IN VIVO STUDY ON THE FREQUENCY OF SISTER CHROMATID EXCHANGE (SCE) AND MICRONUCLEI (MN) INDUCED BY CYCLOPHOSPHAMIDE IN BONE MARROW AND SPLEEN CELLS IN RODENTS

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Abstract. The cytogenetic parameters, i.e. sister chromatid exchange (SCE) as well as micronuclei (MN) in bone marrow and spleen cells, were studied in mice exposed in vivo to cyclophosphamide. The frequency of SCE induced in spleen cells was always considerably and significantly higher as compared to SCE frequency in bone marrow ($p<0.05$). This refers to all the cyclophosphamide doses (1.25—20.0 mg/kg b.w.). Similarly, the micronucleated polychromatic erythrocytes (MN PCE) level in the spleen cells was found to be higher than that in the bone marrow. However, as compared with the SCE test results, the differences between MN PCE frequencies in respective cells were less pronounced. A statistically significant difference ($p<0.05$) was found for all the doses within a 72 hr time period and within any period at doses of 2.5 and 50.0 mg/kg b.w.

INTRODUCTION

Both mutagens and carcinogens have a capacity for inducing cytogenetic lesions within different body organs and tissues, e.g. in peripheral blood lymphocytes, bone marrow, spleen, liver and germinal cells. The cytogenetic lesions which may vary, include pyknosis or nuclear karyorrhexis, mutations, micronucleus formation, sister chromatid exchange, chromosomal aberrations etc. (7, 11). Moreover, the magnitude of effects induced by several chemicals in the cells of various organs may be different (3, 9, 10). An example is diethylnitrosamine which has been found to evoke no chromosomal aberrations either in bone marrow or peripheral blood lymphocytes, slightly increased SCE in bone marrow as well as highly increased MN frequency in hepatocytes (10). In

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view of this, it seems to be justified to use several different modes of testing in order to monitor various biological effects in body organs. This may help to detect the muta/carcinogens and thus contribute to reducing the number of false negative results.

In the present paper the frequencies of SCE and MN induced by cyclophosphamide in bone marrow and spleen cells have been compared.

MATERIAL AND METHOD

Chemical test

The agent tested was cyclophosphamide produced by Germed, VEB Jenapharm, GDR. The dosage was determined according to relevant reports in literature (5, 6).

SCE test

For this study Allen's et al. method was used (1). The test group consisted of 46, 8-10 week male mice, SFIS strain, with mean b.w. of 30 g, from Animal Husbandry at the Institute of Occupational Medicine in Lodz. In mice anaesthetised with ether, 50 mg bromodeoxyuridine tablets (Serva) were implanted subcutaneously. An hour later cyclophosphamide dissolved in distilled water was administered i.p. (0.5 ml/animal), the doses ranging from 1.25 to 20.0 mg/kg b.w. The control were mice in which 0.5 ml distilled water was administered. After the next 21 hours 0.3 ml of 0.01% colcemide solution was given i.p. Two hours later the animals were killed and bone marrow (drawn from both the femurs) as well as splenic tissue samples were collected. After rinsing with Hanks' solution the samples were incubated in 0.075 M KCl and fixed 3 times with methanol:glacial acetic acid (3:1). The slides (bone marrow and spleen cells from each animal) were left to dry and were then stained by the method of Antoshina nad Porjadkova (2).

Micronucleus test

The micronucleus test was performed according to Hayashi et al (4). As in the SCE test, 8-10 week SFIS male mice were used (60 animals, 4/point each). The cyclophosphamide was administered by a single i.p. injection and the doses ranged from 2.5 — 100.0 mg/kg b.w. The animals were killed after 24, 30, 48 and 72 hrs and bone marrow and spleen samples were drawn. Both types of cells were suspended in a little calf serum with a drop of 3.8% sodium citrate, directly on the slide. The preparations were fixed with May-Grünwald reagent and stained with 10% Giemsa solution. In the study the percentage of micro-

nucleated polychromatic erythrocytes (MN PCE) was calculated. Besides, the percentage of polychromatic erythrocytes (PCE) in 1000 analyzed erythrocytes was determined.

Statistical evaluation

The results were evaluated statistically with one way variance analysis using a multiple comparison test. In order to compare the magnitude of effects on different organs, the two way variance analysis with multiple comparison test (Scheffe's method) was used with simple effects testing. For the SCE test results, Whorton's transformation (12) was used to stabilize the variance. The correlation coefficients for the dose-effect relationship were determined. The mean number of SCE as well as standard deviations for the non-transformed results are displayed in the enclosed table.

RESULTS

The results of SCE testing in bone marrow and spleen cells are shown in Table 1. As regards the control, the number of SCE in bone marrow and spleen cells amounted to 4.0/cell and 3.2/cell, respectively, thus differing significantly ($p<0.05$). In all the doses, cyclophosphamide induced increased SCE in both the bone marrow and spleen cells ($p<0.05$) according to the dose administered, with correlation coefficients of 0.99 and 0.94, respectively. The frequency of SCE induced in spleen cells was always considerably higher as compared to SCE frequency in bone marrow ($p<0.05$). This refers to all the cyclophosphamide doses. For the doses of 1.25 and 10.0 mg/kg b.w. the frequency of SCE in spleen cells was 130% and 550% as high as the SCE level in the control group. As for the bone marrow testing, the respective doses of mutagen induced an SCE frequency which was 28% and 350% as high as that in the control.

