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VINYLTOLUENE INDUCED CHANGES IN XENOBIOTIC-METABOLIZING ENZYME ACTIVITIES AND TISSUE GLUTATHIONE CONTENT IN VARIOUS RODENT SPECIES

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Abstract—Present short-term experiments with intraperitoneal injections in rats, mice and Chinese hamsters show time- and dose-dependent changes in reduced glutathione (GSH) content in the liver and kidneys. The depletion of GSH was evident within a few hours after the single injection of vinyltoluene to rats and mice, whereas after 24 and 30 hr the liver GSH concentration levels were even somewhat higher than those of control animals. At the same time, vinyltoluene administration enhanced both the overall drug oxidation reactions (*O*-deethylations of ethoxycoumarin and ethoxyresorufin) and UDP-glucuronosyltransferase activity in the liver. The highest dose of vinyltoluene (500 $mg \cdot kg^{-1}$) decreased acutely (within 6 hr) cytochrome P-450 content and ethoxycoumarin *O*-deethylase activity in mouse liver microsomes. The mouse was also observed to be more vulnerable than the rat as far as vinyltoluene-induced depression of non-protein sulfhydryl groups is concerned. The importance of the vinyl group in vinyltoluene and possible formation of an epoxide intermediate are also underlined by the demonstrated ability of vinyltoluene to bind to cytochrome P-450 with the appearance of type I difference spectrum.

Styrene (vinylbenzene) is widely utilized as a monomer for the manufacture of various plastics and resins, where human exposure can be considerable [1]. The recent information on the health hazards involved in exposure to styrene [1, 2] has increased the pressure to find a substitute without the toxic properties of styrene. Vinyltoluene has been suggested as a possible substitute. Vinyltoluene is also used in special coatings, adhesives and latexes. However, toxicity studies on vinyltoluene are at present almost totally lacking.

To gain insight into the metabolic activation/inactivation of vinyltoluene, we have investigated the effects of intraperitoneal injections on the activities of drug biotransformation enzymes and on GSH concentrations in the liver and kidneys of various rodent species.

MATERIALS AND METHODS

Animals and their treatment. Male rats of Wistar strain (225–260 g), male mice of C57BL/6 (27–35 g) and male Chinese hamsters (21–29 g) were fed on commercial pellet diets. All animals received tap water *ad lib.*

The rats received vinyltoluene (500 $mg \cdot kg^{-1}$ body wt) by intraperitoneal injection (i.p.) dissolved in olive oil. Vinyltoluene (methylstyrene, purum, mixture of isomers) was obtained from Fluka AG, Buchs. Rats were decapitated 1, 3, 6 and 24 hr after the injection. The mice received vinyltoluene (100 $mg \cdot kg^{-1}$ or 500 $mg \cdot kg^{-1}$ body wt) by i.p. injection, and were decapitated 1, 3, 6 and 30 hr after the injection. Vinyltoluene (100 $mg \cdot kg^{-1}$ or 500 $mg \cdot kg^{-1}$ body wt) was administered i.p. in hamsters once daily for 5 consecutive days. The hamsters were decapitated 3 days after the last injection. Control rats, mice and hamsters in each group received i.p. the corresponding volume of olive oil.

After decapitation, livers and kidneys were removed, cooled and stored at −70° for further analyses.

Biochemical analyses. Tissue samples were homogenized in 4 vol. 0.25 M sucrose–0.15 M KCl–10 mM HEPES* buffer, pH 7.4, and calcium aggregated microsomes were obtained as described previously [3]. The microsomal pellet was resuspended in 0.15 M KCl giving a concentration of microsomal protein corresponding to 1 g liver per ml suspension.

Nonprotein sulfhydryl concentration expressed as reduced glutathione (GSH) was assayed from tissue homogenate by the method of Saville [4]. The microsomal protein content was measured by the method of Lowry *et al.* [5]. The content of hepatic cytochrome P-450 was determined from the carbon monoxide difference spectrum of dithionite reduced microsomes with a Cary 219 Varian spectrofotometer according to the method of Omura and Sato [6]. The molar extinction coefficient of the reduced cytochrome P-450–CO complex used was 91 $cm^{-1} \cdot mM^{-1}$. The content of kidney cytochrome P-450 was assayed with the ascorbate-PES† method, as described by Johannesen and DePierre [7], except that we used 0.15 M KCl–0.1 M Na phosphate buffer (pH 7.4) instead of Tris (pH 7.5) and PMS‡ instead of PES†.

