Carcinogenesis vol.18 no.9 pp.1687–1693, 1997

ACCELERATED PAPER

Induction of chromosome-specific aneuploidy and micronuclei in human lymphocytes by metabolites of 1,3-butadiene

Liqiang Xi, Luoping Zhang, Yunxia Wang and Martyn T. Smith[1]

Division of Environmental Health Sciences, School of Public Health, 140 Warren Hall, University of California, Berkeley, CA 94720-7360, USA

[1]To whom correspondence should be addressed

1,3-Butadiene is a carcinogen in rodents, but its potential carcinogenicity to humans remains controversial. Numerous studies have shown that butadiene and its metabolites cause sister chromatid exchanges *in vitro* and *in vivo*. To test for other types of genotoxicity, the micronucleus assay and fluorescence *in situ* hybridization (FISH) have been used to detect chromosome damage in human lymphocytes caused by two reactive metabolites of butadiene, diepoxybutane (DEB) and monoepoxybutene (MEB). DEB (0.5–5.0 μM) significantly increased micronucleus formation 4- to 6-fold ($P < 0.01$) and MEB (1–500 μM) by 2- to 4-fold ($P < 0.01$) over control levels. The ability of DEB and MEB to induce aneuploidy of chromosomes 7, 8, 12, and X was examined using dual-color FISH in both interphase and metaphase cells. These chromosomes were chosen because of their involvement in leukemogenesis. Both DEB and MEB caused dose-dependent increases in hyperdiploidy of chromosomes 12 and X, but had no discernible effect on chromosomes 7 and 8. These results suggest that DEB and MEB cause chromosome-specific aneuploidy in human cells. If formed in sufficient amounts, DEB and MEB may produce chromosome damage of the type found in leukemia following exposure to butadiene.

Introduction

1,3-Butadiene (BD*) is a carcinogen in rodents (1–4), but its potential carcinogenicity to humans remains controversial (5,6). Several epidemiological studies have demonstrated that workers exposed to BD have excess mortality due to lymphatic and hematopoietic cancers (7,8), but other investigators have questioned the methodology used in these studies and have suggested that BD is not carcinogenic to humans (9–11). The International Agency for Research on Cancer (IARC) has concluded, however, that 1,3-butadiene is probably carcinogenic to humans (12), and recent reports support that conclusion (13,14).

BD and its metabolites, 1,2:3,4-diepoxybutane (DEB) and 1,2-monoepoxybutene (MEB) have been shown to be genotoxic in a variety of test systems. In the mouse, *in vivo* for example, BD causes DNA alkylation (15,16), sister chromatid exchanges (17), micronucleus formation (17), chromosomal aberrations (18), and mutations in the *hprt* gene (19). Mutation at *hprt* in circulating T-lymphocytes has also been described in a human population exposed to BD at ~1–3 ppm (20). However, other studies, including our own, have failed to show that BD increases the rate of sister chromatid exchanges, *hprt* mutation, or chromosomal aberrations in the lymphocytes of exposed workers (21–23). Part of the explanation for these negative findings could be that only a sub-group of the workers are sensitive to low level BD exposure. Indeed, when the data of Sorsa *et al.* (22) were reanalysed on the basis of polymorphisms in glutathione S-transferase (GST) enzymes, chromosomal aberrations were significantly increased among workers lacking the GSTT1 gene (24).

DEB and MEB previously have been shown to produce sister chromatid exchanges, chromosomal aberrations, and *hprt* mutation in cultured human cells (25–28). Sensitivity of human cells to DEB and MEB has once again been shown to be dependent on GST genotype (29–31). To expand upon these studies, we have used the antikinetochore antibody modification of the micronucleus (MN) assay in human binucleated lymphocytes to test the ability of DEB and MEB to produce micronuclei and determine the mechanism involved. We have also tested the ability of DEB and MEB to produce chromosome-specific aneuploidy using fluorescence *in situ* hybridization (FISH) of interphase and metaphase cells. Since aneuploidy of chromosomes of 12 and X is commonly observed in lymphocytic leukemias, and aneuploidy of chromosomes 7 and 8 is commonly observed in myeloid leukemias, we have tested the ability of DEB and MEB to produce aneuploidy of these four chromosomes in cultured human lymphocytes. Further, we have compared the effects of the BD metabolites with those of chloral hydrate, a known inducer of micronuclei and aneuploidy.

***Abbreviations**: BD, 1,3-butadiene; DEB, 1,2:3,4-diepoxybutane; MEB, 1,2-monoepoxybutene; CH, chloral hydrate; MN, micronuclei; MN-cells, micronucleated cells; FISH, fluorescence *in situ* hybridization.

Materials and methods

Blood donors, cell culture and slide preparation

The majority of the experiments described here were performed using peripheral blood from a healthy non-smoking male. Peripheral blood from three other male donors was used for additional experiments. The heparinized whole blood (0.4 ml) was cultured in a final volume of 5 ml RPMI 1640 supplemented with 10% fetal bovine serum, 1% phytohemagglutinin (Pharmacia Biotech, Piscataway, NJ), 1% glutamine, and 1% penicillin-streptomycin at 37°C for 72 h in a 5% CO_2 moist atmosphere. Cytochalasin B (Sigma, St Louis, MO) (6 μg/ml) was added at 44 h incubation for the MN assay. Colcemid (0.1 μg/ml) was added to some cultures at 68 h to prepare metaphase spreads. At 72 h, lymphocytes were isolated using a Ficoll–Paque density gradient, washed with PBS, and spun onto glass slides using a cytocentrifuge (Cytospin-II, Shandon, Sewickley, PA). After allowing the slides to air-dry, they were fixed in methanol for 15 min, air-dried, and stored desiccated, under a N_2 atmosphere at –20°C. In FISH assays, at 72 h following the initiation of the culture, the cells were incubated with hypotonic solution (0.075 M KCl) for 20 min at 37°C and fixed three times with methanol:glacial acetic acid (3:1). The fixed cells were dropped onto glass slides, then allowed to air dry, and stored at –20°C under a N_2 atmosphere.

