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In vivo and in vivo/in vitro kinetics of cyclophosphamide-induced sister-chromatid exchanges in mouse bone marrow and spleen cells

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Summary

In several acute and chronic exposures to various chemicals in vivo and in vitro, the average sister-chromatid exchange (SCE) frequencies in human, mouse, rat, and rabbit lymphocytes generally decrease with time following treatment. The rate of this decline varies, but little data have been published pertaining to the comparative kinetics of SCEs both in vivo and in vivo/in vitro (exposure of animals to the test compound and culturing of cells) simultaneously in the same tissues. In this study, a single dose of cyclophosphamide (40 mg/kg) was injected for varying periods (6–48 h) and its effects, as assessed by the induction of SCEs, were analyzed under both in vivo and in vivo/in vitro conditions in mouse bone marrow and spleen cells. In vivo, the cyclophosphamide-induced SCEs increased with increasing time up to 12 h, stayed at approximately the same level until 24 h, and then decreased with increase in post-exposure time. Hoever, the SCE levels remained significantly higher than controls at 48 h post-exposure time in both bone marrow and spleen cells. Under in vivo/in vitro conditions, the SCEs in bone marrow decreased with increase in post-exposure time until reaching control values by 48 h post exposure. However, in pleen cells, the decrease in SCE level was gradual, and by 48 h post-exposure time, the cells still had approximately 6 times higher SCEs than the control values. These results suggest that there are pharmacokinetic differences for cyclophosphamide in mouse bone marrow and spleen. Also, there is a differential SCE response to cyclophosphamide under in vivo and in vivo/in vitro conditions.

The sister-chromatid exchange (SCE) assay is considered to be a good indicator of the relative carcinogenic potencies of several compounds (Latt et al., 1981; Conner et al., 1983; Tice et al., 1984). This assay is now widely used in monitoring of patients treated with drugs (Raposa, 1978) and workers exposed to genotoxic agents (Kucerova et al., 1979).

Cyclophosphamide is an important antineoplastic and immunosuppressive agent. However, it is known to cause secondary neoplasias in cancer patients as well as cancers in noncancer patients (IARC, 1981). Also cyclophosphamide induces a

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variety of changes and/or damages in genetic materials (IARC, 1981; Krishna et al. 1986a). The kinetics and persistence of DNA damage are regarded as important factors in chemical carcinogenesis (Pegg and Nicoll, 1976; Neft et al., 1985; Popescu et al., 1985) It has been speculated that drug-induced SCE lesions in target cells may be involved in the formation of neoplasias (Raposa, 1978; IARC, 1981; Neft et al., 1985; Popescu et al., 1985).

The persistence of cyclophosphamide-induced SCEs has been reported in rodent lymphocytes in the in vivo/in vitro assays (Stetka and Wolff, 1976; DuFrain et al., 1979; Huff et al., 1982; Basiler, 1984; Dearfield et al., 1984; 1985; Takeshita and Conner, 1984). Similar studies for cyclophosphamide have been performed in the in vivo bone marrow assay (Charles et al., 1983; Sozzi et al., 1985). However, a systematic comparative analysis of in vivo and in vivo/in vitro SCE kinetics in the same tissue has not been performed. Such studies are important from the viewpoint of chemotherapy, and proper interpreration of SCEs induced by cyclosphosphamide.

The purpose of this study was to determine and compare the fate of cyclophosphamide-induced lesions that lead to the formation of SCEs in mouse bone marrow and spleen lymphocytes under in vivo and in vivo/in vitro conditions following a single injection of cyclophosphamide. The SCE frequencies were measured at various times from 6 to 48 h following drug treatment.

Materials and methods

Animals

Male CD_1 mice were purchased from Charles River Breeding Laboratories, Wilmington, MA. Animals, 2–3 months old, were housed in a group of 2 per cage. Water and Purina laboratory rodent chow were provided ad libitum throughout the period of animal holding and experimentation.

Drug treatment

Cyclophosphamide (Sigma Chemical Co., St. Louis, MO) was dissolved in phosphate-buffered saline (PBS, pH 7.0) and immediately injected intraperitoneally (i.p.) in a dosage of 40 mg/kg body weight. This concentration was chosen because it caused a significant increase in SCE levels in mice in earlier studies (Krishna et al., 1986b). The control animals received and equivalent volume of PBS. The hours of post-exposure were: 6, 9, 12, 18, 24, 36, and 48 (Fig. 1). These intervals were chosen on the basis of our preliminary studies and those of others (Sozzi et al., 1985; Takeshita and Conner, 1984).