Table 2 shows the results of the micronucleus test. In the control group (4 animals) the MN PCE frequency was similar for both the bone marrow and spleen cells, remaining at the level of 0.18% $\pm$0.05 and 0.20% $\pm$0.27, respectively. Both in the bone marrow and spleen cells the MN PCE frequency induced by cyclophosphamide within a time period of between 24 and 72 hrs was significantly higher than respective values in the control. The highest MN PCE level in both the cell types was found after 48 hrs of exposure to the mutagen. The sum of MN PCEs which were then found in bone marrow and spleen cells amounted to 209 and 229, respectively, decreasing to 125 and 193 within 72 hrs. The MN PCE level in the spleen cells was found to be higher than that in the

TABLE 1. SCE Frequency in Bone Marrow and Spleen Cells in Mice After Cyclophosphamide Administration. The Number of Cells Examined is Given in Brackets

Dose (mg/kg)	Bone marrow cells			Spleen cells		
	No. of animals	SCE/cell ±S.D.	Proportional increase of SCE/cell	No. of animals	SCE/cell ±S.D.	Proportional increase of SCE/cell
0.0	8	4.0±2.1 (400)		6	3.2±1.8 (175)	
1.25	8	5.1±2.6 * (355)	28%	3	7.4±3.3 * (110)	131%
2.5	5	5.4±2.4 * (230)	35%	7	10.8±5.5 * (225)	238%
5.0	8	10.5±4.4 * (400)	165%	4	14.1±5.1 * (110)	341%
10.0	8	18.0±6.1 * (315)	350%	4	20.9±7.0 * (70)	553%
20.0	7	33.3±9.5 * (270)	733%	4	X	

* — the frequency of SCE/cell significantly higher than the control level ($p<0.05$)
X — absence of mitoses

bone marrow but significantly higher only when induced by all the mutagen doses within 72 hrs as well as within all time periods at doses of 2.5 and 50.0 mg/kg b.w. ($p<0.05$).

As regards the PCE level, it decreased considerably in bone marrow and only slightly in the spleen, as the doses of mutagen increased.

DISCUSSION

Recently, cytogenetic tests in vivo have become more widely used for the screening of mutagens and carcinogens. One of the reasons for the popularity of in vivo testing is the possibility of a more accurate estimation of the health risk from exposure to mutagens and carcinogens. The most frequently performed cytogenetic tests in vivo include SCE and micronucleus tests in bone marrow cells in rodents.

Since the distribution of an agent within body organs and different cell sensitivity may affect the frequency of cytogenetic lesions, in the present study, cyclophosphamide activity was tested not only in the

TABLE 2. Number of MN PCEs in Bone Marrow and Spleen Cells in Mice, within Different Time Periods After a Single Cyclophosphamide Administration (Experimental Performed Ac cording to Hayashi's Protocol). In Round Brackets: Percentage Polychromatic Erythrocytes (PCE) in Square Brackets: Percentage of MN PCE

Dose [mg/kg]	Bone marrow cells					Spleen cells				
	24 hrs	30 hrs	48 hrs	72 hrs	Total	24 hrs	30 hrs	48 hrs	72 hrs	Total
2.5	13 (53%)	33 (57%)	14 (53%)	22 (53%)	82 [0.51%]	28 (39%)	37 (36%)	23 (25%)	46 (36%)	134 [0.84%]
25.0	26 (53%)	32 (43%)	48 (41%)	27 (42%)	133 [0.83%]	33 (40%)	30 (23%)	43 (22%)	34 (30%)	140 [0.88%]
50.0	46 (41%)	46 (44%)	69 (38%)	45 (39%)	206 [1.29%]	47 (22%)	48 (22%)	96 (20%)	63 (29%)	254 [1.59%]
100.0	63 (38%)	59 (25%)	78 (19%)	31 (27%)	231 [1.44%]	49 (30%)	54 (20%)	67 (20%)	50 (17%)	220 [1.38%]
Total	148 [0.93%]	170 [1.06%]	209 [1.31%]	125 [0.78%]		157 [0.98%]	169 [1.06%]	229 [1.43%]	193 [1.21%]	

Each result represents total number of MN PCEs found in 4 animals (4000 cells).
MN PCE frequency in the control group: a) in bone marrow cels — 7/4000=0.18%, (60% PCE)
b) in spleen cells — 8/4000=0.20%, (23% PCE).

bone marrow but also in spleen cells. The SCE test revealed that in every animal all the doses of administered mutagen produced a significantly higher level of SCE in the spleen cells as compared to that in the bone marrow (Table 1). In the spleen cells the rate of SCE/cell induced by cyclophosphamide at doses of 1.25 and 10.0 mg/kg b.w. was twice and six times higher than the control level. In the bone marrow cells the SCE frequency was only 0.25 and 4 times as high as in the control.

Our results differ from those obtained by Palitti et al (8) who reported a similar SCE frequency in both the bone marrow and spleen cells. An explanation for these discrepancies may be the different mode of administering bromodeoxyuridine and cyclophosphamide.

In both the bone marrow and spleen cells cyclophosphamide was found to induce increased MN PCE. However, as compared with the SCE test results, the differences between MN frequencies in respective cells were less pronounced and statistically significant only for all the doses within a 72 hr time period and within any period at doses of 2.5 and 50.0 mg/kg b.w.

The study results seem to indicate the necessity of using at least 2 different means of testing chemicals for their mutagenic potentials in different target organs. This would facilitate the detection of chemical mutagens, especially the ones with a weak potential for inducing cytogenetic lesions.

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