The microsomal 7-ethoxycoumarin *O*-deethylase activity was measured by a modified fluorometric method of Aitio [8]. One hundred microliters of

* HEPES = *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Sigma Chem. Co.).

† PES = phenazine ethosulfate.

‡ PMS = phenazine methosulfate (Sigma Chem. Co.).

1.5 mM 7-ethoxycoumarin in acetone was added into the assay tube and then the vehicle was evaporated. The incubation mixture in a final volume of 0.5 ml consisted of 0.3 mM 7-ethoxycoumarin, 125 mM Tris–HCl buffer (pH 7.4), 20 mM KCl, 5 mM $MgCl_2$, 0.005 mM $MnCl_2$, and a NADPH-regenerating system (0.5 mM $NADP^+$ disodium salt, 4.4 mM isocitric acid trisodium salt, 0.0125 unit isocitrate dehydrogenase, EC 1.1.1.42). 7-Ethoxyresorufin *O*-deethylase activity was measured according to Prough *et al.* [9]. 7-Ethoxyresorufin was synthesized by Dr. A. Zitting in our Institute. The microsomal UDP-glucuronosyltransferase (EC 2.4.1.17) was assayed with 0.43 mM *p*-nitrophenol as aglycone and 5.6 mM UDP-glucuronic acid (ammonium salt) as described by Hänninen [10].

Binding studies to cytochrome P-450. The untreated control male rats used were anesthetized lightly with ether. The portal vein was cannulated, the caval vein was incised and the liver was perfused *in situ* with 0.9% NaCl (37°) for about 4 min. The microsomes were gathered by centrifugation at 105,000 *g* for 60 min. The microsomal preparation was kept in an ice bath (maximally 2–3 hr) until the spectral measurements were made. A reaction mixture containing 1 mg of microsomal protein per ml of 0.1 M K-phosphate buffer, pH 7.4, was divided in two cuvettes. Vinyltoluene in acetone (obtained from Merck, *pro anal.*) was added with a Hamilton syringe into the sample cuvette. An equal volume of acetone was added into the reference cuvette. Difference spectra was recorded with a Cary 219 Varian spectrofotometer at room temperature. The concentration of vinyltoluene in the cuvette was 50–700 μM. The maximal concentration of acetone in the cuvette was 165 μmoles per ml.

Statistical analyses were made with Student's *t*-test.

RESULTS

Vinyltoluene was found to bind to hepatic rat cytochrome P-450 producing a type I difference spectrum, with a peak at 385 nm and a trough at 420 nm. The apparent spectral dissociation constant K_s and maximal absorption A_{max} as derived from double reciprocal plots were 0.53 ± 0.15 mM and 0.058 ± 0.011/nmoles P-450, respectively (values are the means ± S.D. from three animals).

Figures 1 and 2 present the dose- and time-dependent changes in GSH contents in liver and kidneys of rat and mouse caused by a single i.p. injection of vinyltoluene. In rat liver the GSH concentration was about 50 per cent of that in controls 3 hr after the administration. In 6 hr the GSH concentration in treated animals was only 26 per cent of that in controls. In 24 hr, however, the GSH level was even slightly higher than in the control liver. The GSH content in rat kidneys was only slightly affected by vinyltoluene injection (Fig. 1).

