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DETOXIFICATION OF CHLOROPRENE (2-CHLORO-1,3-BUTADIENE) WITH GLUTATHIONE IN THE RAT

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SUMMARY

Biotransformation of chloroprene (2-chloro-1,3-butadiene) in the rat or in isolated rat hepatocytes leads to glutathione conjugates with concomitant depletion of hepatic glutathione and increased excretion rates of urinary thioethers. Conjugate formation depends on microsomal enzyme activities suggesting the involvement of an epoxide intermediate.

Chloroprene is a reactive compound which is widely used in the manufacturing of the synthetic rubber neoprene. Its total world production was estimated at 300 000 tons in 1977 (1). An estimated 2500 workers are currently exposed to chloroprene in the USA (2,3). Chloroprene has been suggested to be responsible for the increased incidence of skin and lung cancers in exposed workers in the USSR (4,5). However these data are in question (1) and to date no carcinogenic effects of chloroprene have been noted in animal studies involving several ways of administering the drug (review 6).

More recent data from Bartsch et al. (7,8) demonstrated a slight direct mutagenicity of chloroprene in Salmonella typhimurium strains TA 100 and TA 1530. Mutagenicity was increased about threefold in the presence of S9 fractions of either phenobarbital-treated or untreated mice. These obser-

Abbreviations:

GSH, reduced glutathione; GSSG, oxidized glutathione; DTNB, 5,5'-dithio-bis(2-nitrobenzoic acid); LDH, lactatedehydrogenase.

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vations were considered by the authors to reflect the probable formation of an epoxide intermediate of chloroprene.

In analogy to vinylchloride (9) and vinylidenechloride (10), a subsequent detoxification of chloroprene by conjugation with glutathione (GSH) has been suggested (11). Since in vitro mutagenicity test procedures do not include a sufficient GSH conjugating capacity (12), the apparent discrepancy in the animal experiment may be explainable. To test this hypothesis, we investigated detoxification of chloroprene by GSH conjugation in the rat and in isolated rat hepatocytes.

METHODS

Animals. In vivo studies were performed with male SPF Wistar rats, weighing about 200 g from the GSF breeding station Neuherberg. All animals received water and a standard laboratory diet (Altromin, Lage Lippe, Germany) ad lib. For pretreatment the animals received either a single dose of Clophen A50 (500 mg/kg in olive oil) 5 days prior to the experiment or 0.1% phenobarbital in the drinking water for 10 days. Chloroprene was administered in olive oil (1 ml/kg) by stomach tube.

Preparation of hepatocytes. Liver cells were isolated from male SPF Wistar rats according to a modified method of Berry and Friend (13,14). Trypan blue exclusion of the cells was >92%. Throughout the experimental period of 30 min urea formation from 5 mM NH_4Cl operated linearly at a rate of 1.2 - 1.5 µmol/min x g wet weight of cells. Cells were incubated in Erlenmeyer flasks at 37°C in an atmosphere of 95% O_2/ 5% CO_2 (v/v). Chloroprene was added from stock solutions in ethanol. Due to the 59.4°C boiling point of chloroprene, cell incubation experiments do not reflect true concentrations of chloroprene. Control additions of ethanol did not change the parameters investigated.

Assays. For the determination of hepatic GSH, samples of tissue and cells were homogenized (Ultra-turrax or ultrasonic treatment) in 0.1 M phosphate buffer, pH 7.0. After protein precipitation with perchloric acid (final concentration 10%) and centrifugation, the resulting supernatant was adjusted to pH 5-6 with 1.65 M K_2CO_3 in 1 M triethanolamine hydrochloride. GSH was determined with the coupled assay using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) and GSH reductase (15). Parallel assays using the glyoxylase method, specific for reduced GSH (16), showed that GSH represents more than 95% of the DTNB-reactive thiols.

This relation as well as the hepatic content of glutathione-disulfide (GSSG) was not altered by the treatment with chloroprene. At the higher doses of chloroprene the GSSG content

was rather decreased (not shown). Activity of lactatedehydrogenase in the supernatant of the cells was measured according to Bergmeyer (17). Urinary thioethers were determined as described by Summer et al. (18).

