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Assessment of cytotoxicity for modified nucleosides and nucleotides at their effect on lymphoid millstands cells

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Analogues nitrogenous bases and nucleosides realize their cytotoxic effect by mimicking a natural endogenous nucleosides (after their phosphorylation into nucleotides). The mechanism of action can be associated either with inhibition of enzymes or with the substitution of endogenous nucleo-

sides as substrates during the synthesis of nucleic acid molecules, which leads to DNA and RNA damage and disruption of DNA methylation processes.

Often, the process of triphosphorylation of nucleoside analogues is difficult or impossible due to the high specificity of cell nucleoside- and nucleotide kinases. Nucleoside-5'-monophosphates cannot be used directly because their transport to the cell is extremely limited. In addition, they rapidly degrade to the corresponding nucleosides on the cell membrane.

These reasons arouse wide interest in the synthesis of pronucleotides, i.e. chemically modified nucleoside monophosphates and their analogues, which would have the ability to penetrate into the cell and turn into the corresponding antimetabolites as a result of chemical or enzymatic transformations.

The aim of the work is to evaluate the cytotoxicity of some modified nucleosides and nucleotides when they are exposed to lymphoid cells.

Materials and research methods. Object of study – modified nucleosides: cytarabine (1), cyclocytidine HCl (2), nelarabin (5), and nucleotides: fludarabine phosphate (H^+ – form) (3), fludarabine phosphate emoxipine salt (4),

Peripheral blood was collected in sterile heparinized tubes, diluted 1: 1 with saline, layered on the density gradient of Histopaque-1077 («Sigma », Germany) and centrifuged for 30 min at 1500 on / min at 4°C. The resulting interphase ring mononuclear peripheral blood (MIC) was collected in sterile tubes and washed twice in saline for 10 min at 1500 on / min and 4 °C. Cell culture viability of BMD was determined by flow cytometry using the Annexin A5 FITC / 7-AAD kit (Beckman Coulter, USA).

Results and conclusions. Modified nucleotides (3) and (4), and nucleoside (5) in the studied concentration range of 10^{-5}–10^{-7} M exerted a cytotoxic effect on lymphoid cells mainly due to the induction of early and late apoptosis. It should be noted that at a concentration of 10^{-5} M, these compounds also increased the specific gravity of cells that underwent necrosis (from 6.0 to 7.15%), which is extremely unfavorable at the extrapolation of these results to the human body.

Modified nucleosides (1) and (2) in the investigated range of concentrations [10^{-5} M–10^{-7} M for (2), and a concentration of 10^{-7} M for (1)] does not have a pronounced cytotoxic effect on the lymphocytes. The specific weight of viable cells in this case ranged from 95.51 to 97.15%. Cytarabine (1) at a concentration of 10^{-5}–10^{-6} M induced early cell apoptosis ($p < 0.05$) while maintaining the overall cell viability of up to 90%.