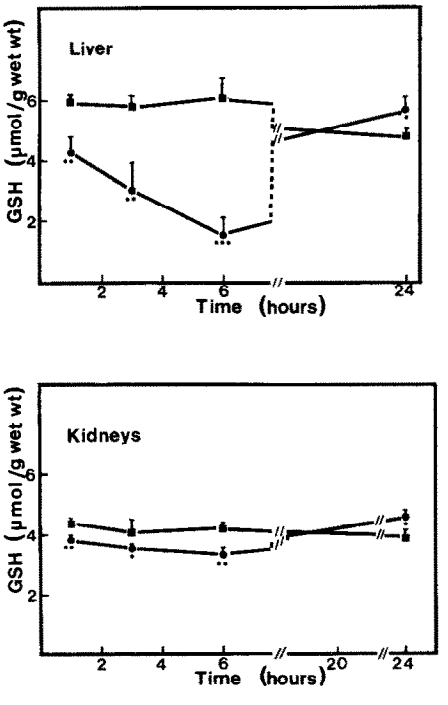

Fig. 1. Effect of a single administration (500 mg·kg^{-1}, i.p.) on reduced glutathione content in rat liver and kidneys. ■——■ = control animals, ●——● = exposed animals. The results are given as the mean ± S.D. value of four animals. Statistical differences from control group: *P < 0.05, **P < 0.01 and ***P < 0.001.

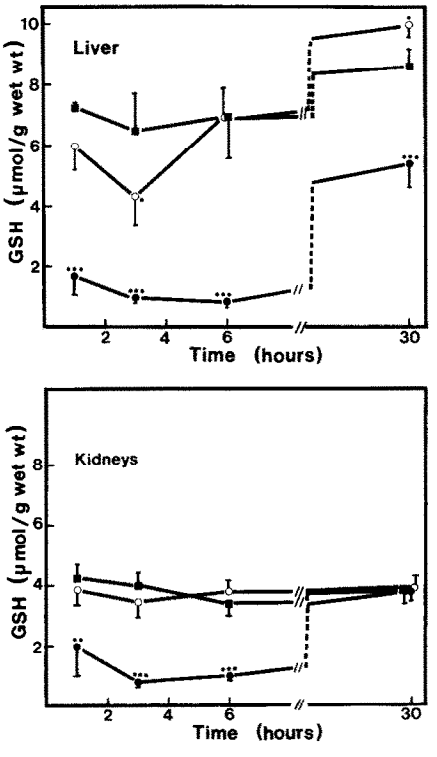

Fig. 2. Effect of a single vinyltoluene administration (100 mg·kg^{-1} or 500 mg·kg^{-1}, i.p.) on reduced glutathione content in mouse liver and kidneys. ■——■ = control, ○——○ = 100 mg·kg^{-1} and ●——● = 500 mg·kg^{-1}. The results are given as the mean ± S.D. values of two to five animals (see the notes in Table 2). Statistical differences from control group: P < 0.05, **P < 0.01 and ***P < 0.001.

Table 1. Effect of vinyltoluene administration (500 mg·kg^{-1}, i.p.) on microsomal cytochrome P-450 content, 7-ethoxycoumarin *O*-deethylase, 7-ethoxyresorufin *O*-deethylase and UDP-glucuronosyltransferase activities in rat liver*

Time after administration (hr)	Cytochrome P-450 (nmoles·mg^{-1})		7-Ethoxycoumarin *O*-deethylase (nmoles·min^{-1}·mg^{-1})		7-Ethoxyresorufin *O*-deethylase (nmoles·min^{-1}·mg^{-1})		UDP-glucuronosyltransferase (nmoles·min^{-1}·mg^{-1})	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
1	0.83 ± 0.03	0.82 ± 0.07	0.87 ± 0.54	1.12 ± 0.09	0.080 ± 0.016	0.082 ± 0.016	0.92 ± 0.16	1.26 ± 0.33
3	0.85 ± 0.09	0.77 ± 0.07	1.10 ± 0.15	1.01 ± 0.005	0.072 ± 0.004	0.096 ± 0.006‡	1.10 ± 0.05	1.48 ± 0.22†
6	0.72 ± 0.09	0.91 ± 0.10†	1.05 ± 0.40	1.12 ± 0.27	0.079 ± 0.028	0.162 ± 0.018‡	1.10 ± 0.15	1.38 ± 0.15†
24	0.80 ± 0.08	0.80 ± 0.06	1.06 ± 0.08	1.61 ± 0.02§	0.083 ± 0.015	0.341 ± 0.135†	1.14 ± 0.08	1.85 ± 0.20‡

* Results are given as the means ± S.D. from four animals.
† Statistical difference from control group $P < 0.05$.
‡ Statistical difference from control group $P < 0.01$.
§ Statistical difference from control group $P < 0.001$.

