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Species differences in the formation of butadiene monoxide from 1,3-butadiene

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Abstract. When 1,3-butadiene is incubated with liver postmitochondrial fractions from mouse, rat, monkey or man and a NADPH-regenerating system, the formation rate of butadiene monoxide is different in the four species. With the exception of the rhesus monkey, the amount of epoxide is proportional to the monooxygenase activity. The sequence of epoxide formation is B6C3F$_1$ mouse, Sprague Dawley rat, man, rhesus monkey. The ratio between mouse and monkey was about 7:1. When 1,3-butadiene is incubated with homogenates from *lung* tissue, only tissues from mouse and rat produce measurable butadiene monoxide concentrations. The monooxygenase activity in lung tissue of the mouse was only 1/30 that in mouse liver. By contrast, lung tissue formed epoxide concentrations comparable to those formed by liver tissue, whereas monkey and human lung tissue did not produce any measurable levels of butadiene monoxide. The data might suggest that the results of recent rodent inhalation studies with 1,3-butadiene could not automatically be extrapolated to man.

Key words: Rodents – Human – Epoxidation – Epoxide hydrolase – 1,3-Butadiene – Butadiene monoxide

Introduction

1,3-Butadiene, a colorless gas, is used as a chemical intermediate or as a monomer in the production of various polymers, e.g., in the synthetic rubber industry, or as a comonomer for ABS-polymers.

Butadiene is mutagenic in the Ames test in the presence of S-9 mix only (de Meester et al. 1979, 1980). In an earlier study (de Meester et al. 1978) probably reported an artefact when butadiene was tested without an activating system but was exposed to volatile intermediates from other plates using butadiene and S-9 mix.

Malvoisin et al. (1979) have demonstrated butadiene monoxide as the main metabolite of butadiene by rat liver microsomes in vitro. Malvoisin and Roberfroid (1982) have studied the roles of microsomal mixed function oxidases and epoxide hydrolases in the metabolic fate of butadiene monoxide. The monoxide has also been detected in exhaled air of rats exposed to butadiene (Bolt et al. 1983; Filser et al. 1984).

Recent long-term inhalation studies in rats (Hazleton 1981; Loeser 1982) and mice (NTP 1984) have demonstrated that rats survive higher butadiene exposure levels than mice. The inhalation exposure of mice to 625 or 1250 ppm butadiene resulted in excessively high mortality in the early stages of the long-term study. Fatal tumors (mainly hemangiosarcomas of the heart, lymphomas, bronchiolar and pulmonary adenomas and carcinomas, tumors in the forestomach, mammary and ovarial tumors) occurred with a high incidence in all exposed groups. Rats, however, survived levels up to 8000 ppm for approximately 2 years, showing minimal toxic or tumor response. A recent epidemiological mortality study (Matanoski et al. 1982) did not detect significant increases in any cause of death or in tumors at any site.

These data suggest species differences in the activating and deactivating steps of butadiene metabolism. Since mutagenicity studies and in vitro and in vivo metabolic studies have demonstrated the formation of the butadiene monoxide as a critical reactive intermediate, we initiated studies to demonstrate possible qualitative and quantitative differences in butadiene epoxidation, inactivation of the epoxide by epoxide hydrolases and/or glutathione transferases, using postmitochondrial preparations from liver and lung tissues of mice, rats, monkey and man. The data obtained may serve to develop a suitable extrapolation from animal data to man.

Materials and methods

Chemicals. 1,3-Butadiene, technical grade, 99.6% purity, was obtained from Erdoelchemie GmbH, Dormagen; butadiene monoxide and butadiene diepoxide, both of 97% purity, were from EGA Chemie. All other chemicals, analytical grade, were obtained commercially.

Tissues of lung and liver. Tissue material was obtained from Sprague Dawley rats (Charles River, Germany), Wistar rats and NMRI-mice (Winkelmann, Germany), B6C3F$_1$-mice (Charles River, Germany), and rhesus monkeys (own breed). Human lung and liver tissue was obtained following surgery.

Tissue homogenates. Animals were sacrificed and liver and lung tissues (1–1.5 g wet weight) were quickly excised. They were placed in ice-cold 0.15 M KCL solution and were homogenized while being chilled. Lung tissue from five mice were pooled. The human material was processed after surgery as quickly as possible. The homogenates were centrifuged at 4° C and 10 000 *g* for 20 min. A 1 ml aliquot

of the obtained supernatant was equivalent to approximately 200 mg tissue wet weight.

