Mutation Research, 206 (1988) 73–82
Elsevier

MTR 01319

Sister-chromatid exchanges induced by triethylenemelamine: in vivo and in vivo/in vitro studies in mouse and Chinese hamster bone marrow and spleen cells *

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(Received 9 November 1987)
(Revision received 10 March 1988)
(Accepted 31 March 1988)

Keywords: Sister-chromatid exchange; Triethylenemelamine.

Summary

This study was designed to obtain sister-chromatid exchange (SCE) frequencies in bone marrow and spleen cells of mice and Chinese hamsters under in vivo and in vivo/in vitro systems following treatment of animals with varying doses (15–405 μg/kg) of triethylenemelamine (TEM). A dose-related SCE response was found in both species, tissues, and systems analyzed following TEM treatment. In vivo, similar responses were noted for both tissues in both species. However, in vivo/in vitro, the response was lower than in vivo and it varied with the tissue.The spleen cells were more sensitive and gave higher numbers of SCEs than bone marrow of both species at the two highest doses tested (135 and 405 μg/kg). These differences may be attributed to cell-culturing effects, type of cells analyzed, species and tissue specificities, and pharmacokinetic properties of the chemical. This study lends support to recently established in vivo/in vitro cell culture methodologies employing mice and Chinese hamsters for comparative cytogenetic analysis.

Sister-chromatid exchange (SCE) represents the interchange of DNA replication products at homologous loci. Although little is known about its molecular mechanism, the exchange process presumably involves chromosomal DNA breakage and reunion. Several known mutagens and carcinogens have been shown to induce SCEs in various systems (Latt et al., 1981; Tice et al., 1984). Studies have demonstrated that SCE formation is correlated with gene mutation (Carrono et al., 1978; Krishna et al., 1984), in vitro cell transformation (Popescu and DiPaolo, 1982; Popescu et al., 1985) and it can serve as a predictor of carcinogenic potential (Latt et al., 1981). SCE assay has also been used as a test system for determining the exposure of human population to genotoxicants in occupational setting (Tice et al., 1984). The simplicity of this technique, the readily scorable chromosomal endpoint, and sensitivity to

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* Results of cooperative investigation of NIOSH and West Virginia Agricultural and Forestry Experiment Station. Published with the approval of the Director of West Virginia Agricultural and forestry Experiment Station as Scientific Paper No. 2092.

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genotoxicants, make this system a useful test for chemically induced chromosomal damage.

Triethylenemelamine (TEM) is an alkylating and antineoplastic agent and has been widely used as a positive control compound in the analysis of chromosomal aberrations and micronuclei formation (Evans and O'Riordan, 1977; Tsuchimoto and Matter, 1979; Preston et al., 1981; Heddle et al., 1983). It has been known to induce SCEs in mice (Wilmer and Soares, 1980) and Chinese hamsters in vivo (Tsuchimoto and Matter, 1979; Neal and Probst, 1984). However, very little information is available on the ability of TEM to induce SCEs in different tissues of mice and Chinese hamsters under in vivo and in vivo/in vitro conditions. In addition to species, strain, and sex of the treated animal, deposition patterns, distribution of test substance, target organ, and the threshold may also affect the TEM-induced SCE responses.

Human cytogenetic monitoring entailing SCE analysis has usually been carried out by collecting lymphocytes and/or bone marrow from the exposed subjects and culturing them for 2 cell cycles by stimulating cells using phytohemagglutinin. Such studies may not reflect the actual in vivo damage since culturing treatments, differences in cell environment, growth of less heavily damaged cells may influence the results (Evans and O'Riordan, 1977). Therefore, it is important to compare actual in vivo and in vivo/in vitro (which is similar to those of human cytogenetic monitoring technique) systems. In rodents, several SCE studies have been performed with peripheral lymphocytes following animal exposure to various chemicals (Takeshita and Conner, 1984; Wilmer et al., 1984; Neft et al., 1985). Such studies have also been reported in rabbit and human lymphocytes (Stetka et al., 1978; Littlefield et al., 1980, 1983; Ashby and Richardson, 1985). However, there is lack of information on actual in vivo and in vivo/in vitro comparison with the same tissues in different species.

