Study of Mutagenic Activity of Dioxidine by the Polyorgan Micronuclear Method

L. P. Sycheva, M. A. Kovalenko, S. M. Sheremet'eva, A. D. Durnev*, and V. S. Zhurkov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 8, pp. 188-190, August, 2004
Original article submitted April 19, 2004

Antibacterial preparation dioxidine administered four times in doses of 10, 100, and 300 mg/kg increased the incidence of micronucleated cells in the bone marrow, lungs, and large intestine of mice. Bone marrow cells were most sensitive, while cells of the lungs and large intestine exhibited lower sensitivity to the cytogenetic effect of dioxidine.

Key Words: *dioxidine; mutagenic effect; polyorgan micronuclear test; lungs; large intestine*

Dioxidine (2,3-di(hydroxymethyl)quinoxaline-1,4-dioxide) is the antibacterial preparation exhibiting a wide range of activity. The bactericidal effect of this preparation is determined by its ability to activate lipid peroxidation in cells. Dioxidine impairs DNA biosynthesis, produces damage to membrane proteins and structural changes in the cytoplasm, and cause death of microbial cells [2].

The mutagenic effect of dioxidine was observed in microorganisms, drosophila, and human lymphocytes *in vitro*. Previous experiments revealed an increase in the incidence of chromosomal aberrations in mouse bone marrow cells and dominant lethal mutations in sex cells of male mice [3,7,10]. Recent studies showed that dioxidine produces DNA damage in cells of the lungs, liver, kidneys (alkaline elution) [1], and liver in mammals (fluorometric study of DNA integrity) [9]. Here we used the polyorgan micronuclear method for studying of the specific cytogenetic effect of dioxidine in various organs of mice.

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice weighing 20-25 g. Dioxidine (Leningrad Chemical-and-Pharmaceutical Company Oktyabr') was *ex tempore* dissolved in sterile distilled water and injected intraperitoneally in daily doses of 10, 100, and 300 mg/kg. Experimental groups included 5-6 mice. Control animals (n=8) received distilled water. The animals were killed 24 h after the last injection.

The cytogenetic effect of dioxidine was studied in polychromatophilic erythrocytes (PCE) of the bone marrow, epithelial cells of the large intestine, and type I and II pneumocytes. Bone marrow cells were isolated as described elsewhere [5]. Samples of the lungs and large intestine were fixed with 10% neutral formalin and suspension preparations were prepared [8]. We examined 1000 PCE, type I and II pneumocytes, and epithelial cells of the large intestine on codified samples.

In addition to micronuclear study, we evaluated the incidence of nuclear protrusions in intestinal cells. They looked like thready, spherical, or more complex structures lying in the cytoplasm and connected with the major nucleus. Most protrusions appear after breaking of anaphase chromosomal or chromatid bridges formed under the influence of mutagens [4]. Accumulation of micronucleated cells with protrusions reflects cytogenetic activity of the preparation. The increase in the mitotic index and number of binucleated or polynucleated cells illustrate the appearance of polyploid structures. The ratio between the count of PCE and total number of erythrocytes in the bone marrow was estimated by examining 200 erythrocytes.

Laboratory of Genetic Monitoring, A. N. Sysin Institute of Human Ecology and Environmental Hygiene, Russian Academy of Medical Sciences; *Laboratory of Mutagenesis, Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** lpsycheva@rambler.ru. Sycheva L. P.

0007-4888/04/1382-0165

The ratio of aberrant cells in treated and control mice was compared by χ^2 test or Fischer exact test. Spearman rank correlation test (R) was used to evaluate the relationship between test indexes and dose of dioxidine.

The doubling dose (DD) of dioxidine was calculated to compare its mutagenic effects in cells of various organs. The preparation in this dose produces a 2-fold increase in the ratio of micronucleated cells (compared to the control). If the ratio of micronucleated cells linearly depends on mutagen concentration of below the minimally effective dose (MED), DD can be calculated by the formula: $DD=MED\times2\times E_C/E_{MED}$, where E_C is the mean ratio of micronucleated cells in the control; and E_{MED} is the mean ratio of micronucleated cells in animals receiving MED. DD was calculated for cytogenetic indexes with pre-estimated MED and dose-dependent effect of the preparation.

RESULTS

The ratio of micronucleated PCE in the bone marrow increased with increasing the dose of dioxidine (R=0.80, df=22, p<0.001, Table 1). MED of the preparation was 100 mg/kg. Administration of dioxidine in doses of 100 and 300 mg/kg was followed by the appearance of binucleated or polynucleated cells. The ratio of PCE decreased with increasing the dose dioxidine. Dioxidine in a dose of 300 mg/kg significantly decreased the ratio of PCE (by 3.6 times compared to the control), which reflects severe disturbances in erythropoiesis (Table 1).

Dioxidine in the maximum dose significantly increased the ratio of epithelial micronucleated cells with protrusions in the large intestine. However, significant differences were revealed only in the total number of micronucleated cells with protrusions (R=0.49, df=22, p<0.05, Table 1). The mean mitotic index in cells of the large intestine in treated mice did not differ from the control.

Dioxidine in the maximum dose significantly increased the ratio of micronucleated cells in the lungs (R=0.51, df=22, p<0.05), but had no effect on the count of binucleated cells.