Monooxygenase activity. Monooxygenase activity was determined in the 10 000 *g* supernatants from liver (≙ 1 mg tissue) and lung (≙ 10 mg tissue) homogenates, using 7-ethoxycoumarin as substrate according to Ullrich and Weber (1972), with modifications introduced by Greenle and Poland (1978) and Aitio (1978). O-Deethylase activities were calculated as nmol 7-hydroxycoumarin per g wet tissue per min.

Epoxide hydrolase. The activity of the epoxide hydrolase was determined according to the method of Guiliano et al. (1980), which uses 3-(*p*-nitrophenoxy)-1,2-propeneoxide as substrate. The hydrolysis product, 3-(*p*-nitrophenoxy)-1,2-propanediol, was determined by HPLC. The epoxide hydrolase activity was calculated as the mean of two determinations as nmol 3-(*p*-nitrophenoxy)-1,2 propanediol per g tissue per min.

Epoxidation of 1,3-butadiene in liver and lung homogenates. Incubation mixtures, in head-space vials (about 30 ml), contained 500 µl tissue homogenate (from 100 mg tissue), 100 µl $MgCl_2$ (5 µmol), 200 µl glucose-6-phosphate (4 µmol), 200 µl NADP (0.4 µmol), 500 µl Sörensen buffer (pH 7.6; 0.06 mol/l); and 20 µl glucose-6-phosphate dehydrogenase solution (Boehringer, Mannheim).

After 10 min preincubation at 37 °C, the headspace vials were capped and 1 ml 1,3-butadiene gas (ca. 2.41 mg) was added with a Hamilton gas syringe. The final concentration in the vial was about 30 000 ppm butadiene. Maximally, three gas samples of 0.5 ml each were withdrawn at different intervals and injected into a gas chromatograph to analyse for butadiene monoxide. The separation was done on a 4-m glass column (diameter ¼", with 10% P 2000) at 70 °C column temperature. Under these conditions (using an FID) the following retention times were recorded: 1,3-butadiene, $r_t = 1.1$ min; butadiene monoxide, $r_t = 5.9$ min. The limit of detection was about 0.1 µg butadiene monoxide/30 ml air, corresponding to 2.5 ppm. The butadiene monoxide was calculated as nmol butadiene monoxide produced per g tissue.

Results

As a monooxygenase marker, ethoxycoumarin-0-deethylase (EOD) was determined in the liver and lung homogenates of the different species examined. Similarly, epoxide hydrolase (EH) activities were measured. The results are compiled in Table 1.

The formation of butadiene monoxide by tissue homogenates from the different species was followed up to 60 min. The data are shown in Fig. 1 (liver) and Fig. 2 (lung) for homogenates from the mouse, rat, monkey and man.

Liver

Female NMRI mouse liver tissue produced more butadiene monoxide than did the tissue of males. This is consistent with higher EOD and lower EH activities in female mice, compared to males. $B6C3F_1$ mice displayed a similar pattern of enzyme activities and butadiene monoxide formation.

Rats showed lower activities of both EOD and EH, which resulted in lower monoxide formation in rats as opposed to mice.

Livers from two *rhesus monkeys* of each sex were also used. The liver homogenates in both sexes contained approximately 5 times the EOD activity of male rats and approximately 16 times the EH activity of male rats. As a result of this, butadiene monoxide appeared only in small amounts in the incubations with monkey liver.

Human liver homogenate showed even lower EOD activities than rhesus monkey liver, but EH activities were comparable in both species. However, butadiene monoxide formation was slightly higher than observed with the monkey tissue.

It must be noted that these results with human tissue should be regarded as preliminary because of the wide variations in drug metabolizing enzymes recognised among individuals.

Table 1. Activities of butadiene oxidase, ethoxycoumarin deethylase and epoxide hydrolase in liver and lung postmitochondrial preparations

