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Inhalation pharmacokinetics of 1,2-epoxybutene-3 reveal species differences between rats and mice sensitive to butadiene-induced carcinogenesis

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Abstract. Comparative investigations of inhalation pharmacokinetics of 1,2-epoxybutene-3 (vinyl oxirane, the primary reactive intermediate of butadiene) revealed major differences in metabolism of this compound between rats and mice. Whereas in rats no indication of saturation kinetics of epoxybutene metabolism could be observed up to exposure concentrations of 5000 ppm, in mice saturation of epoxybutene metabolism becomes apparent at atmospheric concentrations of about 500 ppm. The estimated maximal metabolic rate (V_{max}) in mice for epoxybutene was only 350 μmol × h^{-1} × kg^{-1} (rats: >2600 μmol × h^{-1} × kg^{-1}). In the lower concentration range where first order metabolism applies (up to about 500 ppm) epoxybutene is metabolized by mice at higher rates compared to rats (metabolic clearance per kg body weight, mice: 24900 ml × h^{-1}, rats: 13400 ml × h^{-1}). Under these conditions the steady state concentration of epoxybutene in the mouse is about 10 times that in the rat. When mice are exposed to high concentrations of butadiene (>2000 ppm; conditions of saturation of butadiene metabolism; closed exposure system) epoxybutene is exhaled by the animals, and its concentration in the gas phase increases with exposure time. At about 10 ppm epoxybutene signs of acute toxicity are observed. When rats are exposed to butadiene under similar conditions, the epoxybutene concentration reaches a plateau at about 4 ppm. Under these conditions hepatic non-protein sulfhydryl compounds are virtually depleted in mice but not in rats. We conclude that in addition to the higher rate of metabolism of butadiene in mice, limited detoxification and consequently accumulation of its primary reactive intermediate epoxybutene may be a major determinant for the higher susceptibility of mice to butadiene-induced carcinogenesis.

Key words: Butadiene – Epoxybutene – Inhalation – Species differences – Metabolism – Pharmacokinetics

Introduction

Long-term inhalation studies with rats (Sprague-Dawley; Hazleton Laboratories Europe 1981) and mice (B6C3F1; Huff et al. 1985) have demonstrated remarkable species differences in the carcinogenic potency of 1,3-butadiene. Whereas butadiene was weakly carcinogenic in rats, chronic exposure of mice revealed a considerably higher carcinogenic activity. The reasons are not known.

According to recent publications on metabolism and pharmacokinetics of 1,3-butadiene (Bolt et al. 1983; Filser and Bolt 1984; Schmidt and Loeser 1985; Kreiling et al. 1986a, b; Bond et al. 1986) metabolic elimination of this olefin proceeds entirely via primary epoxidation to 1,2-epoxybutene-3 (vinyl oxirane).

Epoxybutene is a direct acting mutagen in *S. typhimurium* (Gervasi et al. 1985) and is carcinogenic on mouse skin after dermal or subcutaneous administration (van Duuren et al. 1966). After reaction of epoxybutene with DNA in vitro two isomeric 7-N-(hydroxybutenyl)-guanine structures have been identified (Citti et al. 1984). Recently we have demonstrated that reactive butadiene metabolites covalently bind to liver nucleoproteins and to DNA of mice and rats after exposure of the animals to (1,4-^{14}C)1,3-butadiene (Kreiling et al. 1986b).

In a preceding investigation we demonstrated that 1,3-butadiene is metabolized by mice at about twice the rate compared to rats (Kreiling et al. 1986a). The presence of its primary intermediate, epoxybutene, in the blood of rats and mice and in the expired air of rats exposed to butadiene (Bond et al. 1986; Bolt et al. 1983; Filser and Bolt 1984); and its long half-life at physiological pH (13.7 h, Gervasi et al. 1985), along with the formation of tumors at multiple sites (Huff et al. 1985) indicates that this epoxide is systemically available within the animal organism.