Chemical treatment

The cultured cells were treated with DEB (Aldrich, Milwaukee, WI) from 24 to 72 h of culture in T_{25} flasks to allow gas exchange, or with MEB (Aldrich, Milwaukee, WI) from 24 to 44 h of culture in 5.5 ml air-tight glass tubes. DEB and MEB stock solutions were freshly prepared for each experiment and diluted in sterile H_2O. All the treatments were performed in duplicate for each dose. The volume added to each culture was 100 μl. Chloral hydrate

(Sigma, St Louis, MO) and H_2O alone were used as positive and negative controls.

Staining and scoring

MN assay. Treated cells were stained with an antikinetochore antibody (Chemico, Temecula, CA) essentially as described by Eastmond and Tucker (32). Briefly, slides were incubated at 37°C in humidified chamber with the antikinetochore antibody for 1 h, washed twice in PBS containing 0.1% Tween 20 (Fisher, Fair Lawn, NJ), and then incubated with fluoresceinated goat anti-human IgG for 1 h. After washing again, slides were stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma, St Louis, MO) in Vectashield Mounting Medium (Vector, Burlingame, CA).

Randomized and coded slides were scored using a Nikon microscope equipped with epifluorescent illumination and filters for fluorescein (excitation at 470 nm, dichroic at 510 nm, barrier at 520–560 nm) and quinacrine (excitation at 400–440 nm, dichroic at 450 nm, barrier at 470 nm). Scoring criteria and procedures for the measurement of replicative index have been described previously (33).

FISH assay. Rapid dual-color staining of two centromeric chromosomal probes (biotin-labeled chromosome 12 and digoxigenin-labeled chromosome X, biotin-labeled chromosome 8 and digoxigenin-labeled chromosome 7, Oncor Inc., Gaitherburg, MD) was used. Areas with an optimal density of cells in prepared slides were marked. These areas were stained essentially as described by Eastmond and Pinkel (34). Briefly, the cell DNA was denatured in 70% formamide in 2×SSC (0.3 M sodium chloride and 0.03 M sodium citrate, pH 7.0) at 72°C for 3 min. Then the slides were quickly removed to ice-cold 70%, 85%, and 100% ethanol series to dehydrate and air dried. The hybridization cocktail consisted of 1 µl of probe chromosome 12 or 8, 1 µl of probe chromosome X or 7, 1 µl of salmon sperm DNA, and 7 µl of MM2.1 hybridization mix (55% formamide; 1×SSC; 10% dextran sulfate). This probe mixture was denatured at 70°C for 5 min and rapidly removed into ice. The denatured probe was applied to the marked regions on the pre-warmed slides at 37°C on a slide warmer. The slides were then cover-slipped and incubated overnight at 37°C in a humidified chamber. Post hybridization washes were in 0.5×SSC at 72°C for 5 min. The slides were stained in a dual-color detection solution with 20 µg/ml anti-digoxigenin (Boehinger-Mannheim) and 20 µg/ml FITC-avidin (Vector) in 0.1 M phosphate buffer (pH 7.0) for 15 min at 37°C. After three washes in PN buffer (0.1 M sodium phosphate containing 6% 0.1M sodium phosphate monobasic, pH 8.0) of 3 min each with intermittent agitation at room temperature, the hybridization signal was amplified once using a biotinylated goat anti-avidin antibody (Vector, Burlingame, CA) and then fluorescent-conjugated avidin. DAPI (0.25 µg/ml) in Vectashield Mounting Medium (Vector, Burlingame, CA) was used as DNA counterstain.

All scoring was performed from coded slides using a Nikon microscope equipped with epifluorescent illumination, a 100× oil immersion lens and a triple-band pass filter for DAPI/FITC/Texas Red (excitation at 405 nm, 490 nm, and 570 nm; emission at 460 nm, 525 nm, and 635 nm). One thousand cells per dose from each treatment (3000 cells from the control) were scored in two spots on one slide. The nuclei appeared blue with bright green spots (FITC, chromosome 12 or 8) and red spots (Texas red-labeled, chromosome X or 7) indicating the hybridization regions. Both the number of interphase nuclei with 0, 1, 2, 3, and ⩾4 spots of each color and the total number of scored cells were recorded. The standard scoring criteria for interphase (35) and metaphase cells (36) was used as described previously.

Data presentation and statistical analysis

Micronucleated cell and FISH data are presented in tabular form. Statistical analyses were performed using a one-tailed Fisher's exact test to compare the micronucleated cell, hyperdiploidy and monosomy frequencies between control and each chemical treatment. Critical values were determined by a 0.05 probability of type I error.

Micronucleus formation was also expressed in terms of total micronuclei allowing for separation into kinetochore-positive and -negative categories. These data are presented in Figures 1 and 2. To examine for trend in elevated frequencies of total micronuclei, kinetochore-positive and -negative micronuclei, the Generalized Linear Model was used while chemical concentrations were transformed (after adding 1 to each concentration) by log.

Results

Micronucleus induction by DEB and MEB in human lymphocytes

Whole blood was cultured for 24 h and treated with either chloral hydrate at 500 µM or DEB at 0.1–5.0 µM for an additional 48 h. Lymphocytes were harvested using Ficoll isolation. DEB produced a dose-dependent increase in the number of micronuclei per 1000 binucleated cells (r = 0.85, P=0.0001; Figure 1a). This increase was ⩾5-fold over the level found in controls (Figure 1a, Table I). Only at concentrations >5.0 µM DEB were significant toxicity and a decrease in replicative index observed (data not shown). Chloral hydrate, the positive control used in these experiments, produced an ~3-fold increase in micronucleated cells. DEB was, therefore, clearly a powerful inducer of micronuclei in lymphocytes from this human volunteer.

