide hydrase can inactivate it, whereas only that fraction of the formed epoxide which has escaped inactivation by epoxide hydrase and has diffused in the cytosol would be available for binding to glutathione [16]. Extension of this compartmentalized model to *in vivo* covalent binding of the metabolite to liver fractions would suggest that some of the formed metabolite may bind to vicinal microsomal proteins immediately after it is formed, and before epoxide hydrase has any chance to inactivate it; this immediate vicinal microsomal binding should be related to the formation rate only. Some of the formed metabolite may later bind to more distant microsomal proteins or to cytosolic proteins or cytosolic glutathione. Epoxide hydrase may inactivate some of the formed epoxide before it eventually reaches these distant sites. This delayed, distant binding should be influenced both by the formation rate and by the inactivation rate. Inducers, which would increase both the formation rate and the inactivation rate [16], might increase *in vivo* covalent binding to microsomal proteins but have little effect on *in vivo* covalent binding to 100,000 *g* supernatant proteins. On the other hand, SKF 525-A, which inhibits the activity of cytochrome P-450, but not that of epoxide hydrase [17], should reduce similarly the *in vivo* covalent binding to microsomal proteins and the *in vivo* covalent binding to 100,000 *g* supernatant proteins. The purpose of this work was to check if this corollary of our two former hypotheses [16] would be verified or not after i.p. administration of [^{14}C]vinyl chloride.

Male Sprague–Dawley rats, weighing 160–200 g, were obtained from Charles–River, France (Elbeuf, France). Rats were allowed water and food (Autoclave 113, UAR) *ad lib*. Some animals received one of the following pretreatments: (a) SKF 525-A, 75 mg/kg i.p. or (b) DDT, 200 mg/kg i.p. or (c) phenobarbital, 100 mg/kg i.p. daily for 5 days. [^{14}C]vinyl chloride (sp. act., 1 mCi/mmole), labeled on the two carbon atoms, and dissolved in methanol (1 mmole/ml), was purchased from Commissariat à l'Energie Atomique, Saclay, France; its radiopurity, checked by gas–liquid chromatography was higher than 99 per cent. [^{14}C]Vinyl chloride (500 μmoles/kg; 500 μCi/kg) in 500 μl/kg of methanol was administered i.p. to control rats, or 15 min after the single dose of SKF 525-A, or 8 days after that of DDT, or 24 hr after the last dose of phenobarbital. Animals were killed 4 hr later and whole blood samples and tissue fragments were homogenized in 3 vol of 0.154 M KCl, 0.01 M Na^+–K^+ phosphate buffer, pH 7.4. Part of the whole liver homogenate was used to prepare microsomes and 100,000 *g* supernatant. The amount of vinyl chloride metabolite irreversibly bound to whole tissue proteins and to proteins of the liver fractions was determined as reported previously [16]; proteins were

* Abbreviations used: SKF 525-A, 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride; DDT, 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane; TCA, trichloroacetic acid.

Table 1. Vinyl chloride material irreversibly bound to whole tissue proteins*

	Liver	Blood	Kidney	Spleen	Muscle
	(nmol/g tissue)				
Control rats	70 ± 11	23 ± 3	22 ± 10	22 ± 3	2 ± 1
SKF 525-A-pretreated rats	26 ± 7†	8 ± 5†	8 ± 3†	8 ± 5†	1 ± 1†
DDT-pretreated rats	92 ± 6†	26 ± 4	28 ± 2	26 ± 2	3 ± 1†
Phenobarbital-pretreated rats	102 ± 14†	31 ± 19†	36 ± 8†	31 ± 2†	3 ± 1†

* [^{14}C]Vinyl chloride (500 μmoles/kg; 500 μCi/kg) in 500 μl/kg of methanol was administered i.p.; animals were killed 4 hr later and the amount of [^{14}C] material irreversibly bound to tissue proteins was measured; results are mean ± S.D. for four rats.

† Different from that in control rats, $P < 0.05$ (Student's *t* test).

Table 2. Vinyl chloride material irreversibly bound to hepatic microsomal or 100,000 g supernatant proteins*

	Microsomes (nmol/g liver)	(percent change)	100,000 g supernatant (nmol/g liver)	(percent change)
Control rats	19 ± 4		40 ± 8	
SKF 525-A-pretreated rats	6 ± 2†	−68%	15 ± 4†	−62%
DDT-pretreated rats	29 ± 7†	+53%	45 ± 2	+12%
Phenobarbital-pretreated rats	32 ± 5†	+68%	46 ± 9	+15%

* [^{14}C]Vinyl chloride (500 μmoles/kg, 500 μCi/kg) in 500 μl/kg of methanol was administered i.p.; animals were killed 4 hr later; hepatic fractions were prepared and the amount of [^{14}C] material irreversibly bound to proteins was measured; results are mean ± S.D. for four rats.

† Different from that in control rats, $P < 0.05$ (Student's t test).

precipitated with TCA, dried, washed three times with TCA, and repeatedly extracted in various solvents (methanol, heptane, ether); no detectable radioactivity was found in the three last solvent phases; the [^{14}C] material remaining on the proteins was then counted in an Intertechnique ABAC SL40 scintillation counter.