In order to obtain information on the comparative SCE responses to TEM, mice and Chinese hamsters were injected with varying concentrations of TEM. SCEs were analyzed in bone marrow and spleen cells under in vivo and in vivo/in vitro conditions. This study also attempts to validate the recently established bone marrow and spleen cell culture systems in rodents (Krishna et al., 1986a).

Materials and methods

Animals

CD_1 male mice were purchased from Charles River Breeding Laboratories (Wilmington, MA). The male Chinese hamsters were from Chickline Industries, (Vineland, NJ). Animals, 2–3 months old and weighing 25–30 g, were acclimatized to our laboratory for 7–10 days after arrival. Animals were housed in groups of 2 in cages containing hardwood chip bedding and excelsior nesting material. The animal quarters were maintained at $24 \pm 3°C$ with 40% maximum relative humidity and a reverse 5 lighting cycle (dark 13.00–24.00). In total, 4 animals per treatment group were used. Water and Purina laboratory rodent chow were provided ad libitum throughout the period of animal holding and experimentation. Separate groups of animals were used for in vivo and in vivo/in vitro experiments.

Drug treatment

TEM (a gift from Dr. M. Manandhar, American Cyanamide, Pearl River, NY) was dissolved in phosphate-buffered saline (PBS, pH 6.8) and immediately injected intraperitoneally in a volume equivalent to 10 ml/kg body weight (15–405 μg TEM/kg body weight). These concentrations were chosen for comparison because they caused a significant increase of SCEs in both mice and Chinese hamsters in our preliminary studies as well as those of others (Wilmer and Soares, 1980; Neal and Probst, 1984). Control animals were injected with an equivalent volume of PBS. All animals (from both in vivo and in vivo/in vitro studies) were killed by cervical dislocation 20 h after drug injection.

In vivo sister-chromatid exchange assay

Paraffin-coated 5-bromodeoxyuridine (BrdU) tablets (50 mg, Boehringer Mannheim Biochemicals, Indianapolis, IN) were inserted under the skin on the flank (McFee et al., 1983). At 4 h after BrdU implantation, TEM or PBS was injected as described above. At 17 h after chemical treatment, animals were injected with colchicine (4 mg/kg,

Gibco, Grand Island, NY) and were sacrificed by cervical dislocation 3 h later. For bone marrow preparations, both femora and tibia were cleaned with 70% ethyl alcohol and the head cut off with scissors. Marrow from femora and tibia was flushed out with physiological saline into a centrifuge tube, immediately after the animals were sacrificed. All bone marrow preparations were centrifuged within 2 h at $285 \times g$ for 6 min. The supernatant was removed and the pellet was resuspended in hypotonic (0.075 M KCl) solution for 20 min at 37 °C and recentrifuged. The cells were fixed with 2 changes of fixative, 10 min each in methanol–acetic acid (3 : 1). The cells were resuspended in approximately 0.5 ml fixative and dropped onto precleaned, chilled wet slides and air-dried for 24 h.

The spleens, removed from the same animals used for bone marrow studies, were transferred into 15-ml centrifuge tubes, each containing 2 ml Hanks' balanced salt solution (Gibco). The spleens were mashed with a spatula and the debris removed. Cells were treated with hypotonic solution, fixed, and slides prepared for SCE analysis as described for bone marrow cells.