The aim of this study was to comparatively investigate in rats and mice, if in addition to the higher metabolic transformation of butadiene in mice than in rats (Kreiling et al. 1986a) limited detoxification of its reactive epoxide 1,2-epoxybutene-3 may be a factor accounting for the special susceptibility of mice to butadiene-induced carcinogenesis.

Materials and methods

Chemicals. Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid, 99% pure) was purchased from Fluka Chemical (Buchs, CH). Glutathione (GSH) was obtained from Sigma Chemicals Co. (München, FRG), and sulfosalicylic acid from Merck (Darmstadt, FRG). 1,3-Butadiene (99% pure) was purchased from Messer-Griesheim (Düsseldorf, FRG). 1,2-Epoxybutene-3, at a purity of 98%, was from Aldrich Chemie (Steinheim, FRG).

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Animals. Male B6C3F1 mice (25–30 g) and male Sprague-Dawley rats (220–250 g) (Zentralinstitut für Versuchstiere, Hannover, FRG) were used. Previously published equations (Filser and Bolt 1983) were used to relate kinetic parameters to 1 kg body weight.

Gas uptake and kinetic studies. Inhalation pharmacokinetics of expoxybutene in mice were studied using 6.4 l all-glass exposure chambers as already described (Filser and Bolt 1979). Usually eight mice were exposed to different initial concentrations of epoxybutene. Concentration changes in the gas phase of the system were measured by gas chromatography and were recorded for the time periods indicated in the figures. For IP application liquid epoxybutene was injected (without vehicle) into the animals (IP studies: one mouse; 2.8 l desiccator; 63 g soda lime). Pharmacokinetic analysis was performed using the two-compartment open pharmacokinetic model developed previously (Filser and Bolt 1981, 1983).

Gas chromatography. For gas chromatographic analysis a Shimadzu gas chromatograph (GC-8A equipped with gas sampler MGS-4) with a 2 ml gas sample loop and an FID was used. Butadiene and epoxybutene were separated on a 2 m stainless steel 1/8" Carbowax column at an oven temperature of 60° C. The FID temperature was 240° C. Gas flow rates were: carrier gas (N_2), 60 ml/min; hydrogen, 25 ml/min; synthetic air, 240 ml/min. Under these conditions the following retention times were recorded: butadiene 0.5 min; epoxybutene 2.45 min.

Exhalation of epoxybutene and determination of GSH in the liver of butadiene exposed animals. Six mice or two rats were placed in a 6.4 l desiccator system as described above. The animals were exposed to butadiene at concentrations higher than 2000 ppm (2000–4000 ppm) which ensured maximal metabolism to epoxybutene (V_{max}). Concentration changes of butadiene and exhaled epoxybutene in the gas phase of the system were monitored by gas chromatography for up to 17 h. The butadiene concentration was maintained above 2000 ppm by addition of definite amounts of butadiene to the gas phase, every 2–3 h. Control animals were also housed in closed all-glass chambers but were not exposed to 1,3-butadiene.

After an exposure period of 15 h, the animals were removed from the system and immediately decapitated. Hepatic non-protein sulfhydryl content was determined according to Ellman (1959). Liver samples were homogenized in 4 volumes 0.1 M potassium phosphate buffer pH 8. After centrifugation of the homogenate 250 µl sulfosalicylic acid (4%) were added to 250 µl of the supernatant and centrifuged at 4000 *g*; 200 µl of the protein-free supernatant were mixed with 800 µl 5,5-dithiobis(2-nitrobenzoic acid) and allowed to stand at 4° C for 20 min. The molar concentration of non-protein sulfhydryl groups in the sample was calculated using a molar extinction coefficient determined from known concentrations of a glutathione standard.