MATERIALS

Chloroprene (purity >99.7%) was kindly provided by Bayer AG, Leverkusen, Germany. To minimize polymerisation, the unstablized compound was freshly prepared and kept at -80°C. Biochemicals and chemicals were of analytical grade and purchased from Boehringer, Mannheim, Germany and Merck, Darmstadt, Germany, respectively.

RESULTS

In the rat in vivo, chloroprene caused a rapid decrease of hepatic GSH. Three hours after the oral administration of 100 and 200 mg chloroprene per kg to untreated rats, the hepatic GSH level decreased to 55 and 39% of the control level, resp. (Table 1). In Clophen A50-pretreated animals this decrease amounted to 57 and 55%, resp.

In isolated rat hepatocytes cellular GSH decreased to about 50% of the control within 15 min after the addition of 3 mM chloroprene (Fig. 1). This depletion was dose-dependent. In hepatocytes of animals pretreated with phenobarbital or Clophen A50, addition of 3 mM chloroprene almost completely depleted GSH within 30 min.

Table 1

Chloroprene-dependent depletion of hepatic GSH in the rat in vivo three hours after oral administration of the drug.

	hepatic GSH (% of control)	
Dose (mg/kg)	untreated	Clophen A50
100	55 ± 8	57 ± 8
200	39 ± 8	55 ± 6

Mean ± S.D. (n = 4)
Control level of hepatic GSH was 6.8 ± 0.6 µmol/mg prot.

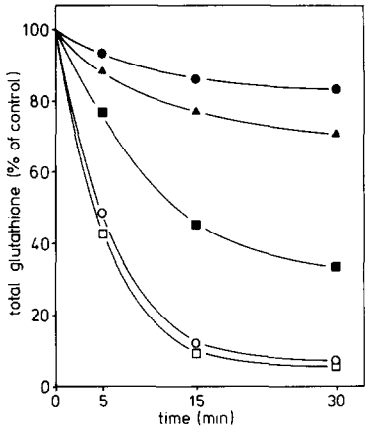

Fig. 1 GSH depletion in isolated rat hepatocytes by chloroprene. Data are from one representative experiment out of three with different cell preparations. Control level of cellular GSH amounted to 19.9 ± 2.1 nmol/mg cell protein. Closed symbols: Hepatocytes (8 mg cell protein/ml) of untreated animals incubated with 0.5 mM (● - ●), 1.0 mM (▲ - ▲) and 3.0 mM (■ - ■) chloroprene. Open symbols: Hepatocytes from either phenobarbital (○ - ○) or Clophen A50 (□ - □) pretreated animals incubated with 3 mM chloroprene. Pretreatment of the animals was performed as described in methods.

As a parameter of acute toxicity, release of lactatedehydrogenase (LDH) into the cell medium was investigated (Table 2). LDH release of the hepatocytes did not rise significantly above control levels until addition of 10 mM chloro-

Table 2

Chloroprene-dependent release of LDH from isolated rat hepatocytes[1]

Chloroprene (mM)	LDH activity (% of total cell activity)[2] 15 min	30 min
0	4	6
0.5	6	7
1.0	6	7
3.0	7	9
7.0	8	10
10.0	46	55

[1] Data are from one representative experiment out of three.
[2] Total cellular LDH activity amounted to 2.01 µmol/min x mg.

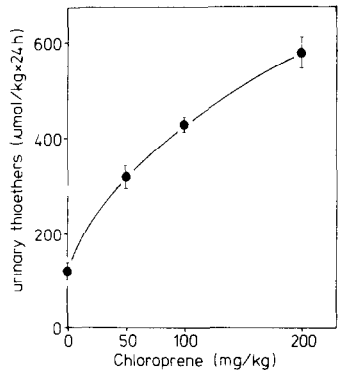

Fig. 2 Chloroprene-dependent excretion of thioethers in the urine of rats. Chloroprene was administered in olive oil by stomach tube. Data represent means ± S.D. from 4 animals. Urinary thioethers were determined as described by Summer et al. (18).

prene. At this concentration 46 and 55% of the total cellular LDH activity was found in the cell medium within 15 and 30 min, resp.