Table 2. Effect of vinyltoluene administration (100 mg·kg^{-1} or 500 mg·kg^{-1}, i.p.) on microsomal cytochrome P-450 content, 7-ethoxycoumarin deethylase and UDP-glucuronosyltransferase activities in mouse liver*

Time after administration (hr)	Cytochrome P-450 (nmoles·mg^{-1})			7-Ethoxycoumarin *O*-deethylase (nmoles·min^{-1}·mg^{-1})			UDP-glucuronosyltransferase (nmoles·min^{-1}·mg^{-1})		
	Controls	100 mg·kg^{-1}	500 mg·kg^{-1}	Controls	100 mg·kg^{-1}	500 mg·kg^{-1}	Controls	100 mg·kg^{-1}	500 mg·kg^{-1}
1	0.78 ± 0.04	0.74 ± 0.08	0.81 ± 0.08	2.53 ± 0.36	2.60 ± 0.32	2.69 ± 0.24	1.57 ± 0.07	1.86 ± 0.14‡	2.00 ± 0.27‡
3	0.76 ± 0.07	0.81 ± 0.04	0.71 ± 0.01	3.29 ± 0.37	3.61 ± 0.44	2.33 ± 0.11‡	2.09 ± 0.14	2.26 ± 0.57	2.31 ± 0.40†
6	0.81 ± 0.07	0.71 ± 0.08	0.56 ± 0.04‡	2.80 ± 0.47	2.88 ± 0.29	1.88 ± 0.18‡	2.34 ± 0.12	2.34 ± 0.21	2.23 ± 0.35
30	0.77 ± 0.09	0.79 ± 0.11	0.81 0.79§	3.24 ± 0.16	3.10 ± 0.27	5.45 3.30§	2.44 ± 0.19	2.50 ± 0.26	2.79 2.87§

* Results are given as the means ± S.D. from four to five animals.
† Statistical difference from control group $P < 0.05$.
‡ Statistical difference from control group $P < 0.01$.
§ The values are from two animals because three of the five animals died during the exposure (between 6 and 30 hr).

Table 3. Effect of vinyltoluene administration (100 or 500 mg·kg^{-1}, i.p., five daily doses, decapitation three days after the last i.p. injection) on GSH content, microsomal cytochrome P-450 content, 7-ethoxycoumarin deethylase, 7-ethoxyresorufin deethylase and UDP-glucuronosyltransferase activity in Chinese hamster liver and GSH content in Chinese hamster kidneys*

	Liver					Kidney
Exposure	GSH (μmoles·g^{-1} wet wt)	Cytochrome P-450 (nmoles·mg^{-1})	7-Ethoxycoumarin O-deethylase (nmoles·min^{-1}·mg^{-1})	7-Ethoxyresorufin O-deethylase (nmoles·min^{-1}·mg^{-1})	UDP-glucuronosyl-transferase (nmoles·min^{-1}·mg^{-1})	GSH (μmoles·g^{-1})
Control	6.22 ± 0.21	0.58 ± 0.05	1.08 ± 0.16	0.28 ± 0.04	4.34 ± 0.81	2.23 ± 0.06
100 mg·kg^{-1}	6.72 ± 0.54	0.69 ± 0.04‡	1.45 ± 0.21†	0.29 ± 0.08	4.58 ± 0.63	2.35 ± 0.08†
500 mg·kg^{-1}	6.90 ± 0.69	0.71 ± 0.13†	1.25 ± 0.44	0.23 ± 0.03†	5.25 ± 0.76†	2.56 ± 0.18‡

* Results are given as the means ± S.D. from four animals.
† Statistical difference from control group $P < 0.05$.
‡ Statistical difference from control group $P < 0.01$.

A single injection of vinyltoluene (500 mg·kg^{-1}) in 1 hr decreased GSH content of liver and kidneys in mouse to 23 and 47 per cent, respectively, of that in controls (Fig. 2). Three of the five animals died 6–30 hr after the injection of 500 mg·kg^{-1} of vinyltoluene. The GSH content in the livers and kidneys of the surviving two mice was increased 30 hr after the vinyltoluene administration. A single injection of 100 mg·kg^{-1} of vinyltoluene caused first a small decrease in GSH content of the liver and kidneys which was subsequently followed by an increase as compared to controls.