Results

Pharmacokinetic analysis

When mice were exposed in a closed chamber to various initial concentrations of epoxybutene (see Fig. 1), the decline curves obtained show a clear saturation behaviour of epoxybutene metabolism. At lower concentrations the elimination of epoxybutene is directly proportional to its concentration in the gas phase of the system. At higher epoxybutene concentrations the slopes of the concentration-time curves decrease, and saturation of epoxybutene metabolism becomes apparent. This is in contrast to studies with rats (Filser and Bolt 1984) where no indication of a saturable metabolism of epoxybutene was found up to exposure concentrations of about 5000 ppm. A biexponential decline of the concentration-time curves obtained with mice should be noted (Fig. 1). This biexponential decline reflects a initial equilibration process for epoxybutene between the gas phase of the closed system and the animal and a second slower phase caused by metabolic elimination of the compound.

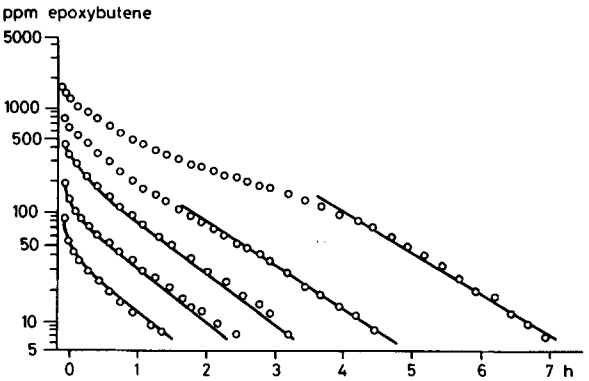

Fig. 1. Time-course of 1,2 epoxbutene-3 concentrations in the gas phase of a closed 6.4 l dessicator jar chamber, occupied by eight animals (B6C3F1 mice)

Thus, the equilibration and elimination processes of the compound could be separately analysed according to previously published procedures (Filser and Bolt 1981).

The pharmacokinetic parameters for distribution and metabolism of epoxybutene obtained for mice and those previously published for rats (Filser and Bolt 1984) are compared in Table 1. Pronounced species differences exist in the "steady state" constant (K_{st}) and in the rate constant for first-order metabolic elimination (k_{el}). From K_{st} it follows that the steady-state concentration of epoxybutene in

Table 1. Pharmacokinetic parameters for distribution and metabolism of inhaled 1,2-epoxybutene-3 in mice and rats (Filser and Bolt 1984) related to 1 kg body weight. (For definition of constants see text and Filser and Bolt 1981)

Parameter	Mouse	Rat	Dimension
$k_{12}V_1$	33 500	13 800	$min \cdot h^{-1}$
k_{21}	0.79	0.37	h^{-1}
K_{eq}	42.5	37	–
K_{st}[a]	10.2	1.16	–
k_{el}[b]	2.3	11.5	h^{-1}
Cl_{tot}[a,b]	24 900	13 400	$ml \cdot h^{-1}$
Metabolic saturation	500	c	ppm

[a] Calculated for $V_1 \to \infty$
[b] Valid for linear range of metabolism
[c] Not observed up to 5000 ppm

mice is about 10 times higher than in rats. The rate constant for first-order metabolic elimination (k_{el}) is about five times lower in mice compared to rats. The approximately twofold higher metabolic clearance (Cl_{tot}, first order metabolism) in mice versus rats results from these values of K_{st} and k_{el}.

In contrast to the data of Fig. 1 only monoexponential decline curves were observed when rats were exposed to epoxybutene concentrations between 10 and 5000 ppm (Filser and Bolt 1984).

In rats (see Table 1), metabolic elimination of inhaled epoxybutene is limited by the influx ($k_{12}V_1$) of epoxybutene into the animal organism. [The nearly identical values in rats for the partial process of uptake ($k_{12}V_1$) and for (first order) metabolic clearance (Cl_{tot}) support this view.] In mice, compared to the high pulmonary uptake ($k_{12}V_1$) a relatively low metabolic clearance (Cl_{tot}) of inhaled epoxybutene is observed. Thus metabolism of epoxybutene in mice is not limited by uptake (and distribution) processes to a greater extent; metabolic elimination is restricted by the low rate constant of (first order) metabolism (k_{el}) expressing the metabolic capacity.