In vivo sister-chromatid exchange assay

Paraffin-coated 5-bromodeoxyuridine (BrdU) tablets (50-mg tablet, Boehringer Mannheim Biochemicals, Indianapolis, IN) were inserted under the skin on the flank of animals for 24 h prior to sacrifice (McFee et al., 1983). 3 h before sacrifice, animals were injected with colchicine (4 mg/kg, Gibco, Gran Island, NY). For bone marrow preparations, both femora and tibia were isolated and marrow was flushed out with physiological saline into a centrifuge tube. After centrifugation at 285 $\times g$ for 6 min, the supernatant fluid was removed, the pellet was resuspended in hypotonic (0.075 M KCl) solution for 20 min at 37°C and recentrifuged. The cells were fixed with two changes of fixative (methanol–acetic acid, 3 : 1) 10 min each. 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 that were used for bone marrow removal were transferred into 15-ml centrifuge tubes, each containing 3 ml Hanks' balanced salt solution (Gibco). Spleens were mashed with a spatula and the debris was removed. Cells were treated with hypotonic solution and fixative, and slides were prepared as described for bone marrow cells.

In vivo / in vitro sister-chromatid exchange assay

The animals in this study did not receive BrdU treatment. Bone marrow cells were obtained as described in the previous section except that bone marrow was flushed out with Ham's F-12 medium (Flow Laboratories, McLean, VA) into a 15-ml centrifuge tube. After 2 washings with the medium, approximately 1.5×10^6 cells were incubated in a 25-cm^2 Falcon flask with 5 ml of the following complete medium: 3.45 ml Ham's F-12, 1 ml fetal bovine serum (FBS, 20%, Gibco), 0.05 ml penicillin and streptomycin (1%, 5000 U/ml and 5000