Table 1 shows that a [^{14}C] material became irreversibly bound to whole tissue proteins in various organs; binding to all organs tested was decreased by SKF 525 A-pretreatment and was increased by phenobarbital-pretreatment; DDT-pretreatment slightly increased the mean value for bound metabolite in all organs but this increase was only significant in the liver and muscle.

Reported results on the effects of phenobarbital-pretreatment on the metabolism of vinyl chloride have been conflicting: it has been reported both that phenobarbital increased [15] and did not modify [9, 16] *in vitro* covalent binding to microsomal proteins, increased *in vivo* covalent binding to whole liver proteins after exposure to 10 ppm [^{14}C]vinyl chloride [15] but did not modify it after exposure to 250 ppm [15], and increased [10] or did not modify [16] the hepatotoxicity of inhaled vinyl chloride. The hypothesis (see above) that inducers enhance both the formation and the inactivation of the metabolite [16] may afford a possible explanation for these apparent discrepancies. Whether the former effect predominates or not over the latter may depend on the pretreatment schedule, the strain of rats, the dose of vinyl chloride, and its route of administration.

Table 2 shows that SKF 525-A-pretreatment decreased similarly the *in vivo* covalent binding to microsomal proteins and that to 100,000 *g* supernatant proteins; DDT- or phenobarbital-pretreatment significantly increased the *in vivo* binding to microsomal proteins but not that to 100,000 *g* supernatant proteins.

These observations are consistent with predictions derived from the compartmentalized model presented above. Although this compartmentalized model obviously remains hypothetical, it has up to now been consistent with the *in vivo* effects of inhaled vinyl chloride and the *in vitro* binding measured in the absence or in the presence of 0.5 mM 1,1,1-trichloropropene 2,3-oxide [16], a potent inhibitor of epoxide hydrase [17], and with the *in vivo* covalent binding to liver fractions after i.p. administration of [^{14}C]vinyl chloride (Table 2). We believe that this model, albeit still hypothetical, may serve as a suitable working hypothesis for studies on the metabolism and the effects of vinyl chloride, and, possibly, of other compounds whose reactive epoxides may be inactivated by epoxide hydrase.

Acknowledgements—This work was supported by Grant 021 R 07 from Faculté de Médecine Xavier Bichat (Université de Paris 7) and ATP 58-78-90 from Institut National de la Santé et de la Recherche Médicale (INSERM).

Unité de Recherches de Physiopathologie Hépatique, INSERM U 24, Hôpital Beaujon, 92118 Clichy, France

DOMINIQUE PESSAYRE
JOA CARLOS WANDSCHEER
VERONIQUE DESCATOIRE
ANDREAS DOLDER
JEAN-PIERRE BENHAMOU

REFERENCES

1. P. D. Berck, J. F. Martin, R. S. Young, J. Creech, I. J. Selikoff, H. Falk, P. Watanabe, H. Popper and L. Thomas, *Ann. intern. Med.* **84**, 717 (1976).
2. T. R. Torkelson, F. Oyen and V. K. Rowe, *Am. ind. Hyg. Ass. J.* **22**, 354 (1961).
3. P. L. Viola, A. Bigotti and A. Caputo, *Cancer Res.* **31**, 516 (1971).
4. C. Maltoni and G. Lefemine, *Ann. N.Y. Acad. Sci.* **246**, 195 (1975).
5. L. Prodan, I. Suciu, V. Pislaru, E. Ilea and L. Pascu, *Ann. N.Y. Acad. Sci.* **246**, 159 (1975).
6. A. Barbin, H. Bresil, A. Croisy, P. Jacquignon, C. Malaveille, R. Montesano and H. Bartsch, *Biochem. biophys. Res. Commun.* **67**, 596 (1975).
7. E. S. Reynolds, M. T. Moslen, S. Szabo and R. Jaeger, *Res. Commun. chem. Pathol. Pharmac.* **12**, 685 (1975).
8. H. M. Bolt, H. Kappus, R. Kaufmann, K. E. Appel, A. Buchter and W. Bolt, *INSERM Symposia Series* **52**, 151 (1976).
9. H. Kappus, H. M. Bolt, A. Buchter and W. Bolt, *Toxicol. appl. Pharmac.* **37**, 461 (1976).
10. E. S. Reynolds, M. T. Moslen, S. Szabo and R. Jaeger, *Panminerva Med.* **18**, 367 (1976).
11. F. P. Guengerich and T. W. Strickland, *Molec. Pharmac.* **13**, 993 (1977).
12. K. M. Ivanetich, I. Aronson and I. D. Katz, *Biochem. biophys. Res. Commun.* **74**, 1411 (1977).
13. S. Osterman-Golkar, D. Hultmark, D. Segerbäck, C. J. Calleman, R. Göthe, L. Ehrenberg and C. A. Wachtmeister, *Biochem. biophys. Res. Commun.* **76**, 259 (1977).
14. P. G. Watanabe, J. A. Zempel, D. G. Pegg and P. J. Gehring, *Toxicol. appl. Pharmac.* **44**, 571 (1978).
15. F. P. Guengerich and P. G. Watanabe, *Biochem. Pharmac.* **28**, 589 (1979).
16. D. Pessayre, J. C. Wandscheer, V. Descatoire, J. Y. Artigou and J. P. Benhamou, *Toxicol. appl. Pharmac.* **49**, 505 (1979).
17. F. Oesch, *Xenobiotica* **3**, 305 (1972).