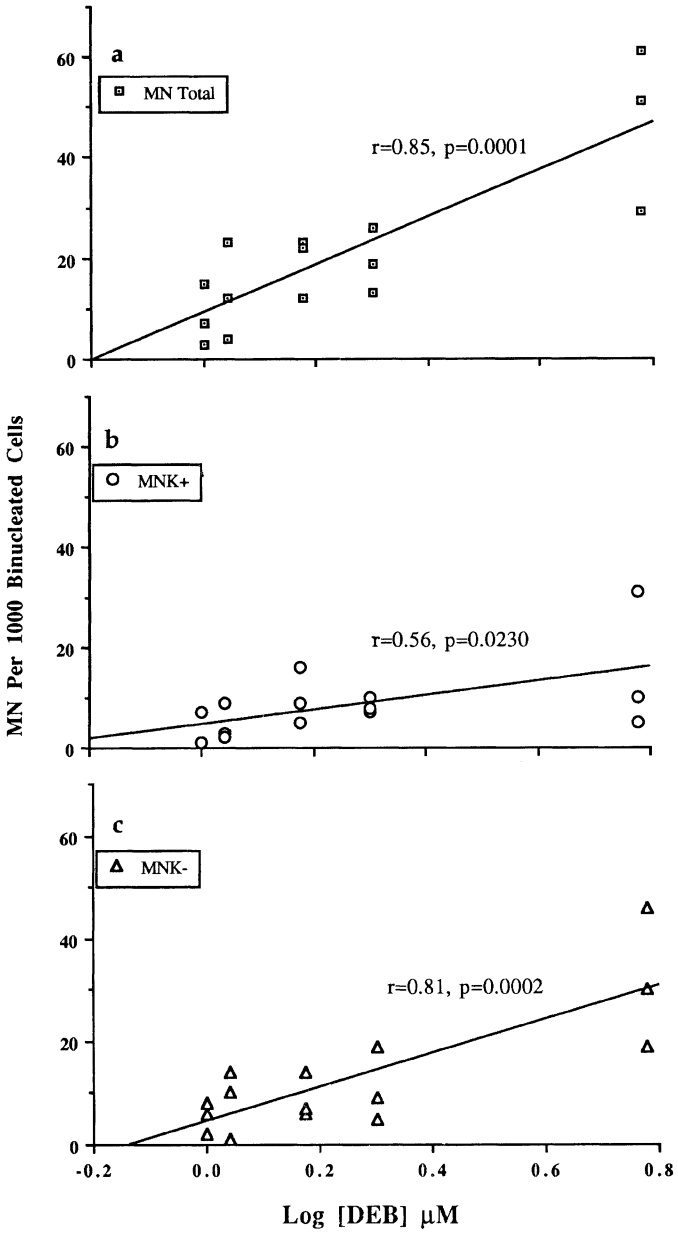

Fig. 1. Effects of 1,2:3,4-diepoxybutane on micronucleus induction in human lymphocytes. (**a**) Total micronuclei; (**b**) kinetochore-positive micronuclei (MNK^+); and (**c**) kinetochore-negative micronuclei (MNK^-). Data from three separate experiments are shown.

The nature of the micronuclei induced by DEB is shown in Figure 1b and c. At lower doses of DEB (<1 µM), the proportion of kinetochore-positive and -negative micronuclei was approximately equal. However, at the highest concentration

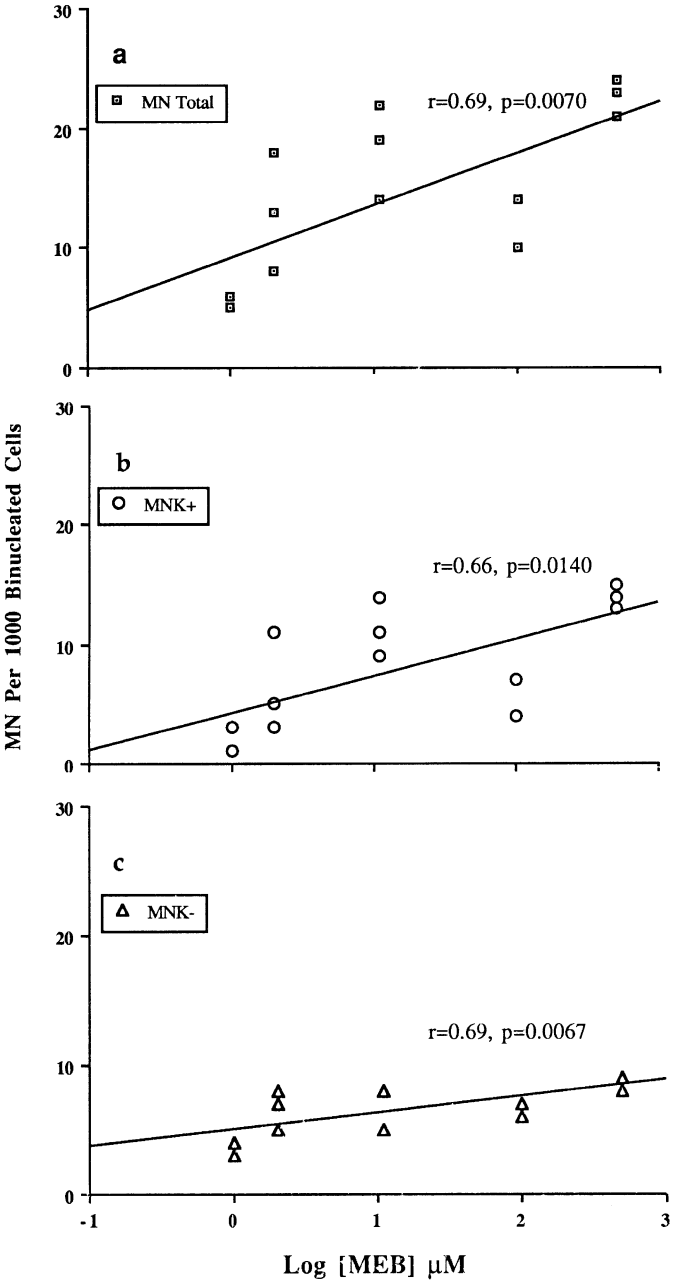

Fig. 2. Effects of 1,2-epoxybutene on micronucleus induction in human lymphocytes. (**a**) Total micronuclei; (**b**) kinetochore-positive micronuclei (MNK^+); and (**c**) kinetochore-negative micronuclei (MNK^-). Data from three separate experiments are shown.