In vivo / in vitro sister-chromatid exchange assay

Mouse and Chinese hamster spleen removal and culture for in vivo / in vitro assay. 20 h following drug treatment, spleens form both species were removed and placed in separate centrifuge tubes, each containing 2 ml RPMI 1640 with L-glutamine and Hepes-buffered medium (Gibco) supplemented with 20% heat-inactivated fetal bovine serum (FBS, Gibco), 2 mM additional L-glutamine (Gibco), 1% penicillin–streptomycin (5000 U/ml and 5000 μg/ml, Flow Laboratories). The spleen was mashed using a sterile spatula. The debris was removed and cells were washed with PBS containing 2% heat-inactivated FBS 3 times by centrifugation at $285 \times g$ for 6 min. Approximately 1.5×10^6 cells were cultured in 5 ml medium consisting of 3.70 ml RPMI 1640 with L-glutamine and Hepes buffer, 1.0 ml heat-inactivated FBS, 0.05 ml penicillin–streptomycin, 0.05 ml of 200 mM L-glutamine, 10 μM 2-mercaptoethanol (Sigma), 20 μM BrdU (Sigma), and 0.2 ml of lipopolysaccharide (*Escherichia coli* serotype 0111 : B4; Sigma, stock of 600 μg/ml in PBS). The cell suspension with the complete medium was dispensed into 25-cm^2 Falcon tissue culture flasks, covered with aluminum foil, and then incubated at 37 °C with 98% relative humidity and 5% CO_2. After 40 h incubation, colchicine (33 μM final concentration, Gibco) was added and cells were harvested 3 h after colchicine treatment.

Mouse and Chinese hamster bone marrow removal and culture for in vivo / in vitro assay. The bone marrow culture procedure was described in previous studies (Krishna et al., 1985, 1986a). In brief, femora and tibia were removed from the same mice and Chinese hamsters as those used for spleen isolation. The marrow was flushed out with Ham's F-12 medium (Flow Laboratories, McLean, VA) into a 15-ml centrifuge tube. After centrifugation the supernatant was removed, washed once, and the pellet resuspended with the remaining medium. The mouse bone marrow cell suspension (approximately 1.5×10^6 cells) was incubated in 25-cm^2 Falcon flasks with 5 ml of the following complete medium: 3.45 Ham's F-12, 1 ml FBS (20%), 0.05 ml penicillin–streptomycin (1%), 0.5 ml pregnant mouse uterus extract (10%), and 20 μM BrdU. The Chinese hamster bone marrow cells were incubated in the same medium but without pregnant mouse uterus extract. Cultures were then covered with aluminum foil and incubated at 37 °C, 5% CO_2, and 98% relative humidity. After 30 h incubation, colchicine was added and cells were harvested 3 h later.

Cell harvest, slide preparation, and staining. Contents of the flask were decanted into 15-ml Falcon centrifuge tubes. The tubes were centrifuged at $285 \times g$ for 6 min and the cell pellet resuspended in 5 ml hypotonic solution (0.075 M KCl at 37 °C) for 20 min and recentrifuged. The cells were fixed twice, each time with 5 ml freshly prepared 3 : 1, methanol : acetic acid. Finally, the cells were resuspended in a few drops of fixative and dropped on precleaned chilled wet slides, which were then air-dried for 24 h.

Staining for SCE analysis was performed according to a modified technique of Perry and Wolff (1974) and Goto et al. (1978). Slides were stained for 15 min with Hoechst 33258 (5 μg/ml)

and exposed to "black" light at 55–60 °C for 15 min at a distance of 1 cm while immersed in Sorenson's buffer (phosphate buffer, pH 6.8). The slides were then rinsed with distilled water and stained with 5% Giemsa (in Sorenson's buffer) for 10–15 min. All slides were coded and 25 cells with at least 38 and 21 chromosomes, in mice and Chinese hamsters respectively, were scored for SCEs by the same individual.

To evaluate replicative index (RI), the frequencies of the first, second, third, and subsequent metaphases were determined in 100 consecutive metaphase cells from each of the 4 animals or cultures. Those cells whose DNA had replicated exclusively before the addition of BrdU could not be distinguished from cells at first metaphase, and those that had gone through 3 or more cell cycles were included as third mitoses. The RI was calculated as follows: $RI = (1M_1 + 2M_2 + 3M_3)/100$, where M_1, M_2, and M_3 represent percentages of first, second, and third division metaphases, respectively (Schneider and Lewis, 1981).