As a further indicator of GSH-dependent detoxification, we investigated the enhanced excretion of thioethers (presumably GSH-conjugates and mercapturic acids) (19) in the urine of rats dosed with chloroprene (Fig. 2). The oral administration of chloroprene to rats resulted in a dose-dependent increase in the excretion of urinary thioethers. This increase was reversible and completed within 24 hours at all dose levels administered (data not shown). At dose levels of 50 and 200 mg chloroprene/kg the additional excretion of urinary thioethers amounted to 196 and 458 µmol/kg x day, resp. The dose-response curve was not linear. At higher doses a decline in the excretion rate of urinary thioethers was observed.

DISCUSSION

Our data indicate that chloroprene is effectively detoxified by conjugation with GSH in the rat and in isolated rat

hepatocytes. Chloroprene does not react with GSH non-enzymatically in vitro nor in the presence of postmitochondrial supernatant of rat liver homogenate which contains GSH and GSH S-transferases (12). Therefore, chloroprene metabolism, most probably the microsome-mediated formation of an epoxide intermediate (11), seems to be a prerequisite for the depletion of cellular GSH. This is also supported by the more pronounced chloroprene-dependent decrease of GSH in hepatocytes from either phenobarbital or Clophen A50 pretreated animals (Fig. 1). In the rat, however, pretreatment with Clophen A50 failed to stimulate hepatic GSH depletion three hours after the administration of 100 and 200 mg chloroprene/kg. This observation cannot yet be explained. It might indicate that, unlike in hepatocytes, organ perfusion is rate-limiting for chloroprene metabolism in vivo. Similar to vinyl methyl ether and 1,1-dichloroethylene (20,21), concentrations of chloroprene at which metabolism is limited by hepatic perfusion should show no difference in the rate of metabolism and subsequent GSH depletion between induced and non-induced animals.

In hepatocytes from phenobarbital-pretreated animals, the initial rate of GSH depletion by 3 mM chloroprene amounted to about 3 nmol/min x mg cell protein. This chloroprene binding capacity is sufficient to assume that chloroprene metabolism and subsequent binding of metabolites with GSH may lead to complete depletion of cellular GSH. This is sustained by the chloroprene-dependent hepatic GSH depletion observed in vivo (Table 1) and the increased excretion of urinary thioethers after the administration of 200 mg chloroprene/kg. At this dose, the urinary thioethers excreted within 24 hours

amounted to about twice the hepatic level of GSH and corresponded to about 20% of the dose of chloroprene. In agreement with a pronounced depletion of GSH, a release of LDH from the cells was observed only at higher doses of chloroprene (Table 2). A drastic GSH depletion may generally result in oxidative damage of cellular membranes with leakage of cytosolic enzymes (22).

In inhalation experiments with rats, Plugge and Jaeger (10) reported a similar effect of chloroprene. The dose-response curve for serum sorbitol dehydrogenase, an enzyme used in the quantitation of liver damage (23,24), did not rise significantly above control values until chloroprene concentrations of 200 ppm and more. Unless reliable information on the effective concentration of chloroprene in a particular tissue becomes available, it will not be possible to compare the inhalation studies with the in vitro experiments.

However, the inconsistency of the data on mutagenicity and carcinogenicity of chloroprene might be explainable by GSH-dependent detoxification. In contrast to the insufficient GSH-dependent detoxification in the bacterial mutagenicity test (12), efficient detoxification of chloroprene in vivo apparently leads to a threshold in the carcinogenic effect. To date, chronic inhalation exposure of animals has not exceeded dose-levels of 100 ppm (6). No tumor inducing effects have been observed in these experiments.

Further experiments will be necessary to evaluate the role of species and tissue variations in the GSH-dependent biotransformation of chloroprene, especially in inhalation studies with higher dose-levels of exposure.

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