tested (5 μM) the proportion of kinetochore-negative micronuclei rose dramatically. This resulted in a highly significant ($P = 0.002$) linear dose–response for the production of kinetochore-negative micronuclei. The dose–response curve for kinetochore-positive micronuclei tended to plateau but still showed a significant linear trend ($P = 0.023$). These data showed that DEB is potentially capable of causing both chromosome breakage (clastogenicity) and alterations in chromosome number (aneuploidy).

MEB is a volatile compound and it was therefore necessary to treat cells in an air-tight tube. This only allowed for a 20 h exposure to MEB as opposed to the 48 h exposure used in experiments with DEB. A simultaneous 20 h positive control

Table I. Micronucleated cells in cultured human lymphocytes treated with 1,2:3,4-diepoxybutane (DEB) and 1,2-epoxybutene (MEB)

Chemical treatment	Concentration (μM)	MN-cells/1000 binucleated cells[b]	Replicative index (RI)[e]
Control (48 h)[a]	0	8.3 ± 6.1[c]	2.16
Chloral hydrate (48 h)	500	23.3 ± 7.0[d]	2.02
DEB (48 h)	0.1	13.0 ± 9.5	2.14
	0.5	18.7 ± 6.7[d]	2.16
	1.0	19.0 ± 6.0 [d]	2.11
	5.0	45.3 ± 14.8 [d]	1.88
Control (20 h)	0	5.3 ± 0.6	1.93
Chloral hydrate (20 h)	500	11.3 ± 2.1[d]	1.82
MEB (20 h)	1	12.3 ± 5.0[d]	1.92
	10	18.0 ± 3.6[d]	1.75
	100	11.7 ± 2.3[d]	1.93
	500	22.7 ± 1.5[d]	1.74

[a]48 h treatment in open culture system, 20 h treatment in air-tight culture system.
[b]Data from three experiments in which 1000 binucleated cells were scored from duplicate cultures per experiment.
[c]Mean ± SD.
[d]$P < 0.01$ in comparison with corresponding control (one-tailed Fisher's exact test).
[e]RI = [(%mononuclear cells) + (2×%binuclear cells + (3×%trinuclear cells) + (4×%tetra- or > tetranuclear cells)]/100.

using chloral hydrate was therefore also employed. It was found that a 20 h exposure to both MEB and chloral hydrate caused a significant increase in the number of micronucleated cells (Table I) and total micronuclei (Figure 2a). With chloral hydrate, the level of micronuclei almost doubled and that obtained with MEB showed a clear dose response ($r = 0.69$, $P = 0.007$; Figure 2a) rising to a 4-fold increase at 500 μM (Table I, Figure 2a). As with DEB, both kinetochore-negative and kinetochore-positive micronuclei were induced by MEB (Figure 2b and c). The concentration of MEB required to induce a significant increase in micronuclei was as low as 1–10 μM. MEB at 10 μM for 20 h induced a similar level of MN as DEB at 0.5 μM for 48 h.

Induction of hyperdiploidy by DEB and MEB in cultured interphase lymphocytes

The demonstrated ability of DEB to induce kinetochore-positive micronuclei strongly suggests that it is also an inducer of aneuploidy. We therefore tested the ability of DEB to induce aneuploidy of chromosomes 7, 8, 12, and X, using dual-color FISH in interphase cells. DEB did not produce significant increases in hyperdiploidy of chromosomes 7 and 8 (Table II), but did cause dose-dependent increases in hyperdiploidy of chromosomes 12 and X. The level of hyperdiploidy induced by DEB at 1.0 and 5.0 μM was approximately the same as that induced by 500 μM chloral hydrate. However, in contrast to DEB, chloral hydrate induced similar levels of aneuploidy of all four chromosomes (Table II).

The effects of MEB were very similar to those of DEB. The levels of hyperdiploidy of chromosomes 7 and 8 were unaltered by MEB exposure. Exposure to chloral hydrate about doubled hyperdiploidy levels of all four chromosomes (Table II). In whole blood cultures exposed to MEB for 20 h there was a significant induction of hyperdiploidy of chromosomes 12 and X. Only the highest concentration of MEB (500 μM) induced a significant increase in hyperdiploidy of chromosome X, but all concentrations of MEB, from 1–500 μM, caused significant increases of hyperdiploidy of

Table II. Frequency of hyperdiploidy of chromosomes 7, 8, 12, and X in interphase cultured human lymphocytes treated with 1,2:3,4-diepoxybutane (DEB) and 1,2-epoxybutene (MEB)