Statistical analysis

The data were obtained in 2 separate experiments performed under identical conditions on 2 animals in each treatment group. The data were analyzed as a split-plot design. When dose was statistically significantly related to a dependent variable, a linear equation was fit. If significant lack of fit was found, a quadratic equation was fit. This was continued until either the test for lack of fit was not statistically significant, or a fourth order polynomial was fit. Because there were only 5 distinct doses, the lack of fit from the fourth order polynomial could not be tested.

Results

The results represent 2 separate experiments performed under identical conditions with 2

TABLE 1

SISTER-CHROMATID EXCHANGE FREQUENCIES AND REPLICATIVE INDICES IN BONE MARROW AND SPLEEN CELLS OF MICE FOLLOWING VARYING CONCENTRATIONS OF TRIETHYLENEMELAMINE IN VIVO [a]

Dose of triethylene-melamine (μg/kg)	Expt.	Bone marrow		Spleen	
		SCEs/cell ± S.D.	RI	SCEs/cell ± S.D.	RI
0 [b]	1	3.04 ± 1.32	1.97	2.98 ± 1.24	1.92
	2	2.96 ± 1.15	1.86	2.76 ± 1.09	1.97
	Mean	3.00 ± 0.31	1.92	2.87 ± 0.17	1.94
15	1	6.72 ± 2.07	1.76	7.72 ± 2.07	2.08
	2	6.06 ± 1.89	2.00	7.52 ± 2.21	1.86
	Mean	6.39 ± 0.51 [c]	1.88	7.62 ± 0.12 [c]	1.97
45	1	11.48 ± 2.00	1.90	12.20 ± 2.35	2.00
	2	11.22 ± 1.77	2.01	11.70 ± 2.26	2.00
	Mean	11.35 ± 0.34 [c]	1.96	11.95 ± 0.31 [c]	2.00
135	1	19.18 ± 3.82	1.92	20.24 ± 2.92	1.90
	2	19.64 ± 3.18	1.96	19.72 ± 3.27	1.96
	Mean	19.41 ± 0.35 [c,d]	1.94	19.98 ± 0.42 [c,d]	1.93
405	1	35.82 ± 5.56	1.95	31.60 ± 4.60	1.49
	2	35.84 ± 5.98	1.95	32.78 ± 4.08	1.40
	Mean	35.83 ± 0.51 [c]	1.95	32.19 ± 1.36 [c]	1.45 [c]

[a] 25 cells for SCE and 100 cells for replicative index were scored per animal.
[b] Solvent control, phosphate-buffered saline, ~ 0.3 ml/animal.
[c] Significantly different from negative control ($p < 0.01$).
[d] Significantly different from Chinese hamster SCE level ($p < 0.01$).

TABLE 2

SISTER-CHROMATID EXCHANGE FREQUENCIES AND REPLICATIVE INDICES IN BONE MARROW AND SPLEEN CELLS OF MICE FOLLOWING VARYING CONCENTRATIONS OF TRIETHYLENEMELAMINE IN VIVO/IN VITRO [a]

Dose of triethylene-melamine (μg/kg)	Expt.	Bone marrow		Spleen	
		SCEs/cell ± S.D.	RI	SCEs/cell ± S.D.	RI
0 [b]	1	6.56 ± 1.56	1.90	6.04 ± 1.82	1.99
	2	6.42 ± 1.60	1.95	6.14 ± 1.85	1.96
	Mean	6.49 ± 0.53 [e]	1.92	6.09 ± 0.37 [e]	1.97
15	1	10.66 ± 2.25	1.97	11.06 ± 2.51	1.85
	2	10.48 ± 2.09	1.97	9.80 ± 2.45	2.03
	Mean	10.57 ± 0.30 [c,d,e]	1.97	10.43 ± 0.81 [c,e]	1.94
45	1	12.14 ± 3.00	1.99	14.34 ± 2.04	2.02
	2	12.80 ± 2.87	2.01	12.76 ± 2.92	1.91
	Mean	12.47 ± 0.99 [c,d]	2.00	13.56 ± 0.98 [c]	1.96
135	1	14.72 ± 4.01	1.81	19.86 ± 3.83	1.87
	2	15.40 ± 3.47	1.92	18.34 ± 3.50	2.09
	Mean	15.06 ± 0.47 [c,d,e,f]	1.86	19.10 ± 0.88 [c,d]	1.98
405	1	17.14 ± 4.65	1.88	27.14 ± 4.67	1.98
	2	17.96 ± 4.37	2.01	25.42 ± 5.12	2.09
	Mean	17.55 ± 0.95 [c,d,e,f]	1.94	26.28 ± 1.31 [c,d,e]	2.03

