Biochemical Pharmacology, Vol. 28, pp. 589–596
© Pergamon Press Ltd. 1979. Printed in Great Britain

0006-2952/79/0301-0589 $02.00/0

METABOLISM OF [^{14}C]- and [^{36}Cl]-LABELED VINYL CHLORIDE *IN VIVO* AND *IN VITRO**

F. PETER GUENGERICH

Department of Biochemistry and Center in Environmental Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232, U.S.A.

PHILIP G. WATANABE

Toxicology Research Laboratory, Health and Environmental Research, The Dow Chemical Co., Midland, MI 48640, U.S.A.

(*Received* 5 *May* 1978; *accepted* 24 *July* 1978)

Abstract—Label from [^{14}C]vinyl chloride was covalently bound to protein and nucleic acids *in vivo* and *in vitro* in the presence of rat liver microsomal fractions or highly purified cytochrome P-450 and NADPH-cytochrome P-450 reductase preparations. The ratio of bound to total non-volatile metabolites increased in going from the *in vivo* to the microsomal to the purified system. [^{36}Cl]vinyl chloride was metabolized by microsomes and highly purified systems; no label was bound and most could be accounted for as chloride ion. Phenobarbital pretreatment of rats did not induce total metabolism of vinyl chloride *in vivo* at either 10 or 250 ppm exposure levels; however, binding to protein and RNA was enhanced at the 10 ppm but not the 250 ppm level. Phenobarbital pretreatment increased the *in vitro* microsomal conversion of vinyl chloride to both total and bound metabolites. A sizeable fraction of the label of [^{14}C]vinyl chloride metabolized *in vivo* was recovered in the microsomal fraction of the liver, but sodium dodecyl sulfate polyacrylamide gel electrophoresis of *in vitro* incubations indicated that the metabolites were distributed among many microsomal proteins and not localized to cytochrome P-450. Evidence was obtained for the metabolism of the suspected vinyl chloride metabolite chloroethylene oxide by microsomal epoxide hydratase. However, the epoxide hydratase inhibitor 3,3,3-trichloropropylene oxide, which blocks the microsomal degradation of chloroethylene oxide, did not enhance the level of vinyl chloride bound to either protein or adenosine.

Vinyl chloride is an industrial chemical that has been demonstrated to be carcinogenic in laboratory animals [1] and humans [2, 3]. The concept that a metabolite(s) of vinyl chloride is responsible for the observed carcinogenicity [4, 5] is supported by the findings that NADPH-dependent microsomal activation is necessary for vinyl chloride-mediated mutagenesis in *Salmonella typhimurium* tester strains [6, 7] and for binding of vinyl chloride to protein and nucleic acids [8–10]. The vinyl chloride metabolite responsible for these activities has been postulated to be the potent electrophile chloroethylene oxide [11].

Liver microsomal cytochrome P-450 has been shown to activate vinyl chloride to a species that destroys the heme of that cytochrome [12]. Cytochrome P-450 has also been suggested, on the basis of CO-inhibition experiments [9], as the enzyme responsible for activating vinyl chloride to metabolites bound to tissue nucleophiles. Spectral evidence [13] for a role of cytochrome P-450 in vinyl chloride metabolism is rather weak when the limitations of such data are considered [14]. Previous workers had reported that induction with phenobarbital increases the ability of the liver to convert vinyl chloride to a P-450 destructive species *in vivo* [15] and *in vitro* [12], observed *in vivo* vinyl chloride hepatotoxicity [16], and the capacity to convert vinyl chloride to mutagenic products *in vitro* [6]; however, induction with phenobarbital had no effect on the levels of vinyl chloride metabolism by rats *in vivo* and *in vitro* [8]. Recently, Watanabe *et al.* [17] reported that, in rats exposed *in vivo* to 100 ppm vinyl chloride, phenobarbital increased binding approximately 2-fold but had no effect on the level of total vinyl chloride metabolism.

The studies described in this report were initiated in order to establish the roles of liver microsomal cytochrome P-450 and epoxide hydratase in the biotransformation of vinyl chloride to metabolites, particularly those bound to protein and nucleic acids.

MATERIALS AND METHODS

Materials. 1, N^6-ethenoadenosine was purchased from P-L Biochemicals (Milwaukee, WI), calf thymus DNA and *Escherichia coli* soluble RNA from Calbiochem (La Jolla, CA), research grade vinyl chloride from Matheson (E. Rutherford, NJ), and 3,3,3-trichloropropylene oxide, (TCPO) from Aldrich (Milwaukee, WI). Chloroethylene oxide was prepared, as described previously [12, 18], on the day of use; identity was confirmed by N.M.R. and mass spectra and by reaction with 4-(*p*-nitrobenzyl)-pyridine [11]. [1,2-^{14}C]vinyl chloride was prepared from [1,2-^{14}C]dichloroethane [19] and was diluted with carrier vinyl chloride; the purity was as described previously [17, 19]. [^{36}Cl]vinyl chloride (0.10 mCi/m-mole) was synthesized from H^{36}Cl (New England Nuclear) and acetylene [20]; the

* This work was supported by National Institute of Environmental Health Sciences Grants ES 01590 and ES 00267 and Biomedical Research Support Grant RR-05424.

Contr. Epidem. Biostatist., vol. 1, pp. 1–22 (Karger, Basel 1979)

Environmental Teratogens

Their Significance and Epidemiologic Methods of Detection

M.A. Klingberg and C.M. Papier

Department of Epidemiology, Israel Institute for Biological Research, Ness-Ziona, and Department of Preventive and Social Medicine, Tel-Aviv University Sackler School of Medicine, Tel-Aviv

Although the past few years have shown a great increase in fundamental research in the field of congenital malformations, particularly the development of new techniques in genetics, biology and biochemistry, progress in the epidemiology of birth defects has been less remarkable.

Following *Gregg*'s discovery of the teratogenic effects of rubella in 1941 (44) it was widely but over-optimistically presumed that the discovery of other environmental agents responsible for malformations would be just around the corner. In the ensuing two decades the number of environmental factors discovered to be crucial determinants of congenital malformations can be counted on the fingers of one hand. After the 1960–61 epidemic of congenital malformations caused by thalidomide there was even more hope regarding the discovery of new environmental teratogens and for establishing new methods of detection and evaluation of teratogens in the human environment. More than 15 years have elapsed since this widely-publicized observation of thalidomide embryopathy in Europe and elsewhere, but even today only about 10% of malformations can be ascribed to environmental factors; those which can be attributed to genetic transmission and chromosomal aberrations account for approximately 25% of the defects. The causes of the great majority remain uncertain (127).

What then is the etiology of the estimated 60–70% of malformations that cannot be pin-pointed to any known causes? Many investigators believe that most presently unaccounted for malformations and syndromes result from the potentiative effect of two or more factors (127). But the thalidomide disaster remains a vivid reminder of the need for developing new methodologies of uncovering the etiologic factors responsible for the majority of birth defects. Epidemiologists must work in conjunction and cooperation with many disciplines in striving together towards the goal of prevention of congenital malformations.