Chemical treatment	Concentration (μM)	Chromosomes (per 1000 cells)[a]			
		7	8	12	X
Control (48 h)[b]	0	14.7 ± 2.1	12.7 ± 4.0	15.7 ± 9.4	15.0 ± 5.6
Chloral hydrate (48 h)	500	33.7 ± 5.5[c]	28.3 ± 3.1[c]	27.0 ± 9.8[c]	27.0 ± 4.6[c]
DEB (48 h)	0.1	14.7 ± 3.2	7.3 ± 3.1	15.7 ± 0.6	11.7 ± 6.7
	0.5	18.3 ± 11.1	13.3 ± 7.0	20.3 ± 8.5	18.7 ± 7.0
	1.0	15.7 ± 2.9	9.3 ± 1.5	24.3 ± 8.5[c]	28.0 ± 4.0[c]
	5.0	14.7 ± 3.1	8.3 ± 3.5	32.7 ± 6.3[c]	28.7 ± 2.1[c]
Control (20 h)	0	12.3 ± 2.9	12.0 ± 1.7	8.8 ± 6.8	13.5 ± 9.4
Chloral hydrate (20 h)	500	27.0 ± 5.6[c]	28.0 ± 6.6[c]	21.0 ± 1.7[c]	24.3 ± 4.0[c]
MEB (20h)	1	14.7 ± 3.1	11.7 ± 1.5	18.0 ± 6.9[c]	15.3 ± 4.0
	10	14.0 ± 3.0	9.3 ± 3.1	24.7 ± 5.5[c]	17.7 ± 2.3
	100	13.7 ± 3.1	12.3 ± 3.8	21.3 ± 10.2[c]	17.0 ± 2.6
	500	11.7 ± 3.5	12.7 ± 6.1	18.0 ± 9.6[c]	22.0 ± 5.3[c]

[a]Data from three experiments in which 1000 cells were scored from duplicate cultures per experiment.
[b]48 h treatment in open culture system, 20 h treatment in air-tight culture system.
[c]$P < 0.01$ in comparison with control (one-tailed Fisher's exact test).

Table III. Aneuploidy of chromosomes 7 and 12 in cultured human lymphocytes at metaphase treated with 1,2:3,4-diepoxybutane

DEB (μM)	Total scored metaphases[a]	Chromosome 7 (per 1000 cells)		Chromosome 12 (per 1000 cells)	
		Monosomy	Hyperdiploidy	Monosomy	Hyperdiploidy
0	3664	20.5	8.2	15.0	12.3
1	683	41.0[b]	8.8	29.3[b]	19.0
5	737	59.7[b]	10.9	36.6[b]	31.2[b]

[a]Data from two duplicate experiments. Metaphase cells were prepared from the same cultures used for the interphase analysis.
[b]$P < 0.01$ in comparison with control (one-tailed Fisher's exact test).

chromosome 12. These results demonstrate that MEB is also able to produce chromosome specific-aneuploidy and has effects of most significance on chromosome 12.

Induction of aneuploidy of chromosome 12, but not 7, by DEB in metaphase cells

In order to further test the specificity of DEB's aneuploidy-inducing effects, we studied the effect of DEB in metaphase cells prepared from the same cultures used for the interphase analysis. Aneuploidy of chromosomes 7 and 12 were studied simultaneously using dual-color FISH. Chromosome 12 was chosen because of its apparent high sensitivity and chromosome 7 because we have found this chromosome to be highly sensitive to the aneuploidy inducing effects of other chemicals (unpublished data). Because metaphase analysis is much more time-consuming than interphase analysis we examined only the effects of DEB at 1 and 5 μM (Table III). The data confirmed our findings in interphase cells, in that DEB had a selective hyperdiploidy-inducing effect on chromosome 12 causing a significant 2–3-fold increase at 5 μM. The same concentration of DEB in the same cells had no significant effect on hyperdiploidy levels of chromosome 7. Monosomy levels of chromosomes 7 and 12 however, were equally affected by DEB, being doubled at 1 μM and tripled at 5 μM (Table III).

Induction of chromosome-specific aneuploidy in donors of different GST genotypes

There was also the possibility that the chromosome-specific effects described above were exclusive to the blood donor used in the experiments. We therefore examined the aneuploidy-inducing effects of DEB in blood from four different donors cultured and treated simultaneously under identical conditions (Table IV). The four donors were of different GSTT1 and GSTM1 genotypes. The principal donor (#1) used in all experiments described above was GSTT1 and M1 null, as determined by PCR–based procedures (37,38). Aneuploidy of chromosomes 8 and 12 was determined using interphase cytogenetics in 1000 cells per dose group from the blood of each donor after 48 h exposure to 0, 1, or 5 μM DEB.

A selective hyperdiploidy inducing effect of DEB on chromosome 12, but not chromosome 8, was observed in three out of the four donors (Table IV). One donor, who was both GSTT1 and M1 positive, showed no increase in hyperdiploidy of either chromosome 8 or 12 following DEB treatment. This donor also failed to respond to DEB with regard to micronucleus induction, whereas the other three donors all responded significantly and about equally (data not shown). The selective hyperdiploidy-inducing effect of DEB on chromosome 12 is therefore not restricted to a single donor.

Discussion

Here, we report clear evidence that two metabolites of 1,3-butadiene, DEB and MEB produce aneuploidy and micronuclei in human cells in addition to their previously described genotoxic effects. Both DEB and MEB were potent inducers of micronuclei and aneuploidy being effective in whole blood cultures at concentrations as low as 1 μM. In other genotoxicity assays MEB has only been shown to be effective at concentrations of ⩾25 μM (27,28,39). Thus, MEB may be especially effective at inducing malsegregation of chromosomes leading

Table IV. Hyperdiploidy of chromosomes 8 and 12 induced by 1,2:3,4-diepoxybutane (DEB) in donors with different GST genotype

GST genotype	DEB (μM)	Chromosome 8 (per 1000 cells)					Chromosome 12 (per 1000 cells)				
		⩽1	2	3	⩾4	Hyperdiploidy	⩽1	2	3	⩾4	Hyperdiploidy
Donor 1[a]	0	19	969	7	5	12	18	968	10	4	14
$T1^{-}M1^{-}$	1	9	978	12	1	13	6	978	15	1	16
	5	19	967	11	3	14	16	946	35	3	38[b]
Donor 2	0	11	975	10	4	14	8	980	8	4	12
$T1^{-}M1^{+}$	1	18	969	8	5	13	20	967	7	6	13
	5	8	979	9	4	13	6	959	30	5	35[b]
Donor 3	0	12	977	9	2	11	10	981	7	2	9
$T1^{+}M1^{-}$	1	13	971	8	8	16	6	977	10	7	17
	5	11	977	4	8	12	13	957	22	8	30[b]
Donor 4	0	5	983	4	8	12	7	981	4	8	12
$T1^{+}M1^{+}$	1	10	977	10	3	13	12	972	13	3	16
	5	8	982	5	5	10	9	977	9	5	14

[a]Donor 1 is the principal donor used in previous experiments.
[b]$P < 0.01$ (one-tailed Fisher's exact test).

to aneuploidy as compared with the induction of other forms of genotoxicity. DEB was somewhat more potent than MEB at inducing aneuploidy and micronuclei because it produced larger-fold increases at similar concentrations. It should be remembered, however, that MEB exposure was for only 20 h as compared to 48 h for DEB, because of the volatility of MEB, and thus direct comparisons of the potency of the two compounds are not really possible in this system.