[a,b,c,d] See Table 1.
[e] Significantly different from in vivo SCE level ($p < 0.01$).
[f] Significantly different from spleen SCE level ($p < 0.01$).

animals in each treatment group. The effect of the experiment was not statistically significant in either species for SCE or RI and thus the data were pooled. Comparative in vivo SCE data for mouse bone marrow and spleen following animal treatment with various doses of TEM are presented in Table 1 and Fig. 1. TEM caused dose-related SCEs in both bone marrow and spleen cells. At

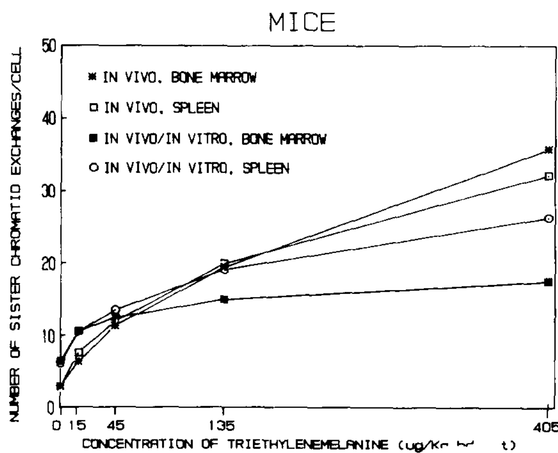

Fig. 1. Triethylenemelamine-induced mean SCE frequencies in bone marrow and spleen cells of mice under in vivo and in vivo/in vitro conditions.

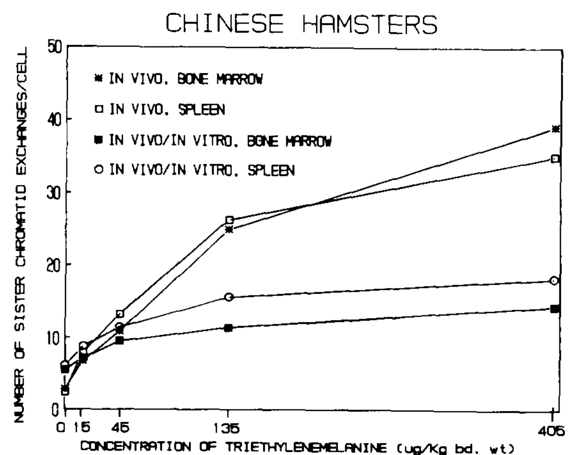

Fig. 2. Triethylenemelamine-induced mean SCE frequencies in bone marrow and spleen cells of Chinese hamsters under in vivo and in vivo/in vitro conditions.

the highest concentration tested, 405 μg/kg body weight, TEM caused over 11-fold increase in SCEs in comparison with control levels in both tissues analyzed. A cubic equation was required for bone marrow, and a quadratic equation was required for spleen. The RI data did not differ significantly between treated and controls except for spleen cells at the highest dose tested, wherein it caused significant cell cycle delay.

Data for mouse bone marrow and spleen cells in in vivo/in vitro studies are shown in Table 2 and Fig. 1. The results indicate that TEM also induced dose-related SCEs in both tissues analyzed. At the highest concentration tested, 405 μg/kg body weight, it caused approximately a 3-fold and a 4-fold increase in SCEs over controls, in bone marrow and spleen cells, respectively. The required equation was quadratic for bone marrow and cubic for spleen. The cell cycle kinetics data did not indicate any delay in cell cycle progression in culture.