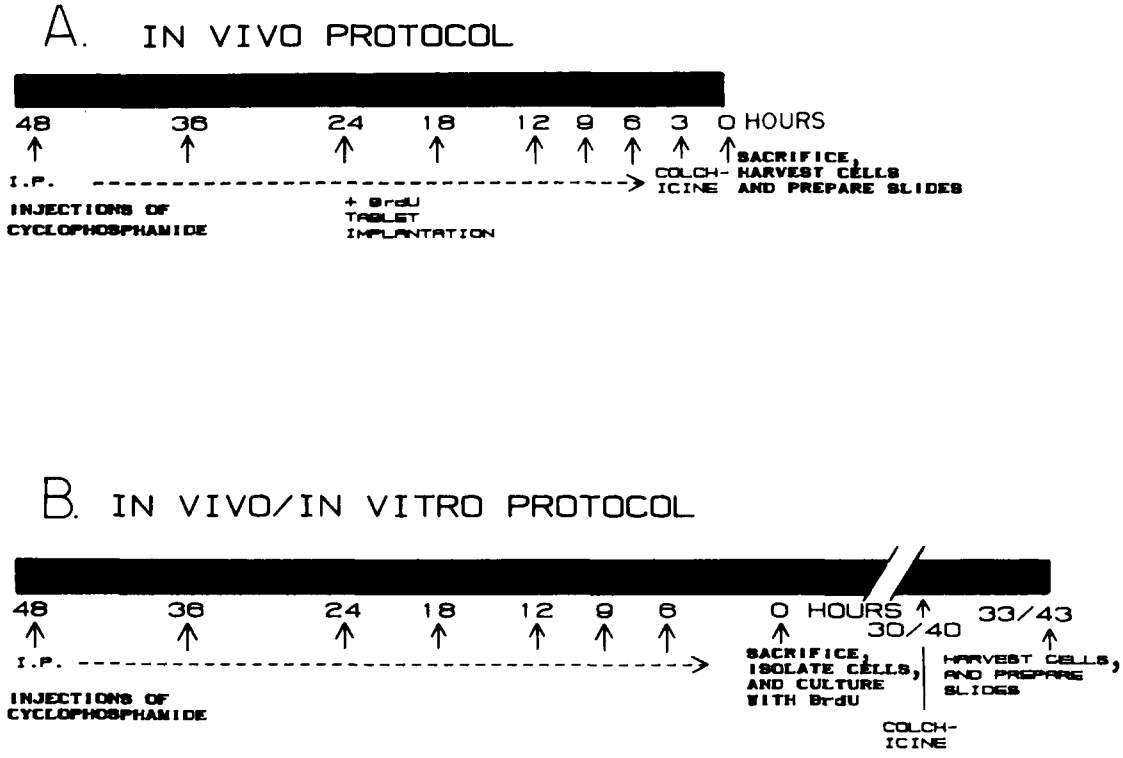

Fig. 1. (A) In vivo protocol: An intraperitoneal injection of cyclophosphamide was given at 48, 36, 24, 18, 12, 9 and 6 h before sacrificing. At 24 h, BrdU tablets were implanted in all animals. At 3 h before sacrificing, colchicine (i.p.) was injected. (B) In vivo/in vitro protocol: An intraperitoneal injection of cyclophosphamide was given at 48, 36, 24, 18, 12, 9 and 6 h before sacrificing. Animals were sacrificed at 0 h, bone marrow and spleen cells were isolated and cultured in the presence of BrdU for 30/40 h at which time colchidine was added and cells were harvested 3 h later as described in Materials and Methods.

μg/ml, Flow Laboratories), 0.5 ml pregnant mouse uterus extract (10%), and 20 μM BrdU (Sigma). Cultures were covered with aluminum foil, incubated at 37° C at 98% relative humidity and 5% CO_2. After 30 h of incubation, colchicine (33 μM final concentration) was added and cells were harvested 3 h later (Krishna et al., 1986c).

Spleens obtained from the same mice used for bone marrow isolation were transferred into centrifuge tubes, each containing 2 ml RPMI 1640 with L-glutamine and Hepes-buffered medium (Gibco) supplemented with 20% heat-inactivated FBS, 2 mM additional L-glutamine (Gibco), and 1% penicillin and streptomycin. Spleens were mashed using a sterile spatula. The debris was removed and the cells were washed 3 times with PBS containing 2% heat-inactivated FBS by centrifugation at 285 × g for 6 min. The final cell suspension (approximately 1.5×10^6 cells) was cultured in 5 ml medium consisting of: 3.70 ml RPMI with L-glutamine and Hepes buffer, 1 ml heat-inactivated FBS (20%), 0.05 ml penicillin and streptomycin (1%), 0.05 ml of 200 mM L-glutamine (1%), 10 μM 2-mercaptoethanol (Sigma), 20 μM BrdU, and 0.2 ml of lipopolysaccharide (*Escherichia coli* serotype 0111 : B4; Sigma, stock of 600 μg/ml in PBS). The culture flask was covered with aluminum foil, incubated at 37° C at 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 later (Krishna et al., 1986a; Neft et al., 1985).

Harvesting, staining and scoring

The cultures were harvested by decanting the contents of the flasks into 15-ml Falcon centrifuge tubes. Each flask was rinsed with 3 ml Hanks' balanced salt solution which was also transferred

to the centrifuge tube. The tubes were centrifuged at 285 × *g* for 6 min and cells processed for slide preparation as described previously.

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 Sørenson's buffer (phosphate buffer, pH 6.8). The slides were then rinsed with distilled water and stained with 5% Giemsa (in Sørenson's buffer) for 10–15 min. All slides were coded and cells with at least 38 chromosomes were analyzed for the SCEs.

For calculating replicative indices (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 per treatment. 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:

$$RI = \frac{1M_1 + 2M_2 + 3M_3}{100}$$

where M_1, M_2, and M_3 represent percentages of first, second, and third metaphases, respectively (Schneider and Lewis, 1981; Krishna et al. 1985).

Statistical analysis

SCEs in 25 cells from each organ of each animal were analyzed employing an analysis of variance appropriate for split plot designs (with tissue being the only main effect in the subplot). Differences among times were tested using Fischer's least significant difference test. Due to unequal variances, the log transformation was employed. All testing was performed using $\alpha = 0.05$ as the probability of Type I error. The RIs were analyzed similarly except that no transformation was used.