In rat liver the microsomal overall drug oxidation reactions, *O*-deethylations of 7-ethoxycoumarin and 7-ethoxyresorufin, were significantly increased (1.5- and 4-fold, respectively) 24 hr after a single vinyltoluene injection (500 mg·kg^{-1}).7-Ethoxyresorufin *O*-deethylase activity was already doubled 6 hr after the administration of vinyltoluene. The microsomal UDP-glucuronosyltransferase activity also increased 1.8-fold in 24 hr. No significant changes in microsomal cytochrome P-450 level could be detected within 24 hr. Neither were the cytochrome P-450 level or 7-ethoxycoumarin *O*-deethylase and UDP-glucuronosyltransferase activities affected in kidneys (data not shown).

In mouse liver the administration of 100 mg·kg^{-1} of vinyltoluene caused no significant effect in microsomal 7-ethoxycoumarin *O*-deethylase activity, whereas 500 mg·kg^{-1}-injection decreased the activity in 3 and 6 hr to about 70 per cent of that in the controls (Table 2). The decrease levelled off within 30 hr. The microsomal UDP-glucuronosyltransferase activity was only slightly increased in mice liver 30 hr after the administration of 500 mg·kg^{-1} of vinyltoluene. The significant decrease in cytochrome P-450 content was detected 6 hr after the administration of vinyltoluene (500 mg·kg^{-1}).

Short-term treatment (five subsequent daily injections) of Chinese hamsters with vinyltoluene enhanced the hepatic drug metabolizing enzymes (Table 3). Cytochrome P-450 level was slightly increased together with the 7-ethoxycoumarin *O*-deethylase and UDP-glucuronosyltransferase activities. The hepatic GSH concentrations were slightly increased both in the liver and kidney due to the several subsequent vinyltoluene doses (Table 3).

DISCUSSION

The changes in GSH in rats and mice after vinyltoluene administration are dose- and time-dependent. The changes of GSH concentration show that vinyltoluene or its reactive metabolite, probably epoxide, interacts with this endogenous nucleophile. A single injection of vinyltoluene caused a depression in the hepatic free GSH content. This is similar to the data obtained in the case of styrene [11]. Both with vinyltoluene and styrene the mouse appeared to be the most susceptible rodent species as far as GSH depletion is concerned. In the case of styrene, the GSH decrease has been suggested to be due to the formation of a reactive metabolite styrene oxide, and its interaction with GSH [12]. Vinyltoluene is also suggested to metabolize to a reactive metabolite which then can bind to GSH.

The importance of the vinyl group in vinyltoluene and the possible formation of an epoxide are also underlined by the demonstrated ability of vinyltoluene to bind to cytochrome P-450. The appearance of type I difference spectra following the addition of vinyltoluene to hepatic microsomes indicates that vinyltoluene binds to hepatic microsomal cytochrome P-450 in the manner characteristic of the binding of substrates to this enzyme [13]. The epoxides formed from vinyl compounds interact with cellular molecules such as glutathione or proteins. When bound to the apoprotein of cytochrome P-450, the catalytic function of the cytochrome may be impaired [14].

In long-term exposures, many compounds that acutely decrease the tissue GSH level may later elevate it [14–16]. Hepatic GSH levels can rapidly decrease and then rebound similarly to the result observed with vinyltoluene, after the administration of various unsaturated compounds which are excreted as glutathione conjugates [12, 15, 17].

In mouse, with the largest dose used a decrease in monooxygenase activity as well as in cytochrome P-450 content was evident. This is most probably due to the unfavorable monooxygenase/epoxide hydratase ration in mouse liver [18]. An enhancement of drug biotransformation reactions was also observed in rats (within 6 hr) and in mice (within 30 hr) after a single dose of vinyltoluene. In Chinese hamsters, repeated injections of vinyltoluene had no major effect on drug monooxygenation and conjugation reactions. A small increase in GSH content of the kidneys was, however, observed.

The vinyltoluene-mediated decrease in GSH concentration and the possibility for reactive epoxide intermediate formation strongly suggest that thorough toxicological studies on vinyltoluene are warranted.

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