The in vivo data on the induction of SCEs by TEM in Chinese hamster bone marrow and spleen cells are given in Table 3 and Fig. 2. As in the mouse study, a clear dose response in bone marrow and spleen cells was observed. TEM treatment yielded approximately a 14-fold increase in SCEs over controls in both tissues and quadratic equations were required for both tissues. It did not cause cell cycle delay in treated animals, except at the highest dose (405 μg/kg body weight) in spleen, wherein a cell cycle delay was evident.

Under in vivo/in vitro conditions, TEM also caused a dose-related SCE response in bone marrow and spleen cells of Chinese hamsters (Table 4, Fig. 2). Approximately 3-fold increase in SCEs was noted in both tissues analyzed. The required equations were cubic for bone marrow and spleen. The RI data did not differ between treated and control animals.

A comparison of results between in vivo and in vivo/in vitro indicated a statistically significant difference and it varied with the organ and dose. The SCE frequencies from in vivo experiments, at the highest concentrations tested (405 μg/kg), were relatively higher than those from in vivo/in vitro

TABLE 3

SISTER-CHROMATID EXCHANGE FREQUENCIES AND REPLICATIVE INDICES IN BONE MARROW AND SPLEEN CELLS OF CHINESE HAMSTERS FOLLOWING VARYING CONCENTRATIONS OF TRIETHYLENEMELAMINE IN VIVO [a]

Dose of triethylene-melamine (μg/kg)	Expt.	Bone marrow		Spleen	
		SCEs/cell ± SD	RI	SCEs/cell ± SD	RI
0 [b]	1	2.88 ± 1.32	2.06	2.38 ± 0.93	2.00
	2	2.76 ± 1.03	1.98	2.48 ± 0.98	1.98
	Mean	2.82 ± 0.16	2.02	2.43 ± 0.07	1.99
15	1	6.60 ± 2.03	1.96	8.48 ± 2.63	1.88
	2	6.88 ± 2.47	2.06	7.38 ± 2.24	1.91
	Mean	6.74 ± 0.19 [c]	2.01	7.93 ± 0.82 [c]	1.89
45	1	10.78 ± 1.89	1.98	13.60 ± 2.95	1.90
	2	10.98 ± 1.93	1.92	12.70 ± 2.75	2.00
	Mean	10.88 ± 0.31 [c]	1.95	13.15 ± 0.64 [c]	1.95
135	1	24.58 ± 6.17	2.05	25.82 ± 4.43	1.96
	2	25.28 ± 5.89	1.85	26.72 ± 4.29	2.07
	Mean	24.93 ± 0.46 [c]	1.95	26.27 ± 0.75 [c]	2.02
405	1	39.78 ± 7.10	2.07	36.00 ± 7.36	1.45
	2	38.56 ± 6.83	1.97	34.20 ± 6.36	1.37
	Mean	39.17 ± 0.71 [c]	2.02	35.10 ± 1.41 [c]	1.41 [c]

[a,b,c] See Table 1.

TABLE 4

SISTER-CHROMATID EXCHANGE FREQUENCIES AND REPLICATIVE INDICES IN BONE MARROW AND SPLEEN CELLS OF CHINESE HAMSTERS FOLLOWING VARYING CONCENTRATIONS OF TRIETHYLENEMELAMINE IN VIVO/IN VITRO [a]