Figure 2 shows the metabolic elimination rates of epoxybutene, as calculated from individual experiments with mice (see Fig. 1) and rats (Filser and Bolt 1984). These were calculated for conditions of exposure in an "open" ($V_1 \rightarrow \infty$) system and plotted versus the atmospheric concentrations of the compound. A comparison of the metabolic elimination rates in both species reveals (Fig. 2) that at lower exposure concentrations mice show a higher metabolic rate for epoxybutene than rats. Whereas metabolic elimination of inhaled epoxybutene in rats is linearly dependent on the atmospheric concentration at least up to exposure concentrations of about 5000 ppm (V_{max} >2600 μmol $\times$ h^{-1} $\times$ kg^{-1}), in mice saturation of epoxybutene metabolism becomes apparent at about 500 ppm. A calculation of the maximal (V_{max}) metabolic rate for epoxybutene in mice reveals a value of 350 μmol $\times$ h^{-1} $\times$ kg^{-1}. Therefore, with increasing exposure concentrations the metabolic capacity for epoxybutene becomes rate limiting in mice.

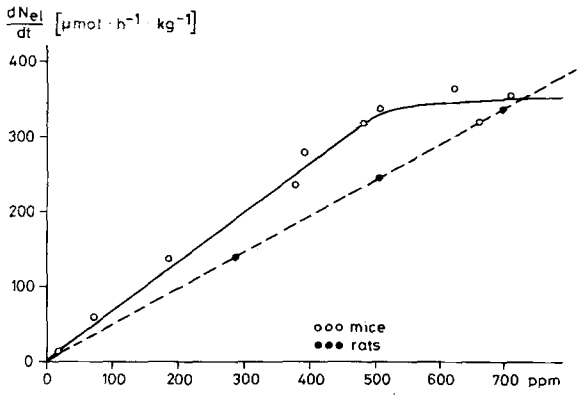

Fig. 2. Metabolic elimination rates per kg body weight (dN_{el}/dt) of mice (*open circles*) and rats (*closed circles*) depending on atmospheric concentrations of 1,2-epoxybutene-3. Metabolic elimination of epoxybutene in rats (*dotted line*) is linearly dependent on the atmospheric concentration of the compound up to 5000 ppm (not shown). Saturation of epoxybutene metabolism in mice becomes apparent at atmospheric concentrations of about 500 ppm

Exhalation of epoxybutene formed endogenously from 1,3-butadiene

Exhalation of epoxybutene is observed when mice are exposed to butadiene in a closed exposure system. When butadiene concentrations were maintained above 2000 ppm (by repeated addition of definite amounts into the gas phase of the closed system, see Fig. 3c) a time-dependent increase in epoxybutene concentration is noticed (Fig. 3a). For comparison, the results of a similar experiment with rats (Filser and Bolt 1984) are included in Fig. 3a.

In both experiments metabolism of butadiene proceeded under saturation conditions. Remarkable differences are obvious between both species (Fig. 3a). Whereas epoxybutene exhaled by rats reaches a plateau concentration of about 4 ppm, epoxybutene exhalation by mice leads to an increase in concentration until a peak concentration of about 10 ppm in the system is reached after 10 h.

The subsequent decline in atmospheric epoxide concentration in the experiment with mice is due to a decrease in butadiene metabolism (see Fig. 3b), as calculated from the concentration-time courses of butadiene (Fig. 3c). From about 12 h onwards mice show signs of acute toxicity, and lethality occurs when the butadiene exposure is prolonged over 15 h. No toxicity was observed with rats using the same experimental protocol.

The differences in epoxybutene exhalation and in toxicity of butadiene between mice and rats can be easily explained on the basis of the differences in pharmacokinetics. As metabolic elimination of epoxybutene in mice is a saturable process, the concentration of epoxybutene metabolically generated from butadiene gradually increases in the animal organism (under saturation conditions of butadiene metabolism), i. e. K_{st} for epoxybutene approximates K_{eq} (see Filser and Bolt 1981). Because exhalation of a volatile compound is proportional to its concentration in the animal this also results in an increase in epoxybutene concentration in the atmosphere of the closed exposure system.

