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SHORT COMMUNICATIONS

Organ Specificity of the Genotoxic Effects of Cyclophosphane and Dioxidine: An Alkaline Comet Assay Study

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Abstract—The applicability of alkaline comet assay to studying the organ specificity of the genotoxic effects of drugs has been estimated using cells from four organs of mice (the liver, lungs, spleen, and brain). It has been found that cyclophosphamide damages DNA in all the four organs; and dioxidine, in all organs except the brain. It is concluded that this method can be used for studying the organ specificity of the DNA-damaging effects of various substances.

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Cytogenetic analysis of rat and mouse bone marrow cells is currently the main experimental method for estimating the mutagenic potential of various substances in mammals. Mutagenicity is an important prognostic characteristic of the carcinogenic activity of chemical compounds. The estimates of genotoxicity of substances in different organs of mammals are important for the prediction of carcinogenic activity.

Analysis of the database on the carcinogenic activities of chemical environmental factors has shown that the detected carcinogenic effects depend on both the species of the experimental animals (rats or mice) and the organ of the animals used [1]. The organ specificity of the genotoxic effect is currently studied using polyorgan micronucleus assay [2], DNA alkaline elution assay [3], single cell gel electrophoresis assay (comet assay) [4], and transgenic mouse assay [5]. They differ from one another not only in the principles of the methods, but also in the detected genetic effects and reliability. Polyorgan micronucleus assay is the most reliable (valid) of them [6]. While micronucleus assay detects all induced micronuclei in the cells that have completed mitosis after the exposure to a genotoxic factor, DNA alkaline elution and comet assays detect breaks and other DNA lesions converted into breaks in an alkaline medium.

We studied the organ specificity of the DNA-damaging (genotoxic) effects of the antibacterial drug dioxidine and the antitumor drug cyclophosphamide on mice with the use of alkaline comet assay.

The experiment was performed on male BALB/c mice aged three to four months with a mean body weight of 25 g kept under the standard conditions of a vivarium. The drugs were injected once intraperitoneally. Cyclophosphamide was injected at a dose of 50 mg/kg (prepared ex tempore); dioxidine, at a dose of 200 mg/kg (a commercial solution for injections). The control group was administered water for injections. Each group consisted of five mice. The animals were sacrificed by cervical dislocation 5 h after the injection. The following organs were used for the study: the liver, lungs, spleen, and brain.

The degree of DNA damage in these organs was estimated as recommended in [7]. The cells were immobilized in 0.5% (for brain cells, 2%) warm (37°C) low-melting-point agarose. The resultant cell suspension was applied onto prepared slides (1% normal-melting-point agarose dissolved in water), and the cells were lysed for 1 h at 4°C (2.5 mM NaCl, 100 mM Na_2EDTA, 10 mM Tris-HCl, with DMSO and Triton X100 (10 and 1% of the final volume, respectively) added on the day of the experiment). The slides with the cell lisate layered on them were put into an alkaline buffer solution (1 mM Na_2EDTA, 300 mM NaOH, pH 13) for 20 min to con-

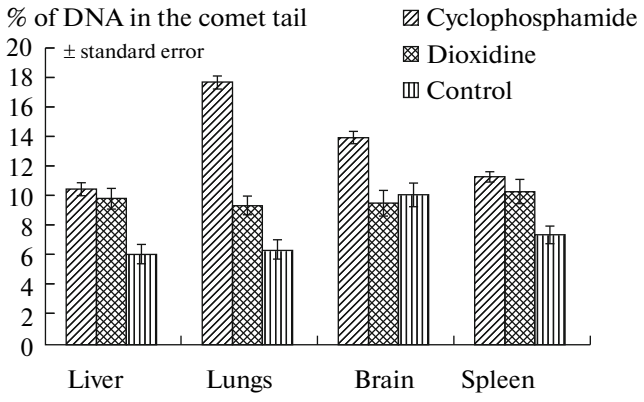

Fig. 1. The DNA-damaging effects of cyclophosphamide and dioxidine in cells of some organs of mice.