Dose of triethylene-melamine (μg/kg)	Expt.	Bone marrow		Spleen	
		SCEs/cell ± S.D.	RI	SCEs/cell ± S.D.	RI
0 [b]	1	5.44 ± 1.94	1.95	6.14 ± 1.99	1.91
	2	5.58 ± 1.65	2.01	6.10 ± 1.58	2.05
	Mean	5.51 ± 0.18 [e]	1.98	6.12 ± 0.25 [e]	1.98
15	1	7.14 ± 1.63	1.92	8.60 ± 1.77	1.81
	2	7.20 ± 1.80	2.00	8.98 ± 2.67	2.02
	Mean	7.17 ± 0.24 [c]	1.96	8.79 ± 0.36 [c]	.191
45	1	9.82 ± 3.60	2.01	12.04 ± 2.53	2.06
	2	9.16 ± 2.50	1.94	10.82 ± 2.60	1.91
	Mean	9.49 ± 0.90 [c]	1.98	11.43 ± 0.82 [c]	1.99
135	1	11.44 ± 3.19	2.07	15.86 ± 3.14	1.97
	2	11.32 ± 2.84	1.92	15.26 ± 3.62	1.99
	Mean	11.38 ± 0.15 [c,e,f]	1.99	15.56 ± 1.26 [c,e]	1.98
405	1	14.88 ± 3.95	1.91	18.86 ± 4.07	1.80
	2	13.92 ± 4.38	1.94	17.68 ± 3.53	1.96
	Mean	14.40 ± 0.67 [c,e,f]	1.92	18.27 ± 0.95 [c,e]	1.88

[a,b,c,e,f] See Tables 1 and 2.

experiments in both species and tissues analyzed. Also, within in vivo, the tissues did not differ significantly in their SCE response. However, in in vivo/in vitro experiments, the spleen cells had a relatively higher frequency of SCEs than bone marrow cells especially at 135 and 405 μg/kg doses.

In general, though varying doses of TEM yielded similar patterns of SCE responses in both mice and Chinese hamsters, the magnitude of SCE response was significantly different and varied with tissues and systems studied. In vivo, at 135 μg/kg TEM, Chinese hamsters showed higher SCE values than mice. Under in vivo/in vitro conditions, mouse bone marrow exhibited more SCEs than Chinese hamsters at doses 15, 45, 135 and 405 μg/kg TEM; however, mouse spleen had higher SCE values only at 2 high doses.

Discussion

Analysis of SCE formation has been proposed as a sensitive indicator of genotoxic damage of the type leading to mutation and cancer (Tice et al., 1984). SCE data in in vivo mouse bone marrow in the present study following treatment with TEM are in agreement with the result of the study of Wilmer and Soares (1980). TEM has also been shown to cause a dose-related increase in SCEs in Chinese hamster bone marrow in earlier studies (Tsuchimoto and Matter, 1979; Neal and Probst, 1984). However, in these studies, the genotoxicity of TEM was not evaluated in spleen cells. TEM has also been shown to induce SCEs and gene mutations at the HGPRT locus in cultured Chinese hamster lung cells (Nishi et al., 1984). TEM possesses 3 aziridinyl residues and is known to function as a polyfunctional alkylating agent (Wilmer and Soares, 1980). In the present study, TEM caused approximately equivalent SCE responses in both bone marrow and spleen cells in vivo. Similar equivalent SCE responses have been noted for both tissues of mice for cyclophosphamide and mitomycin C (Krishna et al., 1986b) and for cyclophosphamide in Chinese hamsters (Krishna et al., 1986c). TEM at the highest concentration,

405 μg/kg body weight, caused cell cycle delay in spleen cells, indicating toxicity at this dose. However, the same dose was not toxic to bone marrow suggesting differential sensitivity of the tissues to TEM. TEM has been widely used as a positive control in many chromosomal aberration and micronucleus analyses, since it is a potent inducer of chromosomal damage due to its polyfunctional alkylating activity (Preston et al., 1981; Heddle et al., 1983).

Under in vivo/in vitro conditions, though the degree of SCE increase was relatively lower than in vivo, especially at high doses, TEM caused a dose-related increase in SCEs in both species and tissues studied. This indicated that some of the TEM-induced SCE lesions in vivo can be detected after 2 cell cycles in culture. Under these conditions, spleen cells had relatively higher SCE levels than bone marrow cells. Bone marrow is an actively cycling population with cells at various stages of differentiation. However, the spleen consists of slow-cycling cell population with low mitotic index, wherein SCE-forming lesions may persist longer than bone marrow cells. It is possible that spleen cells stimulated by lipopolysaccharide in culture undergo minimal divisions in vivo. These cells when cultured may reveal higher SCEs than bone marrow. Similar differential sensitivity of bone marrow and lipopolysaccharide-stimulated B lymphocytes has been reported in Chinese hamsters (Krishna et al., 1986c), and in mouse T and B peripheral lymphocytes (Wilmer et al., 1984; Wierda and Pazdernik, 1979) following cyclophosphamide treatment.