Results

The in vivo SCE frequencies in bone marrow and spleen cells following a single injection of cyclophosphamide (40 mg/kg body weight) are presented in Table 1 and Fig. 2. The data represent 2 separate experiments performed under identical conditions with 2 animals in each experiment for each data point. Because the method * time * tissue interaction was significant, each factor was analyzed at each combination of the other two. Cyclophosphamide induced significantly higher numbers of SCEs in both tissues starting with 6 h of post-exposure, the earliest time tested. The number of SCEs increased with increase in time of post-exposure until 12 h in both bone marrow and spleen cells in an equivalent manner, except at 9 h post exposure, spleen cells produced higher SCEs than bone marrow cells ($p < 0.01$). Between 12 and 24 h, the SCE level remained relatively unchanged, even though tissue differences existed (spleen cells yielded significantly higher SCEs than bone marrow at 24 h, $p < 0.01$). After 24 h, the SCE levels dropped markedly in both tissues and the decrease was more drastic in spleen than in bone marrow at 36 h post exposure ($p < 0.01$). At the last sampling time point (48 h post exposure), the SCEs reached minimal levels which were still significantly higher than the baseline levels ($p < 0.01$). In general, the first, second, third and subsequent cell divisions were in close approximation in the study and thus, the RI data did not differ significantly between treatments and controls. In fact, the only variable

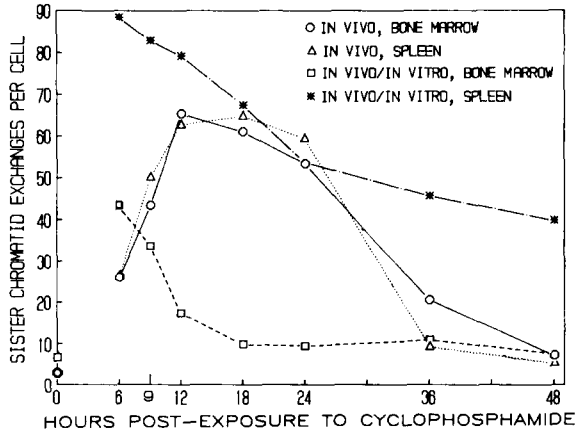

Fig. 2. In vivo and in vivo/in vitro kinetics of cyclophosphamide-induced sister-chromatid exchanges in mouse bone marrow and spleen cells.

TABLE 1

SISTER-CHROMATID EXCHANGE FREQUENCIES AND REPLICATIVE INDICES IN BONE MARROW AND SPLEEN CELLS OF MICE ANALYZED AT VARIOUS TIMES FOLLOWING SINGLE INTRAPERITONEAL INJECTION OF CYCLOPHOSPHAMIDE (40 mg/kg) IN VIVO [a]

Hours post exposure	Experiment	Bone marrow			Spleen		
		SCEs/cell ± S.D.	RI	Range (per cell)	SCEs/cell ± S.D.	RI	Range (per cell)
Negative	1	2.86 ± 1.19	2.01	1– 5	2.70 ± 0.94	2.01	1– 5
	2	2.82 ± 1.00	2.06	1– 4	2.54 ± 1.08	1.87	1– 5
	Mean	2.84 ± 1.10	2.04		2.62 ± 1.01		
6	1	26.60 ± 5.52	1.98	18–40	26.14 ± 5.09	1.84	18–41
	2	25.10 ± 4.10	2.09	18–36	26.52 ± 5.17	1.99	17–40
	Mean	25.85 ± 4.86 [b]	2.04		26.33 ± 5.13 [b]	1.92	
9	1	43.60 ± 9.73	1.94	28–64	49.42 ± 10.17	1.86	28–64
	2	43.12 ± 10.50	2.02	25–69	50.72 ± 10.67	2.02	30–71
	Mean	43.36 ± 10.12 [b,c]	1.98		50.07 ± 10.42 [b]	1.94	
12	1	66.02 ± 8.39	2.02	45–92	61.72 ± 7.46	1.88	39–72
	2	64.48 ± 8.00	1.97	48–75	63.40 ± 6.31	1.84	44–74
	Mean	65.25 ± 8.20 [b]	2.00		62.56 ± 6.91 [b]	1.86	
18	1	61.88 ± 6.14	1.88	50–74	65.50 ± 6.89	1.86	45–79
	2	60.14 ± 7.17	1.97	40–72	63.78 ± 5.70	1.96	49–74
	Mean	61.01 ± 6.67 [b]	1.93		64.64 ± 6.32 [b]	1.91	
24	1	55.16 ± 7.15	1.96	44–65	58.38 ± 7.33	1.84	39–71
	2	51.38 ± 10.01	1.91	23–67	60.26 ± 6.65	1.95	40–74
	Mean	53.27 ± 8.70 [b,c]	1.94		59.32 ± 7.00 [b]	1.90	
36	1	19.88 ± 10.44	1.89	7–48	8.06 ± 3.14	1.85	2–15
	2	21.06 ± 10.78	1.90	7–45	10.38 ± 4.81	1.99	4–29
	Mean	20.47 ± 10.61 [b,c]	1.90		9.22 ± 4.06 [b]	1.92	
48	1	7.74 ± 3.43	2.00	3–16	5.30 ± 2.62	1.81	2–10
	2	6.60 ± 2.48	1.98	3–15	5.12 ± 2.48	1.92	2–10
	Mean	7.17 ± 2.99 [b,c]	1.99		5.21 ± 2.55 [b]	1.87	

