# Evaluation of Mutagenic Activity of Dioxazid by the Polyorgan Micronuclear Method in Experiments on Rats

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The mutagenic effect of antituberculous drug dioxazid was studied on rats receiving this preparation in a dose of 25 mg/kg (in conversion to dioxidine) via inhalation route for 3 months. The percentage of cells with micronuclei and the content of polychromatophilic erythrocytes among all bone marrow erythrocytes and percentage of cells with micronuclei, protrusions, and binucleated cells in the lungs and urinary bladder were evaluated. Dioxazid caused no changes in organs, except the increase in the percentage of binucleated cells in bladder epithelium, which attests to minor cytotoxic effect of the drug for this organ.

**Key Words:** *dioxazid; mutagenic effect; polyorgan micronuclear test; lungs; urinary bladder*

We evaluated the side (mutagenic) effects of dioxazid, an effective antituberculous drug developed at Chemical Pharmaceutical Institute and based on a combination of dioxidine (2,3-di(hydroxymethyl)quinoxaline-1,4-dioxide) and isoniazid (hydraside 4-pyridine-carbonic acid) for inhalations. Both initial components are mutagenic. Mutagenic effect of dioxidine was demonstrated on various test objects *in vitro*, in experimental mammals *in vivo*, and in humans [5,7]. DNA damaging effect of dioxidine was detected in mammalian cerebral, gastric, cystic, rectal, pulmonary, hepatic, and renal cells [1,3, 8]. Cytogenetic effect of dioxidine was demonstrated on polychromatophilic bone marrow erythrocytes, pulmonary and rectal cells in mice [7]. Isoniazid exhibited a slight mutagenic activity in Ames test without metabolic activation, induced chromosome aberrations and sister chromatid exchanges in rodent cells *in vitro*, but exhibited no mutagenic activity in mammals *in vivo* [10].

Mutagenic activities of the initial components and prospective use of dioxazid for inhalation treatment necessitated the study of the drug mutagenic effect during inhalations in the organ of its entry (lungs) and discharge (urinary bladder).

## MATERIALS AND METHODS

Experiment was carried out on male outbred rats (350 g by the end of experiment) from Kryukovo Breeding Center. The animals were kept under conditions of 12:12 h day:night regimen with free access to water and food. Each group consisted of 5 animals. Experimental rats received dioxazid inhalations daily for 3 months, the dose corresponded to 25 mg/kg in conversion to dioxidine. The inhalation mixture corresponded to prospective mixture for the treatment of patients (100 mg dioxidine+

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250 mg isoniazid). Control rats were kept under the same conditions, but received no treatment.

The cytogenetic effect of dioxazid was evaluated by the polyorgan micronuclear method developed at our laboratory [6], including the traditional micronuclear method on bone marrow polychromatophilic erythrocytes (PCE) [12] and evaluation of the percentage of cells with microinuclei, protrusions, and of binucleated cells at the site of the drug administration (in the lungs) and in the organ of drug discharge (urinary bladder). Bone marrow cell preparations were made as described previously [4]. Fragments of the lungs and urinary bladder were fixed in 10% neutral formalin. Suspensions of these organs were prepared as described previously [4]. Microscopic analysis was carried out at magnification 1000. A total of 2000 PCE, 1000 lung cells (a sum of types I and II pneumocytes and alveolar macrophages), and cystic epithelial cells per preparation (coded) were analyzed. The cytotoxicity for the bone marrow was evaluated by estimating the percentage of PCE in the total number of all erythrocytes (200 erythrocytes per preparation were analyzed). The percentage of nucleus protrusions and binucleated cells, in addition to the percentage of micronuclei, was evaluated in pulmonary and cystic cells. Protrusions are thread-like or spherical structures beyond the main nucleus in the cytoplasm, connected to the nucleus with a cord. Various mechanisms of protrusion formation are hypothesized. It seems that protrusions form (similarly as the micronuclei) after completion of the aberrant cell mitosis by chromosome fragments; by disrupted fragments of chromosome bridges formed by dicentrics; by whole chromosomes lagged behind after impairment of the division spindle. Possible budding of interphase nuclei during elimination of amplified DNA [11,13] or DNA reparation complexes [9] is also hypothesized. All these mechanisms indicate that the formation of protrusions, similarly as of micronuclei, characterizes the cytogenetic effect of the drug. Increased count of binucleated cells indicates modified process of nucleus division.

The results were statistically processed using Statistica software. The parameters were compared using $\chi^2$ test.

## RESULTS

The percentage of cells with micronuclei and nucleus protrusions in the lungs and urinary bladder of rats slightly increased, but the difference for these main cytogenetic parameters was statistically negligible (Table 1). Variations in the incidence of cells with micronuclei in the experimental groups were within the range of the mean basal values [4]. The absence of cytogenetic effect of a mixture of substances with cytogenetic effects was determined by the active dose (25 mg/kg for dioxidine). According to published data, the cytogenetic effect of dioxidine manifests in mouse bone marrow cells only at doses close to $^1/_5$ $LD_{50}$ (100-150 mg/kg) and higher. Isoniazid (component of dioxazid) exhibited no genotoxic effect *in vivo* [10].

No cytotoxic effect of dioxazid was detected in the analysis of the percentage of PCE among all erythrocytes and of the percentage of binucleated cells in the lungs. However, this effect was detected in the urinary bladder epithelium. The percentage of binucleated cells in this organ increased significantly (by about 60%). The detected effect can be explained by the toxicokinetics of dioxazid components. About 30% administered isoniazid is discharged with the urine, half of this volume is excreted unchanged. Dioxidine is virtually not metabolized and is discharged mainly through the kidneys. High bactericidal concentrations of the drug are detected in the urine within 8 h after intravenous injection of dioxidine in a therapeutic dose (10 mg/kg) [2]. Increased count of binucleated cells is explained by intensification of the compensatory processes in the organ under the effect of the toxic agent, which disturbs the functioning of some cells or causes their death. This leads to more intensive renewal of the cell population.

Hence, dioxazid exhibited no cytogenetic effect on bone marrow, lung, and urinary bladder cells of rats receiving inhalations of the drug for 3 months in a dose corresponding to 25 mg/kg dioxi-

**TABLE 1.** Cytogenetic and Cytotoxic Parameters of PCE, Pulmonary and Bladder Cells of Rats after Inhalations of Dioxazid for 3 Months ($X_{mean}\pm m$; $n$=5)

| Parameter | Control | Experiment |
|---|---|---|
| Bone marrow | | |
| Cells with micronuclei, ‰ | 1.50±0.15 | 1.10±0.33 |
| PCE/all erythrocytes, % | 0.47±0.02 | 0.48±0.02 |
| Lungs | | |
| Cells with micronuclei, ‰ | 0.20±0.20 | 0.40±0.40 |
| Cells with protrusions, ‰ | 0.40±0.24 | 1.20±0.58 |
| Binucleated cells, ‰ | 12.60±1.60 | 11.80±1.07 |
| Urinary bladder | | |
| Cells with micronuclei, ‰ | 0.00 | 0.75±0.75 |
| Cells with protrusions, ‰ | 2.40±1.80 | 3.50±1.19 |
| Binucleated cells, ‰ | 15.6±4.69 | 25.75±4.13* |

**Note.** *$p$<0.001 compared to the control ($\chi^2$ test).

dine. A slight increase in the percentage of binucleated cells in the bladder epithelium can indicate a slight cytotoxic effect of the drug in this organ.

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