	Liver									Lung								
	BMO[c]			EOD[d]			EH[e]			BMO[c]			EOD[d]			EH[e]		
	[nmol / g · 45 min]			[nmol / g · min]			[nmol / g · min]			[nmol / g · 45 min]			[nmol / g · min]			[nmol / g · min]		
	N	$\bar{x}$	± SD	*N*	$\bar{x}$	± SD	*N*	$\bar{x}$	± SD	*N*	$\bar{x}$	± SD	*N*	$\bar{x}$	± SD	*N*	$\bar{x}$	± SD
Mice, male	5	125	49	5	17	7	5	430	74	2[a]	251	28	2	2.3	0.1	2	40	6
Mice, female	5	217	33	5	37	6	5	340	26	4[a]	198	14	2	1.3	0.4	–	–	–
Rat, male	5	82	45	5	15	4	5	320	133	4	41	11	4	1.4	0.2	2	20	4
Rat, female	5	67	11	5	7	2	5	220	102	3	27	12	3	1.2	0	1	30	–
Monkey, male	2	32	3	2	75	27	2	5150	700	2	n.d.[b]	–	2	0.02	–	2	80	33
Monkey, female	2	34	6	2	79	20	2	6200	600	3	n.d.[b]	–	3	0.02	–	3	110	38
Human	1	79	–	1	17	–	1	6800	–	1	n.d.[b]	–	1	0.01	–	1	100	–

[a] Five lungs each were pooled
[b] n.d. = not detectable
[c] BMO = Butadiene monoxidase
[d] EOD = Ethoxycoumarin deethylase
[e] EH = Epoxide hydrolase

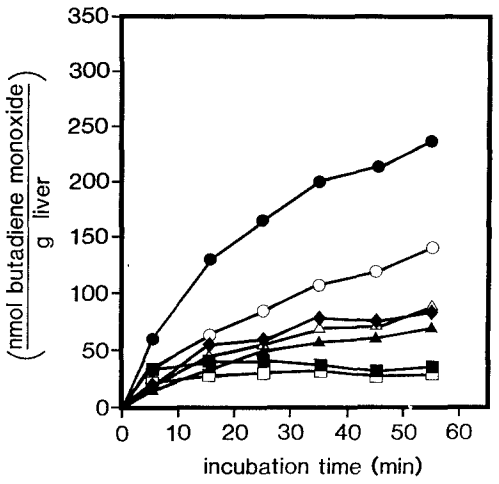

Fig. 1. Formation of butadiene monoxide from 1,3-butadiene by liver tissue (10 000 *g* fraction) of the rat △ male, ▲ female), mouse (○ male, ● female), monkey (□ male, ■ female), man (◆)

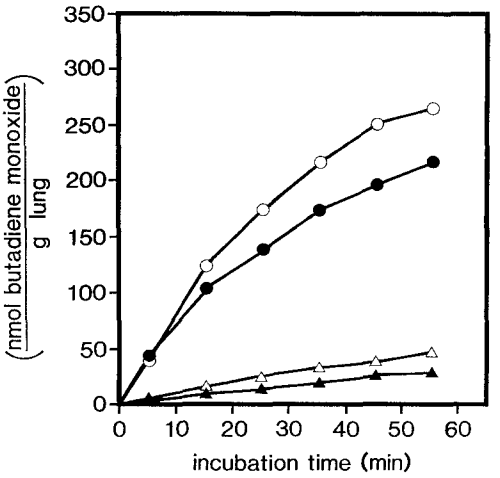

Fig. 2. Formation of butadiene monoxide from 1,3-butadiene by lung tissue (10 000 *g* fraction) of the rat (△ male, ▲ female) and mouse (○ male, ● female)

Lungs

EOD and EH activities were markedly (approximately 10 times) lower in lung tissue of NMRI mice than in the liver of the same species. Unexpectedly, butadiene monoxide formation in vitro did not correlate with these enzyme activities. In the lung of male mice, twice the amount of butadiene monoxide was produced compared with liver, and in female mice production was similar in both tissues.

Similarly, lung EOD and EH activities in Sprague-Dawley *rats* amounted to about one tenth of the respective activities in the liver. The EOD activities in rat lung of both sexes were similar to those in mice; however, monoxide formation was 5–6 times lower than in mouse lung.

The EOD activities in the lungs of four *rhesus monkeys* were lower by a factor of approximately 1000 than in the liver of this species, whereas EH in lung tissue was less active than in liver, by a factor of about 60. Probably as a result of the low monooxygenase activity in rhesus monkey lung, no butadiene monoxide could be detected from incubations with butadiene.

Enzyme activities in *human* lung tissue were similar to those in rhesus monkeys, i.e., no monoxide formation was detected.

Interspecies comparison

From an interspecies comparison (Fig. 1) it appears that female mouse liver produced the highest amounts of butadiene monoxide, followed by male mouse liver. The formation of the monoxide in rat and human liver was less, but was similar in both these species. Rhesus monkey liver produced the lowest amounts of epoxide among the species tested.