[a] cells for SCE and 100 cells for replicative index were scored per animal. Numbers shown in each experiment are averages of 2 animals.

[b] Significantly different from controls ($p < 0.01$).

[c] Significantly different from spleen SCE level ($p < 0.01$).

which was significantly related to RI was tissue for which bone marrow had a higher RI than spleen (1.96 vs. 1.92, $p < 0.05$), when averaged over both in vivo and in vivo/in vitro data.

The cyclophosphamide-induced in vivo/in vitro SCE data in mouse bone marrow and spleen cells are shown in Table 2 and Fig. 2. At 6 h post exposure, a marked increase in SCE levels was noted in both bone marrow and spleen cells over controls ($p < 0.01$). The spleen cells yielded a much higher incidence of SCEs than bone marrow ($p < 0.01$). After 6 h, the SCE levels decreased markedly in bone marrow and leveled off by 18 h. By the last sampling point, 48 h, the SCE levels were similar to those of baseline levels. However, in spleen cells, the decrease in SCE levels with increase in post-exposure time was gradual, and at 48 h the SCE levels were approximately 6 times higher than the baseline levels. The cell cycle kinetic data were similar in both treated and control animals.

A comparison of in vivo and in vivo/in vitro data indicates that the in vivo bone marrow cells gave significantly higher SCE values at 12, 18, 24

TABLE 2

SISTER-CHROMATID EXCHANGE FREQUENCIES AND REPLICATIVE INDICES IN BONE MARROW AND SPLEEN CELLS OF MICE ANALYZED AT VARIOUS TIMES FOLLOWING SINGLE INTRAPERITONEAL INJECTION OF CYCLOPHOSPHAMIDE (40 mg/kg) IN VIVO/IN VITRO [a]