Thus, dioxidine increased the count of micronucleated cells in the bone marrow, lungs, and large intestine. However, MED of dioxidine and type of changes produced by this preparation differed in cells of various organs.

The cytogenetic effect of dioxidine was most pronounced in bone marrow cells (Table 2). Administration of dioxidine in a dose of 100 mg/kg was followed by the appearance of binucleated or polynucleated cells. Dioxidine in the maximum dose produced severe disturbances in erythropoiesis. Our results are consistent with published data on the ratio of chromosomal aberrations in mouse bone marrow cells [8,10].

Cytogenetic activity of dioxidine in cells of the lungs and large intestine was lower than in the bone marrow by 5 and 10 times respectively. The mutagenic effect of dioxidine in lung cells confirm the results of an alkaline dilution study that intraperitoneal injection of the preparation produces DNA damage in mice [1]. It was reported that MED of dioxidine in cells of the liver and kidneys (200 mg/kg) surpasses that in the lungs (100 mg/kg).

Our study revealed differences in the mutagenic effect of dioxidine in cells of various organs. The

TABLE 1. Cytogenetic Effect of Dioxidine in Cells of Various Organs in Mice ($X\pm SE$)

Organ, index	Control (n=8)	Dose of dioxidine, mg/kg		
		10 (n=6)	100 (n=5)	300 (n=5)
Bone marrow				
micronucleated PCE, ‰	3.13±0.61	3.83±1.19	33.00±7.48***	47.40±7.62***
binucleated and polynucleated PCE, ‰	0.00	0.00	1.00±0.45*	6.40±1.17***
PCE/total erythrocyte number	47.9±2.6	45.7±2.7	41.5±4.9	13.2±3.4*
Large intestine				
micronucleated cells, ‰	1.13±0.55	0.50±0.34	0.60±0.40	4.80±2.85*
cells with protrusions, ‰	1.50±0.82	0.50±0.34	2.40±0.68	3.40±1.57*
micronucleated cells with protrusions, ‰	2.63±1.32	1.00±0.52	3.00±0.63	8.20±4.24***
mitotic index	0.74±0.11	0.60±0.11	0.56±0.18	0.64±0.28
Lungs				
micronucleated cells, ‰	0.25±0.25	0.00	0.40±0.24	1.60±0.68*
binucleated cells, ‰	9.33±2.14	12.67±2.14	8.60±2.86	11.80±2.78

Note. *p<0.05 and **p<0.001 compared to the control.

TABLE 2. DD of Dioxidine by the Incidence of Cytogenetic Damage to Cells of Various Organs in Mice

Organ	Index	MED, mg/kg	Surpassing the control, times	DD, mg/kg
Bone marrow	Ratio of micronucleated PCE	100	10.5	19.0
Large intestine	Ratio of micronucleated cells	300	3.1	192.4
Lungs	Ratio of micronucleated cells with protrusions	300	6.4	93.8

organ specificity of dioxidine is probably related to the existence of a specific molecular mechanism underlying the effect this preparation. Dioxidine is rapidly accumulated in various organs and tissues [2] and is excreted in unmodified form without metabolic transformations [6]. The mutagenic effect of dioxidine is not modified by microsomal monooxygenases. In the Ames test, dioxidine induces mutations not accompanied by metabolic activation. Some investigators believe that the mutagenic effect of dioxidine is related to the induction of free oxygen radicals [7]. The influence of dioxidine on the bone marrow is probably associated with the presence of macrophages and neutrophils in considerable amounts. These cells act as a major inductor of free oxygen radicals. Dioxidine probably initiates generation of reactive oxygen species in these cells. The mutagenic effect of dioxidine in the lungs is related to oxygen saturation of this tissue, which contributes to free radical generation [1]. Our results and published data [1] show that dioxidine exhibits lower mutagenic activity in cells of the liver, kidneys, large intestine, and gonads, which is associated with low-intensity generation of free radicals under the influence of this preparation in high dose.

REFERENCES

1. S. K. Abilev and M. M. Abdrazakov, *Genetika*, **27**, No. 11, 2039-2041 (1991).
2. *Manual on Antibacterial Therapy*, Eds. L. S. Strachunskii *et al.* [in Russian], Moscow (2000).
3. T. S. Bakai and L. M. Fronshtein, *Mol. Genet.*, No. 4, 35-39 (1987).
4. S. N. Kuzovatov, V. Yu. Kravtsov, and Yu. B. Vakhtin, *Tsitologiya*, **42**, No. 11, 1097-1102 (2000).
5. *Study of Mutagenic Activity of Chemical Substances by the Micronuclear Method: Methodical Recommendations* [in Russian], Moscow (1984).
6. E. A. Rudzit, A. M. Bespalov, and R. F. Tagirov, *Farmakol. Toksikol.*, No. 4, 472-473 (1974).
7. S. B. Seredinin and A. D. Durnev, *Pharmacological Protection of Genome* [in Russian], Moscow (1992).
8. L. P. Sycheva, *Byull. Eksp. Biol. Med.*, **131**, No. 4, 445-447 (2001).
9. M. V. Usol'tsev, A. D. Durnev, and S. B. Seredinin, *Eksper. Klin. Farmakol.*, **63**, No. 2, 60-62 (2000).
10. L. M. Fonshtein, Yu. A. Revazova, G. N. Zolotareva, *et al.*, *Genetika*, **15**, No. 6, 901-908 (1978).