Short report
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Gordana JOKSIĆ
Miroslava STANKOVIĆ
Sandra RADOVANOVIĆ
Milena DRAGIĆ

The use of cytochalasin block (CB) micronucleus test to identify clastogenic and antiproliferative action of tiazofurin

KEY WORDS: *Micronucleus tests; Lymphocytes; Drug Toxicity; Antineoplastic Agents*

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MEDICAL PROTECTION CENTER, INSTITUTE OF NUCLEAR SCIENCES VINČA, BELGRADE, YUGOSLAVIA

Genotoxicity testing aims to detect a large range of genetic damage endpoints and evaluate such results in the context of cell survival. The most often used endpoint in routine genotoxicity testing is the induction of chromosomal aberrations, however the cytochalasin block (CB) micronucleus test is also suitable to monitor genotoxicity in mammalian cells. It is easier and faster to perform than the analysis of chromosomal aberrations and has a better potential for automation. Micronuclei are chromatin-containing structures in the cytoplasm surrounded by a membrane without any detectable link to the cell nucleus. They are formed by exclusion of whole chromosomes or chromatin fragments during cell division. Micronuclei can be detected using different DNA dyes and their frequency can be quantified microscopically. Enumeration of micronuclei in mitogen-stimulated lymphocytes provides a simpler and statistically more precise method for quantification of chromosomal damage (1-4). At the same time the test enables estimation of cytotoxicity using frequency of cytokinesis-blocked binucleated and polynucleated cells as good estimators of the mitotic rate. The cytokinesis block micronucleus test offers the advantage of providing information on both chromosome aberrations and cell cycle progression simultaneously. Most often a cytokinesis block proliferation index (CBPI) is used that indicates the average number of cell cycles undergone by a given cell (5,6) calculated using the following formula: CBPI=[MI+2MII+3(MIII+MIV)]/total, where MI to MIV represent the number of cells with one to four nuclei.

The present investigation was undertaken to study the effect of various concentrations of tiazofurin on micronucleus formation and in vitro proliferation in phytohemaglutinin-stimulated human lymphocytes. The aim of our investigation was to estimate the applicability of CB micronucleus test in assessment of cell effects of this novel synthesised drug.

Human lymphocytes obtained from blood samples of three healthy adults were exposed to tiazofurin concentrations of 0.08, 0.11, 0.2, 0.42, 0.76 and 0.90μmol/ml at two different treatment periods; two and seventeen hours after stimulation with phytohemaglutinin. About one million lymphocytes per each culture were treated with the tiazofurin concentrations referred above. The concentrations of tiazofurin were chosen according to our previous study of its chemical analogue (1,β-D ribofuranosyl-1,2,4 triazole-3-carboxamide) ribavirin (7). After the treatment, the medium containing the drug was removed and replaced with a fresh one. Harvesting of cells was continued during the next 72 hours, according to the method of Fenech and Morley (8). The induction of micronuclei was evaluated by scoring a total of 1000 binucleated cells. Toxicity was evaluated by classifying 1000 cells from the same slides according to the number of nuclei (mononucleated, binucleated and polinucleated).

Results of the study demonstrated that proliferation potential of cells treated two hours with 0.42, 0.76 and 0.90μmol/ml of tiazofurin was significantly reduced (with significance ranging from 0.009 to 0.1 respectively), while only the 0.76μmol/ml concentration significantly increased the yield of tiazofurin-induced micronuclei ($t=-10.4$, $p<0.009$) (Figure1). The accumulation of mononucleated cells and reduced portion of polinucleated cells was observed with increased dosage of tiazofurin.

Prolonged treatment with tiazofurin (17 hours) reduced the proliferation potential of cells at all concentrations (with significance ranging from $p<0,01$ to $p<0.001$), enhancing the yield of tiazofurin-induced micronuclei at concentrations of 0.08, 011 and 0,22 mmol/ml ($t=-5.2$, $p<0.03$; $t=-5.9$, $p<0.02$ and $t=-5.8$, $p<0.02$ respectively) (Figure 2).

Proliferation of cells through the second cell division was nearly stopped at tiazofurin concentrations higher than 0.08mmol/ml as evident from the total absence of polinucleated cells.