Hours post exposure	Experiment	Bone marrow			Spleen		
		SCEs/cell ± SD	RI	Range (per cell)	SCEs/cell ± SD	RI	Range (per cell)
Negative	1	6.54 ± 1.55	1.92	4– 9	6.74 ± 1.59	2.12	4– 10
	2	6.44 ± 1.84	1.97	3–10	6.30 ± 1.38	1.84	4– 9
	Mean	6.49 ± 1.70	1.95		6.52 ± 1.49	1.98	
6	1	42.56 ± 9.92	2.00	20–64	89.10 ± 15.40	1.95	60–120
	2	44.18 ± 10.98	1.96	18–62	87.58 ± 12.20	1.93	56–113
	Mean	43.37 ± 10.46 [b,c]	1.98		88.34 ± 13.89 [b]	1.94	
9	1	30.20 ± 12.87	1.88	12–56	82.94 ± 11.33	2.06	61–112
	2	36.92 ± 12.15	1.88	16–66	82.76 ± 10.54	1.86	63–106
	Mean	33.56 ± 12.52 [b,c]	1.88		82.85 ± 10.94 [b]	1.96	
12	1	17.72 ± 7.79	1.95	7–38	81.40 ± 14.06	1.96	50–114
	2	16.62 ± 8.80	1.98	6–37	76.84 ± 11.28	1.94	55–102
	Mean	17.17 ± 8.31 [b,c]	1.96		79.12 ± 12.75 [b]	1.95	
18	1	9.42 ± 3.78	1.92	5–20	67.74 ± 9.82	2.02	50– 95
	2	10.00 ± 3.97	1.92	5–21	66.96 ± 10.76	1.80	47– 94
	Mean	9.71 ± 3.87 [b,c]	1.92		67.35 ± 10.30 [b]	1.91	
24	1	10.10 ± 4.06	1.88	4–22	51.54 ± 9.22	2.07	36– 72
	2	8.58 ± 3.18	1.96	4–17	55.76 ± 9.03	1.89	39– 72
	Mean	9.34 ± 3.65 [b,c]	1.92		53.65 ± 9.13 [b]	1.98	
36	1	12.10 ± 3.69	1.91	6–21	45.30 ± 9.49	1.88	20– 66
	2	9.50 ± 2.86	2.04	5–17	45.76 ± 10.97	1.99	24– 79
	Mean	10.80 ± 3.30 [b,c]	1.98		45.53 ± 10.26 [b]	1.94	
48	1	6.94 ± 1.60	2.06	3–10	37.92 ± 7.52	1.89	20– 52
	2	7.92 ± 2.17	2.00	4–12	41.62 ± 11.10	1.90	10– 81
	Mean	7.43 ± 1.91 [c]	2.03		39.77 ± 9.48	1.90	

[a,b,c] See footnotes in Table 1.

and 36 h post exposure than in vivo/in vitro ($p < 0.01$). However, such difference was not observed in spleen cells. The numbers of SCEs were higher in in vivo/in vitro than in vivo for bone marrow at 0 and 6 h and for spleen at 0, 6, 9, 12, 36 and 48 h post exposure ($p < 0.01$). The two methods yielded similar SCE values at other times.

Discussion

In the present in vivo study, the SCEs increased rapidly following cyclophosphamide treatment in both bone marrow and spleen cells. The SCEs reached a peak by about 12 h post exposure and remained relatively unchanged until 24 h and then decreased with increase in time. The rapid increase in SCE response produced in this study parallels the reported rapid production of active metabolites following cyclophosphamide administration in rodents (Torkelson et al., 1974; Natarajan et al., 1983). Similar SCE response pattern in bone marrow following 10 and 15 mg cyclophosphamide/kg body weight has been reported (Charles et al., 1983; Sozzi et al., 1985). The high SCE levels between 12 and 18 h post exposure and gradual decrease with increase in time in the present study, are in agreement with other studies (Charles et al., 1983; Sozzi et al., 1985) which did

not include shorter periods of post-exposure. The initial post-exposure times, 6, 9 and 12 h, showed a time-dependent increase in SCEs, indicating the importance of length of time for complete metabolic activation of cyclophosphamide which would influence the kinetics of the process. The primary factor in the lack of accumulation of SCEs with longer periods of time is the proliferative nature of the bone marrow and spleen populations sampled for SCE analysis and the resultant dilution of DNA damage with time. An almost similar response of bone marrow and spleen cells to cyclophosphamide, as evidenced by the number of SCEs, indicates a similar effect of the drug in vivo.

The SCE levels by 48 h post exposure of animals to cyclophosphamide were slightly above the control values. The difference was statistically significant. The range of SCEs at this sampling time was also larger than the control range, perhaps indicating the presence of a few cells containing higher numbers of SCEs. Similar persistence of bone marrow SCEs following ethyl carbamate treatment (Conner and Cheng, 1983) and γ-irradiation (Morales-Ramirez et al., 1984) has been reported. The cyclophosphamide-induced SCEs in these cells may be related to its tumorigenic activity (IARC, 1981; Popescu et al., 1985). However, with lower doses of cyclophosphamide (10 and 15 mg/kg body weight) in earlier studies (Charles et al., 1983; Sozzi et al., 1985), the SCE levels in mouse bone marrow cells of various strains reached their respective control levels by 48 h post exposure. Kram et al. (1981) and Conner et al. (1983) analyzed bone marrow cells of mice injected with mitomycin C and diepoxybutane, respectively, and observed that SCEs did not persist for more than 2 cell cycles after treatment, concluding that the drug-induced DNA damage was short lived. The differences among various studies may be due to the differences in the metabolic patterns (biotransformation) of the test drug, the amount of the drug, its half-life, and its excretion in urine. The presence of alkylating metabolities has been shown within 10–15 min in mouse blood after administration of cyclophosphamide. Also, mice excrete approximately 60% of the cyclophosphamide in their urine within 24 h (Torkelson et al., 1974). The SCE persistence results of the present study in in vivo are comparable to the biotransformation pattern of cyclophosphamide in mice (Torkelson et al., 1974; Goetz et al., 1975).