Our findings with DEB and MEB are consistent with the recent observations of Xiao *et al.* (40) in mice. These workers used centromeric DNA probes to characterize micronuclei in mouse bone marrow and concluded that butadiene, DEB, and MEB had not only strong clastogenic (chromosome-breaking) properties, but were also aneugens (aneuploidy-inducing compounds) in mice. In the present study, we demonstrate that DEB and MEB induce significant increases in both kinetochore-negative and -positive micronuclei showing that they have both clastogenic and aneugenic properties in cultured human lymphocytes.

Since both DEB and MEB produced significant dose-dependent increases in kinetochore-positive micronuclei, we investigated their ability to produce aneuploidy by FISH. Initially, their effect on chromosomes 7, 8, 12, and X was examined using centromeric probes in interphase cells. We chose these particular chromosomes because of their reported involvement in the development of leukemias. Monosomy of chromosome 7 and trisomy 8 are commonly found in myeloid leukemias (41,42); trisomy 7 in multiple myeloma (43); and trisomy 12 and X in lymphocytic leukemias (42,44). To our surprise we found that both DEB and MEB significantly altered the level of hyperdiploidy of chromosomes 12 and X but had no discernible effect on chromosomes 7 and 8. We switched the color labeling of the probes and still found the same selective effect (data not shown). The selective effect of DEB and MEB on chromosomes 12 and X was in stark contrast to the effects of the known aneuploidogen chloral hydrate, which produced equal increases in the hyperdiploidy rates of all four chromosomes examined. If the selective effect of the butadiene metabolites is real and not an artifact of the FISH procedure, it suggests that DEB and MEB produce aneuploidy by a different mechanism to chloral hydrate, which is considered a spindle poison.

Mechanisms by which DEB and MEB could induce aneuploidy, other than spindle poisoning, are numerous. Since they are both also clastogens and cause strand breakage, it is possible they cause chromosome 'stickiness' or the formation of unresolved recombination structures. It is also of interest that topoisomerase II inhibitors have recently been shown to cause aneuploidy as well as chromosome breakage (45). It would therefore be of interest to test the ability of DEB and MEB to inhibit topoisomerase II.

We were concerned that the selective effect observed in interphase cells was an artifact of the FISH procedure. This caused us to examine metaphase spreads prepared from the same blood cultures. Using dual-color FISH, the effects of DEB on chromosomes 7 and 12 were compared. In agreement with the interphase data, we found that DEB produced a highly significant increase in hyperdiploidy of chromosome 12 but had no effect on hyperdiploidy of chromosome 7. Interestingly, monosomy (the loss of one chromosome) of both chromosomes 7 and 12 was increased equally by DEB. It is not possible to determine monosomy rates in interphase cells by FISH because of probe overlap (34), and so comparison with metaphase data is not possible. We were also concerned that the selective hyperdiploidy-inducing effects of DEB on chromosome 12 may be specific to the male donor used. We therefore tested the effects of DEB on hyperdiploidy of chromosomes 8 and 12 in blood from four donors of different GST genotype. Similar selective effects of DEB on chromosome 12 were observed in other donors, supporting our belief that the selective effect is real.

The potential reasons why DEB should (i) selectively elevate the rate of hyperdiploidy of chromosome 12 but not 7 or 8; and (ii) have equal effects on loss of chromosomes 7 and 12 are unclear to us at this time. It is of interest that hyperdiploidy of chromosome 12 is most commonly associated with lymphocytic leukemias, lymphoma, and seminoma (46). These forms of cancer have been associated with exposure to 1,3-butadiene (5). Further, to the best of our knowledge this is the first report of a chemical having a highly specific, selective aneuploidy-inducing effect on some chromosomes but not on others. Just how selective this effect is must await further study by spectral karyotyping or some similar technique and, if found to be real, mechanistic explanations must be sought. This is a goal of future research in our laboratory.

Acknowledgements

We thank Dr John Wiencke for genotyping the donors with respect to GST polymorphisms and for his advice on technical aspects of this work. This work was supported by a fellowship from the National Cancer Institute to L.Xi, Subcontract B118-05 from NCI and NIH grant P42ES04705 (to M.T.Smith) from the National Institute for Environmental Health Sciences, using funds provided by EPA.