A comparison between in vivo and in vivo/in vitro assays indicated significant differences. A relatively higher SCE response was noted in vivo than in vivo/in vitro in both species as well as tissues analyzed. These differences may be partly related to culturing and non-culturing differences (Freshney, 1983). It is possible that the subpopulations of cells analyzed for SCEs in vivo and in vivo/in vitro may have different susceptibilities to the effects of TEM. It should be pointed out that the in vivo/in vitro system involved 2 additional cell divisions, which might have added to the dilution of SCE lesions in culture (Iijima and Morimot, 1986; Bochkov et al., 1984). Differences in the metabolism of TEM in bone marrow and spleen of mice and Chinese hamsters under in vivo and in vivo/in vitro conditions may also add to the observed differences in SCE levels. However, the actual enzyme activities responsible for such differences have not yet been identified. It may be important to consider the differential effect of TEM on BrdU-substituted (in vivo) and non-substituted (in vivo/in vitro) cells in animals. It may be noted that interspecies, intertissue/cell, and different assay system variations are determined not only by a karyologic characteristic of an individual species, but also by pharmacokinetic effects of the drug and its half-life in the rodent species and subsequently in the culture. It is also possible that cells sustaining more damage are less able to survive the stress of transfer to in vitro culture, which further depends on the threshold of damage to each cell type.

In vivo, Chinese hamsters were relatively more susceptible to the effects of TEM (at least at 135 μg/kg) than mice. However, under in vivo/in vitro conditions, mouse cells produced higher SCEs at 135 and 405 μg/kg than Chinese hamsters. These results are comparable to the earlier studies pertaining to species and tissue specificities, and differences in the pharmacokinetic properties of a test compound under varying systems (Streisinger, 1983).

In vivo, both bone marrow and spleen cells were prepared for SCE analyses at 24 h following BrdU tablet implantation. However, such preparations yielded lower mitotic figures in spleen than bone marrow. Similar RI values in both spleen and bone marrow (except at 405 μg/kg TEM, in vivo) indicate that a small portion of spleen cells (subpopulation) seems to be actively dividing (like bone marrow) and had normal distribution of first, second and third mitoses. Under in vivo/in vitro conditions, bone marrow cells were cultured for 33 h and spleen cells for 43 h to obtain maximum number of second division cells and high mitotic yields, based on earlier studies. Because of these differences in harvest times among systems and tissues, the comparison of RIs had been made only between treatment and their respective controls to gain an indication of toxicity of treatments. The data on RI under in vivo/in vitro conditions indicated that TEM at the concentrations tested was not toxic and did not cause

cell cycle delay in culture. The highest concentration tested (405 μg/kg body weight) was toxic in vivo to spleen, but not in vivo/in vitro. This observation suggests that in vivo/in vitro, a less heavily damaged cell population might have multiplied and/or the time gap between chemical injection and cell harvest may have played a role in cell recovery from the toxic effects of the drug.

Thus, this study shows that TEM can induce dose-related SCEs in bone marrow and spleen cells of mice and Chinese hamsters in both in vivo and in vivo/in vitro assays. However, the SCE frequencies vary with the dose, tissue, species, and system employed. The test compound, TEM, caused more SCEs in in vivo than in vivo/in vitro assays, in both species analyzed. The study also suggests that the previously reported in vivo/in vitro assays for these species and tissues may be useful for comparative cytogenetic analyses, which are similar to in vivo cytogenetic monitoring techniques used in humans. However, it should be noted that the degree of response and relationship among tissues and systems may vary with pharmacokinetic properties of the test compound.

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