Various treatment periods significantly changed the incidence of

Address correspondence to:
Dr. Gordana Joksić, Medical Protection Center, Institute of Nuclear Sciences Vinča, POB 522, 11000 Belgrade, Yugoslavia

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micronuclei. At lower concentration of tiazofurin the expression of clastogenic properties took longer. However, the lowest concentration of tiazofurin markedly changed the frequency of mono and multinucleated cells with binucleated cells declining significantly.

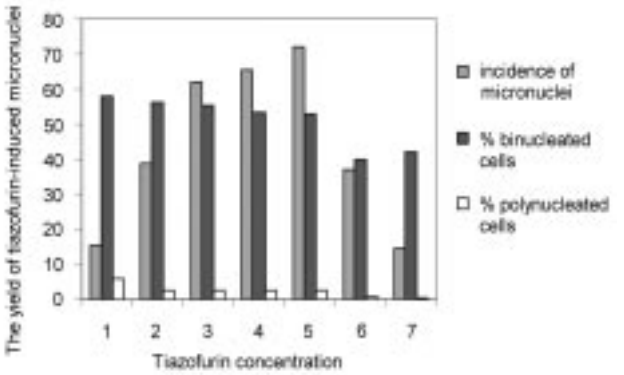

Figure 1. The incidence of micronuclei and percentages of binucleated and polinucleated cells in harvested lymphocytes treated two hours with different tiazofurin concentrations (ranged from control to 0.90μmol/ml)
Y axis: No 1 corresponds to control; No 2 corresponds to tiazofurin concentration of 0.08μmol/ml; No 3 corresponds to tiazofurin concentration of 0.11μmol/ml; No 4 corresponds to tiazofurin concentration of 0.22μmol/ml; No 5 corresponds to tiazofurin concentration of 0.42μmol/ml; No 6 corresponds to tiazofurin concentration of 0.76μmol/ml; No 7 corresponds to tiazofurin concentration of 0.90μmol/ml

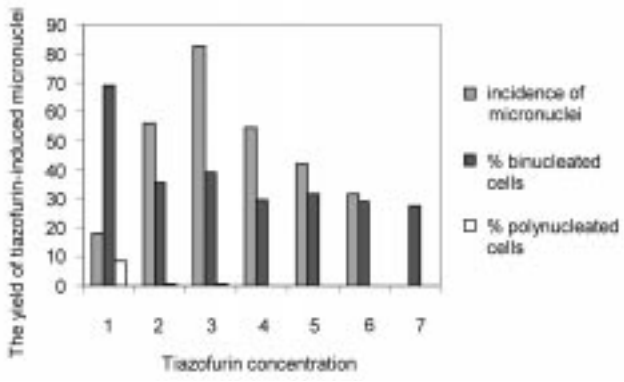

Figure 2. The incidence of micronuclei and percentages of binucleated and polinucleated cells in harvested lymphocytes treated 17 hors with different tiazofurin concentrations (ranged from control to 0.90μmol/ml)
Y-axis: No 1 corresponds to control; No 2 corresponds to 0.08μmol/ml of tiazofurin; No 3 corresponds to 0.11μmol/ml of tiazofurin; No 4 corresponds to 0.22μmol/ml of tiazofurin; No 5 corresponds to 0.42μmol/ml of tiazofurin; No 6 corresponds to 0.76μmol/ml of tiazofurin; No 7 corresponds to 0.90μmol/ml of tiazofurin

The decline in the frequency of binucleated cells was accompanied with an accumulation of mononucleated cells. Treatment of cells with the lowest concentration of tiazofurin (0.08 μmol/ml) reduced the number of first division cells by approximately 50% and prevented the second cell division as evidenced through the significantly reduced number of polynucleated cells. Higher tiazofurin concentrations altered the speed of first cell division that is evidenced through the significantly reduced number of binucleated cells accompanied with the accumulation of mononucleated ones.

Tiazofurin is a very efficient inducer of alterations in cell divisions. All tested concentrations of tiazofurin displayed antiproliferative properties after the treatment of two hours, while the clastogenic properties of tiazofurin were evidenced at the longest treatment period.

In summary our research has produced evidence for the clastogenic and antiproliferative properties of tiazofurin and demonstrated that CB micronucleus assay is a simple and rapid method that can be used to assess the cell effects of novel synthesised drugs. More knowledge about the mode of action of novel drugs on cells will contribute to the treatment of target cells and reduce the toxic effect in other tissues.

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