The final decline in butadiene metabolism in the mouse experiment (Fig. 3) which is associated with a reduction in epoxide exhalation can be attributed to a toxic action of epoxybutene. This is supported by the fact that at the end of the 15-h exposure period the hepatic non-protein sulfhydryl content is reduced to about 4% of that of non-exposed animals (see Table 2). Under similar conditions of butadiene exposure in rats the hepatic non-protein

Table 2. Hepatic non-protein sulfhydryl content in mice and rats after a 15-h exposure to butadiene in the closed exposure system under conditions of saturation of butadiene metabolism (see Fig. 3a–c). Controls were exposed to air only. Data represent means (six mice ± SD or two rats)

	Hepatic non-protein sulfhydryl content			
	Mice ($B_6C_3F_1$)		Rats (Sprague-Dawley)	
	μmol/g	%	μmol/g	%
0 h control	5.5 ± 0.2	100	4.2	100
15 h control	3.9 ± 0.2	72	3.7	88
15 h exposed	0.2 ± 0.05	4	3.2	76

sulfhydryl content is practically maintained at the level of control animals.

The initial biphasic increase in epoxybutene exhalation by mice is difficult to explain and could be a result of distribution processes, and/or delayed availability of an enzyme or substrate for detoxification.

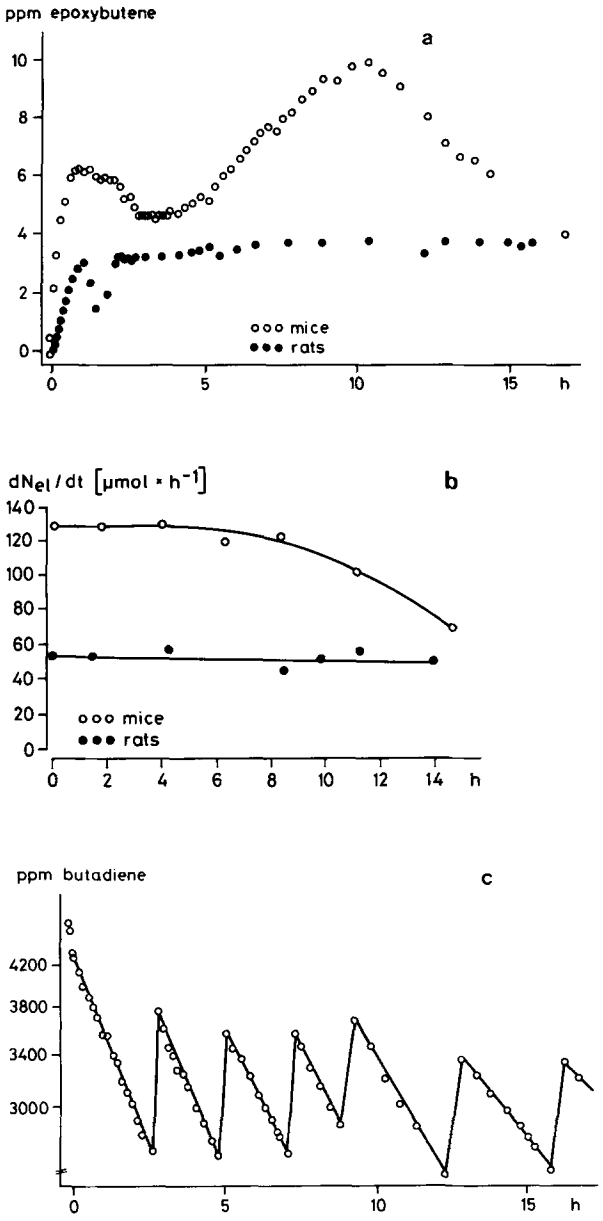

Fig. 3.a Time course of epoxybutene concentrations in the gas phase of the closed exposure system (6.4 l), occupied by two rats (*closed circles*) or six mice (*open circles*) upon eposure to butadiene at concentrations (> 2000 ppm) which cause maximal metabolism to epoxybutene for both species. **b** Metabolic elimination rates (not standardized) for butadiene in mice (*open circles*) and rats (*closed circles*) under conditions of exposure of the animals as indicated in Fig. 3a and calculated from the concentration-time courses of butadiene in the closed exposure system (see Fig. 3c). **c** Concentration-time course of butadiene in the gas phase of the closed exposure system occupied by mice (see Fig. 3). Repeated addition of definite amounts of butadiene into the gas phase of the closed exposure system ensured butadiene concentrations which cause maximal metabolism to epoxybutene

Discussion

Previous investigations of inhalation pharmacokinetics of butadiene in rats and in mice (Filser and Bolt 1984; Kreiling et al. 1986a) revealed that butadiene is metabolized by mice at about twice the rate of rats.

In both species metabolism of butadiene follows saturation kinetics and proceeds via oxidation to epoxybutene as the primary reactive intermediate. Thus differences in metabolism ("detoxification") of expoxybutene between both species may play a crucial role for the differences observed in butadiene carcinogenicity (mice versus rats).