EH activities in monkey and human liver were some 20 times higher than those measured in the liver of rodents.

With the exception of monkey liver, there was agreement between the relative EOD activities and monoxide formation in vitro. However, monkey liver was exceptionally inactive in producing butadiene monoxide, despite very high EOD activities in this tissue. This might suggest dissimilar patterns of isoenzymes of monooxygenases and/or epoxide hydrolases in monkey and man.

Although the EOD activities were quite similar in the lung tissues of rats and mice, butadiene monoxide formation in mouse lung homogenates was some 5–6 times higher than in rat lung preparations. In agreement with the very low EOD activities in monkey and human lung tissue, no butadiene monoxide could be detected on incubation of butadiene with these tissues.

Discussion

Incubation of rat liver microsomes with 1,3-butadiene resulted in the formation of the monoxide of 1,3-butadiene, as previously described by several authors (Malvoisin et al. 1979, 1980, 1982; Malvoisin and Roberfroid 1982; Bolt et al. 1983; Filser and Bolt 1984). In the present study, the kinetics of butadiene monoxide formation differed in the species investigated (rat, mouse, monkey, man), but correlated, with the exception of the rhesus monkey, with monooxygenase activities as determined by the EOD assay. Among the livers of the species examined, those of female mice formed the highest amounts of epoxide; those of male monkeys produced the lowest amounts. The ratio of activities in these species was 7 : 1.

Between the livers of Sprague-Dawley rats on one hand and B6C3F$_1$ mice, the species used for testing the carcinogenity of 1,3-butadiene (Loeser 1982; NTP 1984), on the other, there was a ratio of 1 : 1.5 (males) or 1 : 3.2 (females) for butadiene monoxide formation. Similar species differences have been demonstrated in vivo (Kreiling and Laib 1985) and in experiments on the formation of epoxide in vitro (Gervasi et al. 1984). In Ames tests with butadiene and S-9 mix from mouse and rat liver, the mutagenic response with the preparation from mice was about twice as high as that with rat tissue (Poncelet et al. 1980). This also suggests a slower formation of reactive butadiene intermediates in rat than in mouse liver.

Although there were 20-fold higher epoxide hydrolase activities in primate (monkey and human) liver compared to those of rodents, the levels of butadiene epoxide pro-

duced by primate livers in vitro were close to those in the experiments with rat liver. The EOD activity of the human liver sample was slightly higher than expected from published data (Ullrich 1977; Schoene 1972).

The EOD activity in the lung of rats and mice was only 1/10 or 1/30 of the activity in liver in these species, which is consistent with data of Aitio (1978). Similarly, the EH activity in lung of both species was lower by a factor of 10 than in liver. That butadiene monoxide formed by the lung of (female) mice was 5 times higher compared to (male) rats (whereas the EOD activity was similar) suggests that 1,3-butadiene may be a "better substrate" for some isoenzyme(s) of cytochrome P-450 present particularly in the lung of mice.

The ratio of butadiene monoxide formation by lung tissue of Sprague-Dawley rats versus NMRI mice was between 1:6 and 1:7.

In baboons Pacifici et al. (1983) have found that microsomal EH activity (styrene oxide as a substrate) was lower by a factor of 32 in lung than in liver. Our data (rhesus monkey) show only 1/60 of the EH activity in the lung compared to liver. EOD activity was at the limit of detection, similar to results reported by Lorenz et al. (1979). At such a low EOD activity no monoxide of butadiene could be detected. Similar data were obtained with the human lung sample.

The formation of butadiene di-epoxide by a second microsomal oxidation could not be demonstrated in the present study.

In conclusion, the present data demonstrate a high capability of liver and especially lung postmitochondrial preparations of mice to produce butadiene monoxide after incubation with butadiene. With rat liver and lung, significantly less butadiene monoxide was produced, which may account for the different responses in toxicity and carcinogenicity of both species in outstanding long-term bioassays. Monkey and human postmitochondrial liver preparations catalysed only slow formation of the epoxide; with lung preparations no epoxide was detected. These results could be of some importance in suggesting that rodent studies with 1,3-butadiene, especially those with mice, may not accurately reflect the human situation. Further studies are therefore necessary to quantitate the possible formation of butadiene epoxide in various species in vivo and to evaluate the contribution of different tissues, e.g., lung and liver, to the bioactivation of butadiene.

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