References

1. Owen,P.E., Glaister,J.R., Gaunt,I.F. and Pullinger,D.H. (1987) Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. *Am. Ind. Hyg. Assoc. J.*, **48**, 407–413.
2. Miller,R.A. and Boorman,G.A. (1990) Morphology of neoplastic lesions induced by 1,3–butadiene in B6C3F1 mice. *Environ. Health Perspect.*, **86**, 37–48.
3. Melnick,R.L., Huff,J.E., Roycroft,J.H., Chou,B.J. and Miller,R.A. (1990) Inhalation toxicology and carcinogenicity of 1,3-butadiene in B6C3F1 mice following 65 weeks of exposure. *Environ. Health Perspect.*, **86**, 27–36.
4. Owen,P.E. and Glaister,J.R. (1990) Inhalation toxicity and carcinogenicity of 1,3-butadiene in Sprague–Dawley rats. *Environ. Health Perspect.*, **86**, 19–25.
5. Melnick,R.L. and Kohn,M.C. (1995) Mechanistic data indicate that 1,3-butadiene is a human carcinogen. *Carcinogenesis*, **16**, 157–163.
6. Bond,J.A., Recio,L. and Andjelkovich,D. (1995) Epidemiological and mechanistic data suggest that 1,3-butadiene will not be carcinogenic to humans at exposures likely to be encountered in the environment or workplace. *Carcinogenesis*, **16**, 165–171.
7. Matanoski,G.M., Santos-Burgoa,C. and Schwartz,L. (1990) Mortality of a cohort of workers in the styrene–butadiene polymer manufacturing industry (1943–1982). *Environ. Health Perspect.*, **86**, 107–117.
8. Santos-Burgoa,C., Matanoski,G.M., Zeger,S. and Schwartz,L. (1992) Lymphohematopoietic cancer in styrene–butadiene polymerization workers [see comments]. *Am. J. Epidemiol.*, **136**, 843–854.
9. Cole,P., Delzell,E. and Acquavella,J. (1993) Exposure to butadiene and lymphatic and hematopoietic cancer [see comments]. *Epidemiology*, **4**, 96–103.
10. Matanoski,G.M. and Santos-Burgoa,C. (1994) Butadiene and lymphatic and hematopoietic cancer (letter; comment). *Epidemiology*, **5**, 261–263.
11. Cowles,S.R., Tsai,S.P., Snyder,P.J. and Ross,C.E. (1994) Mortality, morbidity, and haematological results from a cohort of long-term workers involved in 1,3-butadiene monomer production. *Occup. Environ. Med.*, **51**, 323–329.
12. IARC (1992) 1,3-Butadiene. *IARC Monog. Eval. Carcino. Risks Hum.*, **54**, 237–85.
13. Macaluso,M., Larson,R., Delzell,E., Sathiakumar,N., Hovinga,M., Julian,J., Muir,D. and Cole,P. (1996) Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. *Toxicology*, **113**, 190–202.
14. Delzell,E., Sathiakumar,N., Hovinga,M., Macaluso,M., Julian,J., Larson,R., Cole,P. and Muir,D.C. (1996) A follow-up study of synthetic rubber workers. *Toxicology*, **113**, 182–189.
15. Jelitto,B., Vangala,R.R. and Laib,R.J. (1989) Species differences in DNA damage by butadiene: role of diepoxybutane. *Arch. Toxicol. Suppl.*, **13**, 246–249.
16. Leuratti,C., Jones,N.J., Marafante,E., Kostiainen,R., Peltonen,K. and Waters,R. (1994) DNA damage induced by the environmental carcinogen butadiene: identification of a diepoxybutane–adenine adduct and its detection by ^{32}P-postlabelling. *Carcinogenesis*, **15**, 1903–1910.
17. Tice,R.R., Boucher,R., Luke,C.A. and Shelby,M.D. (1987) Comparative cytogenetic analysis of bone marrow damage induced in male B6C3F1 mice by multiple exposures to gaseous 1,3-butadiene. *Environ. Mutagen.*, **9**, 235–250.
18. Irons,R.D., Oshimura,M. and Barrett,J.C. (1987) Chromosome aberrations in mouse bone marrow cells following *in vivo* exposure to 1,3-butadiene. *Carcinogenesis*, **8**, 1711–1714.
19. Cochrane,J.E. and Skopek,T.R. (1994) Mutagenicity of butadiene and its epoxide metabolites: II. Mutational spectra of butadiene, 1,2-epoxybutene and diepoxybutane at the hprt locus in splenic T cells from exposed B6C3F1 mice. *Carcinogenesis*, **15**, 719–723.
20. Ward,J.B.,Jr., Ammenheuser,M.M., Bechtold,W.E., Whorton,E.B.,Jr. and Legator,M.S. (1994) Hprt mutant lymphocyte frequencies in workers at a 1,3-butadiene production plant. *Environ. Health Perspect.*, **102**, 79–85.
21. Ahlberg,R.W., Sorsa,M., Pfafli,P., Maki-Paakanen,J. and Viinanen,R. (1992) Genotoxicity and exposure assessment in the manufacture of 1,3-butadiene, *WHO Report on Occupational Health in the Chemical Industry.* WHO, Copenhagen, pp. 199–204.
22. Sorsa,M., Autio,K., Demopoulos,N.A., Jarventaus,H., Rossner,P., Sram,R.J., Stephanou,G. and Vlachodimitropoulos,D. (1994) Human cytogenetic biomonitoring of occupational exposure to 1,3-butadiene. *Mutat. Res.*, **309**, 321–326.
23. Hayes,R.B., Xi,L., Bechtold,W.E., Rothman,N., Yao,M., Henderson,R., Zhang,L., Smith,M.T., Zhang,D., Wiemels,J., Dosemeci,M., Yin,S. and O'Neill,J.P. (1996) Hprt mutation frequency among workers exposed to 1,3-butadiene in China. *Toxicology*, **113**, 100–105.
24. Sorsa,M., Osterman-Golkar,S., Peltonen,K., Saarikoski,S.T. and Sram,R. (1996) Assessment of exposure to butadiene in the process industry. *Toxicology*, **113**, 77–83.
25. Wiencke,J.K., Christiani,D.C. and Kelsey,K.T. (1991) Bimodal distribution of sensitivity to SCE induction by diepoxybutane in human lymphocytes. I. Correlation with chromosomal aberrations. *Mutat. Res.*, **248**, 17–26.
26. Kelsey,K.T., Christiani,D.C. and Wiencke,J.K. (1991) Bimodal distribution of sensitivity to SCE induction by diepoxybutane in human lymphocytes. II. Relationship to baseline SCE frequency. *Mutat. Res.*, **248**, 27–33.
27. Sasiadek,M., Norppa,H. and Sorsa,M. (1991) 1,3-Butadiene and its epoxides induce sister-chromatid exchanges in human lymphocytes *in vitro*. *Mutat. Res.*, **261**, 117–121.
28. Cochrane,J.E. and Skopek,T.R. (1994) Mutagenicity of butadiene and its epoxide metabolites: I. Mutagenic potential of 1,2-epoxybutene, 1,2,3,4-diepoxybutane and 3,4-epoxy-1,2-butanediol in cultured human lymphoblasts. *Carcinogenesis*, **15**, 713–717.
29. Kelsey,K.T., Wiencke,J.K., Ward,J., Bechtold,W. and Fajen,J. (1995) Sister-chromatid exchanges, glutathione S-transferase theta deletion and cytogenetic sensitivity to diepoxybutane in lymphocytes from butadiene monomer production workers. *Mutat. Res.*, **335**, 267–273.
30. Norppa,H., Hirvonen,A., Jarventaus,H., Uuskula,M., Tasa,G., Ojajarvi,A. and Sorsa,M. (1995) Role of GSTT1 and GSTM1 genotypes in determining individual sensitivity to sister chromatid exchange induction by diepoxybutane in cultured human lymphocytes. *Carcinogenesis*, **16**, 1261–1264.
31. Uuskula,M., Jarventaus,H., Hirvonen,A., Sorsa,M. and Norppa,H. (1995) Influence of GSTM1 genotype on sister chromatid exchange induction by styrene-7,8-oxide and 1,2-epoxy-3-butene in cultured human lymphocytes. *Carcinogenesis*, **16**, 947–950.
32. Eastmond,D.A. and Tucker,J.D. (1989) Identification of aneuploidy-inducing agents using cytokinesis-blocked human lymphocytes and an antikinetochore antibody. *Environ. Mol. Mutagenesis*, **13**, 34–43.
33. Zhang,L., Robertson,M.L., Kolachana,P., Davison,A.J. and Smith,M.T. (1993) Benzene metabolite, 1,2-benzenetriol, induces micronuclei and oxidative DNA damage in human lymphocytes and HL60 cells. *Environ. Mol. Mutagen.*, **21**, 339–348.
34. Eastmond,D.A. and Pinkel,D. (1990) Detection of aneuploidy and aneuploidy-inducing agents in human lymphocytes using fluorescence in situ hybridization with chromosome-specific DNA probes. *Mutat. Res.*, **234**, 303–318.
35. Zhang,L., Venkatesh,P., Creek,M.L. and Smith,M.T. (1994) Detection of 1,2,4-benzenetriol induced aneuploidy and microtubule disruption by fluorescence *in situ* hybridization and immunocytochemistry. *Mutat. Res.*, **320**, 315–327.
36. Tucker,J.D., Lee,D.A., Ramsey,M.J., Briner,J., Olsen,L. and Moore,D.H.N. (1994) On the frequency of chromosome exchanges in a control population measured by chromosome painting. *Mutat. Res.*, **313**, 193–202.
37. Wiencke,J.K., Kelsey,K.T., Lamela,R.A. and Toscano,W.A.,Jr. (1990) Human glutathione S-transferase deficiency as a marker of susceptibility to epoxide-induced cytogenetic damage. *Cancer Res.*, **50**, 1585–1590.
38. Warwick,A., Sarhanis,P., Redman,C., Pemble,S., Taylor,J.B., Ketterer,B., Jones,P., Alldersea,J., Gilford,J., Yengi,L. *et al.* (1994) Theta class glutathione S-transferase GSTT1 genotypes and susceptibility to cervical neoplasia: interactions with GSTM1, CYP2D6 and smoking. *Carcinogenesis*, **15**, 2841–2845.
39. Wiencke,J.K. and Kelsey,K.T. (1993) Susceptibility to induction of chromosomal damage by metabolites of 1,3-butadiene and its relationship to 'spontaneous' sister chromatid exchange frequencies in human lymphocytes. *IARC Sci. Publ.*, **1993**, 265–273.
40. Xiao,Y., de Stoppelaar,J.M., Hoebee,B., Schriever-Schwemmer,G., Adler,I.D. and Tates,A.D. (1996) Analysis of micronuclei induced by 1,3-butadiene and its metabolites using fluorescence *in situ* hybridization. *Mutat. Res.*, **354**, 49–57.
41. Le Beau,M.M. (1990) Chromosomal abnormalities in hematologic malignant diseases. In Mendelsohn, M.L. and Albertini, R.J. (eds) *Progress in Clinical and Biological Research: Mutation and the Environment*. Wiley-Liss, New York, Vol. 340, pp. 325–335.