Our data show that, in addition to the higher metabolic rate of butadiene in mice (Kreiling et al. 1986a), detoxification of its primary reactive metabolite is saturable in this species. Saturation of epoxybutene metabolism in mice becomes apparent at atmospheric concentrations of about 300 ppm, whereas in rats no indication of saturation kinetics of epoxybutene metabolism could be observed up to exposure concentrations of 5000 ppm (Filser and Bolt 1984).

When the animals are exposed to butadiene in the closed exposure system (see Fig. 3), epoxybutene concentration generated by mice is 2–3 times that of rats. This confirms recent data of Bond et al. (1986), who observed a 2–5 times higher epoxybutene concentration in the blood of mice versus rats during exposure of the animals to butadiene.

A comparison of the coefficients of dynamic distribution (K_{st}) for epoxybutene metabolism in rats and mice, for exposures under conditions where first-order elimination applies, shows that the steady-state concentration of epoxybutene in the mouse organism is about 10 times that in the rat under comparable conditions of butadiene exposure.

Furthermore, the hepatic non-protein sulfhydryl content is practically depleted in mice by a 15-h exposure to butadiene (conditions of metabolic saturation). By contrast, the hepatic non-protein sulfhydryl content in rats upon exposure to butadiene (conditions of metabolic saturation) remains at the level of air-exposed controls.

With regard to the concentration of epoxybutene in the organism of mice and to the depletion of hepatocellular non-protein sulfhydryls in this species, upon exposure to butadiene, we feel that epoxybutene endogenously produced from butadiene might be processed by two main routes of "detoxification" which show quantitative differences for rats and mice at high epoxybutene concentrations. In rats, epoxybutene metabolism could proceed mainly via epoxide hydrolase (and in part via glutathione-S-transferase). In mice this balance of epoxybutene metabolism should be far on the side of glutathione-S-transferase. The depletion of the hepatic non-protein sulfhydryl content in mice, the lower specific activity of epoxide hydrolase, and the higher specific activity of glutathione-S-transferase in liver tissue preparations of mice when compared to rats (Lorenz et al. 1984; Schmidt and Loeser 1985) are supportive of this view.

That epoxybutene, in principle, can be metabolized by epoxide hydrolase and glutathione-S-transferase could be shown in studies with liver microsomal preparations in vitro (Bolt et al. 1983).

We conclude, that in addition to the higher metabolism of butadiene in mice, limited detoxification and thus accu-

mulation of its primary reactive intermediate 1,2-epoxybutene-3 may be a major determinant for the higher susceptibility of mice to butadiene-induced carcinogenesis.

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