In the in vivo/in vitro study, there was a rapid increase in SCE levels following 6 h post exposure. Similar results have been noted in mouse, rat, rabbit, and human peripheral lymphocytes in in vivo/in vitro assays (Ohtsuru et al., 1982; Basiler, 1984; Huff et al., 1982; Takeshita and Conner, 1984). However, there was a marked difference in the SCE frequency between bone marrow and spleen cells at various sampling times. In the bone marrow, there was a rapid decrease in SCE levels from the first sampling time onwards which reached the control values by 48 h post exposure. In the spleen, there was a gradual decrease with time, but, the SCE levels remained at significantly higher levels until 48 h post exposure, the last sampling time analyzed. The higher SCE response in spleen than bone marrow cells may indicate that the lipopolysaccharide-responsive B lymphocytes are more sensitive to cyclophosphamide than bone marrow cells and the drug-induced lesions could persist for a long period (Ropke et al., 1975). It should be noted that the spleen cells are mostly in G_0 during treatment while the bone marrow cells are rapidly cycling without BrdU during the initial 6 or more hours after treatment. Thus the spleen cells may accumulate damage while the bone marrow cells lose lesions through cell cycling. This may account for the difference observed in the two tissues. Differential response of bone marrow and spleen to cyclophosphamide in mice (Wierda and Pazdernik, 1979; Wilmer et al., 1984) and Chinese hamsters (Krishna et al., 1986a) has been reported previously.

Comparison between in vivo and in vivo/in vitro SCE kinetics data revealed a significant difference, and it varied with the tissue. These differences can be attributed to culturing and/or cell cycling effects since in vivo/in vitro cells had additional opportunity to undergo two extra cycles in culture. Many of the differences in the cell behavior between cultured cells and their counterparts in vivo stem from the dissociation of cells from an intact animal and their propagation on a substrate where specific cell interaction characteristics of the tissue are lost. The culture environment also lacks the systemic components involved in homeostatic regulation in vivo (Freshney, 1983).

In spleen, since there is a lack of information on the cell types analyzed in vivo, it is possible that the cell types in in vivo and in vivo/in vitro conditions were not the same. In a recent study, Wilmer et al. (1984) noted a similar SCE response in B and T blood lymphocytes to cyclophosphamide in vitro. However, under in vivo/in vitro conditions, B lymphocytes seemed somewhat more sensitive than T lymphocytes. In addition, increased SCEs under in vivo/in vitro conditions may result from a carry-over of the residual chemical or its metabolites (even after washing) into the culture so that additional lesions would continue to be induced during the culture period. The carry-over of the chemical may depend on cell type and cell cycle length. The possible synergistic effects between cyclophosphamide and BrdU, which was present for 24 h in vivo and was present only in culture under in vivo/in vitro conditions, may also have contributed to the differences in systems.

The range of SCEs among cells at various times of post-treatment was examined. Between 6 and 12 h post treatment, all the cells had a higher frequency of SCEs than control cells. As the overall SCE frequency declined with time so did the number of cells with high levels of SCEs. In vivo, at 48 h post exposure, there were very few cells with high SCE frequencies. Similar shifts in the distribution of SCEs in cells with time have been noted in several other studies (see Tice et al., 1984, for references). The RI of treatments in the present study did not differ significantly from the controls, thus indicating nontoxicity of cyclophosphamide administered under both in vivo and in vivo/in vitro conditions.

Thus, this study indicates that the in vivo/in vitro results are similar to those in vivo, in being positive for cyclophophamide. However, such in vivo/in vitro results may not reflect the actual extent of in vivo damage. The results could vary with the drug's pharmacokinetic properties, sampling time after chemical exposure and the tissue analyzed. These factors should be considered in cytogenic monitoring of patients under chemotherapeutic treatment.

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