42. Sandberg,A.A. (1990) *The Chromosomes in Human Cancer and Leukemia*, 2nd edn. Elsevier, New York.
43. Drach,J., Schuster,J., Nowotny,H., Angerler,J., Rosenthal,F., Fiegl,M., Rothermundt,C., Gsur,A., Jager,U., Heinz,R. *et al*. (1995) Multiple myeloma: high incidence of chromosomal aneuploidy as detected by interphase fluorescence *in situ* hybridization. *Cancer Res.*, **55**, 3854–3859.
44. Losada,A.P., Wessman,M., Tiainen,M., Hopman,A.H., Willard,H.F., Sole,F., Caballin,M.R., Woessner,S. and Knuutila,S. (1991) Trisomy 12 in chronic lymphocytic leukemia: an interphase cytogenetic study. *Blood*, **78**, 775–779.
45. Ferguson,L.R. and Baguley,B.C. (1994) Topoisomerase II enzymes and mutagenicity. *Environ. Mol. Mutagen.*, **24**, 245–261.
46. Sandberg,A.A. (1987) Role of chromosome change in carcinogenesis. In Barrett, J.C. (ed.) *Mechanisms of Environmental Carcinogenesis*. CRC Press, Inc., Baca Raton, Florida, Vol. 1, pp. 97–111.

Received on March 26, 1997; revised on June 16, 1997; accepted on June 24, 1997