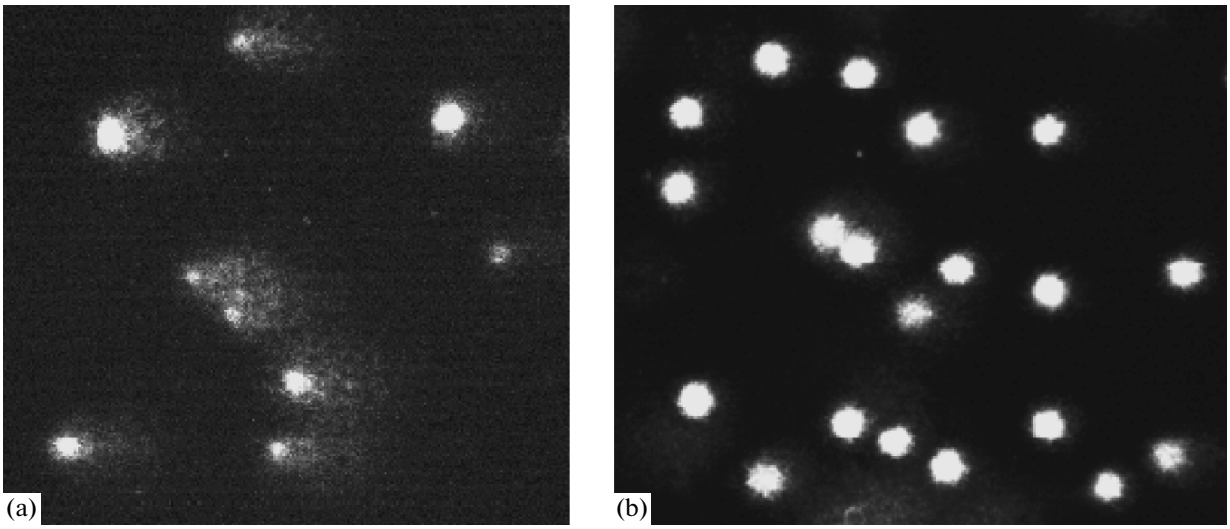

Fig. 2. (a) Cyclophosphamide-treated and (b) control mouse lung cells.

vert alkali-labile sites into single-strand breaks. After that, we performed electrophoresis (0.7 V/cm, 200 mA, 10 min), and the slides were consecutively neutralized in a neutralizing buffer solution (Tris-HCl, pH 7.5), dried at room temperature, and stained with acridine orange. The stained slides were examined under a Zeiss fluorescent microscope, and the DNA content in the comet tail (in percent) was calculated using the Comet Score software. The parameter *DNA* percentage in the comet *tail* reflects the amount of low-molecular-weight DNA in the form of single-strand fragments resulting from breaks and alkali-labile sites converted into breaks; these fragments migrated towards the anode during electrophoresis.

The Win Stat software (an application of Excel) was used for statistical treatment of the results.

As evident from Fig. 1, dioxidine did not damage brain cells and had equal DNA-damaging effects in the cells of the other organs studied, i.e., the liver, lungs, and spleen (9.87, 9.37, and 10.29% of DNA in the comet tail versus control values of 6.13, 6.40, and 7.38%, respectively). Cyclophosphamide had a stronger DNA-damaging effect than dioxidine and was active in all organs studied, including the brain. Its effect was the strongest in lung cells. The photograph in Fig. 2 shows a part of a slide containing mouse lung cells treated with cyclophosphamide as a typical example of the images obtained by means of comet assay of cells with damaged DNA.

Our study was the first to demonstrate that cyclophosphamide can damage DNA in mammalian brain cells. Apparently, active metabolites of cyclophosphamide easily penetrate through the blood–brain barrier.

Dioxidine is not subjected to biotransformation in the body and is excreted almost unchanged [2]. It has been shown to enhance intracellular free-radical processes leading to DNA lesions [7]. In our experiments, dioxidine was equally potent in inducing DNA lesions in mouse liver, lung, and spleen cells. However, in contrast to cyclophosphamide, it had no effect on brain cells, although it was earlier found to be able to penetrate through the blood–brain barrier [2].

Dioxidine is widely used in clinical practice as an antibacterial agent for treating purulent infections [8]. Dioxidine is mutagenic; it induces mutations in bacterial cells, chromosomal aberrations and micronuclei in mouse bone marrow cells [9, 10], and dominant lethal mutations in mouse male sex cells [9]. Cyclophosphamide is an antitumor drug. Its cytotoxicity and mutagenicity are accounted for by the formation of highly reactive alkylating metabolites in a mammalian body [11, 12].

Thus, our experiments using comet assay have demonstrated that the antibacterial drug dioxidine and the antitumor drug cyclophosphamide may induce DNA damage in some organs of mice. Comet assay makes it possible to quantitatively estimate the degree of DNA damage in the organs studies and may be used for analyzing the organ specificity of the genotoxic effects of a number of substances.

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