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Study of the antiviral activity of the nucleoside analogue 2-(*beta*-D-ribofuranosyl)-5-amino-1,2,4-triazine-3(2H)-one against Epstein-Barr virus

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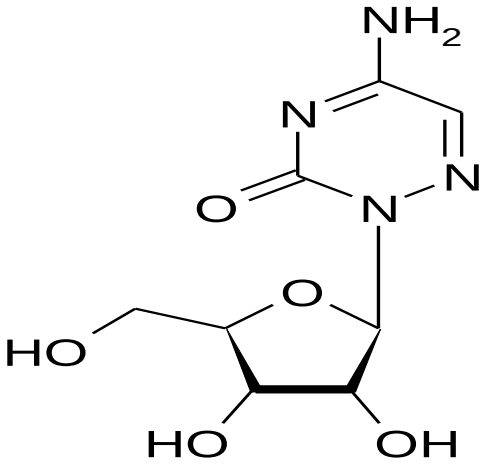
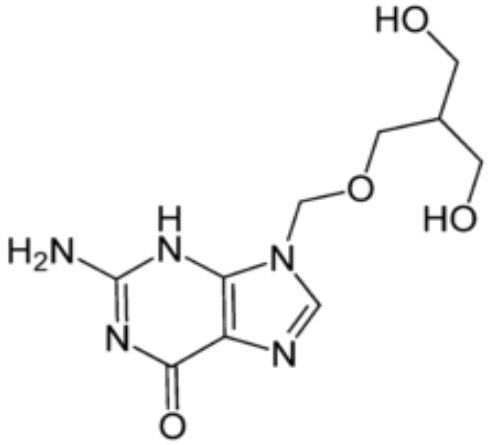


2-(*beta*-D-ribofuranosyl)-5-amino-1,2,4-triazine-3(2H)-one



Ganciclovir

IC_{50}

Acute EBV infection model

0.5 ug/ml **1.3 ug/ml**

Chronic model of EBV infection

0.76 ug/ml **< 0.5 g/ml**

Abstract:
Study of the antiviral action of the abnormal nucleoside analogue 2-(*beta*-D-ribofuranosyl)-5-amino-1,2,4-triazine-3(2H)-one was performed with Epstein-Barr virus (EBV) positive human B-cells (Raji) superinfected by EBV, as an acute infection model, and EBV-producing tamarin (*Saguinus oedipus*) B-cell line (B95-8), as a chronic infection model. The antiviral activity of the compound was determined by RT-PCR. It was shown that the IC_{50} values (concentration of compound that inhibits the accumulation of the viral genome at 50 %) for 2-(*beta*-D-ribofuranosyl-5-amino-1,2,4-triazine-3(2H)-one were 0.5 ug/ml for the acute infection of EBV infected Raji cells and 0.76 ug/ml for the model of chronic infection. The anti-EBV action of Ganciclovir (the official anti-herpetic drug) was studied for comparison. IC_{50} values for Ganciclovir were 1.3 ug/ml for the acute model and below 0.5 ug/ml for the chronic model of EBV infections. Thus abnormal nucleoside 2-(*beta*-D-ribofuranosyl)-5-amino-1,2,4-triazine-3(2H)-one is a promising anti-EBV compound deserving further research.
Keywords: Epstein-Barr virus; abnormal nucleoside; antiviral action; acute EBV infection; chronic EBV infection

Introduction

Epstein-Barr virus (EBV) or herpesvirus of 4th type is capable to infect almost all organs and systems of the host, causing latent, acute and chronic forms of infections. EBV is associated with a variety of cancers, mainly lymphoproliferative (nasopharyngeal carcinoma, Burkitt's lymphoma, 10-20% of all cancers of the stomach), and autoimmune diseases (rheumatic diseases, vasculitis, ulcerative colitis, etc.). To date, herpes infection is treated with antiviral drugs with directed effect, such as abnormal nucleosides, whose mechanism of action is associated with inhibition of viral DNA synthesis and replication of viruses by competitive inhibition of viral DNA polymerase. However, prolonged use of drugs leads to the development of drug resistance of the virus. We have investigated a new antiviral nucleoside analog 2-(*beta*-D-ribofuranosyl)-5-amino-1,2,4-triazine-3(2H)-one (RFAT) on acute and chronic forms of EBV infection.

Results and discussion

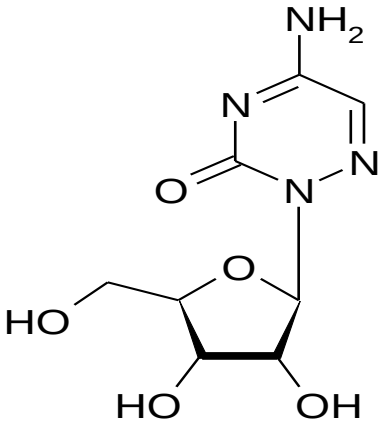
Acute EBV infection model:

Lymphoblastoid human B-cells Raji infected with EBV. Raji cell culture is a line of human cells from Burkitt's lymphoma, which contain an episomal genome of the Epstein-Barr virus (about 30 copies per cell).

Chronic model of EBV infection:

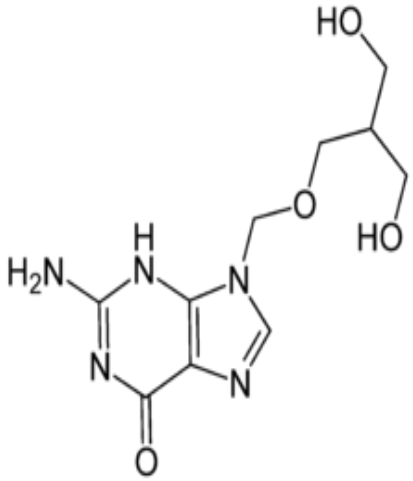
Lymphoblastoid tamarine (*Saguinus oedipus*) B-cells B95-8. B95-8 cell culture is lymphocytes of tamaryn monkey, EBV transformed and characterized by production of infectious virus.

The compounds under study:



RFAT - 2-(*beta*-D-ribofuranosyl)-5-amino-1,2,4-triazine-3(2H)-one

Mr – 244,2



Ganciclovir – 2-amino-9-{[(1,3-dihydroxypropan-2-yl)oxy]methyl}-6,9-dihydro-3H-purin-6-one (Cymevene (Roche, Switzerland)); Mr – 255,23

Antiviral activity of the test-agents was assessed by the degree of inhibition of EBV reproduction by quantitative RT- PCR method (Amplificatory qTOWER 2.2., Germany) at concentrations 0.1-16.0 ug/ml and 0.5-10.0 ug/ml respectively for Raji and B95-8 cell cultures. Reference-drug Ganciclovir was tested in similar way at concentrations 0.1-10.0 ug/ml and 0.5-10.0 ug/ml respectively for Raji and B95-8 cells.

Samples for analysis were taken after 48 h since this time point was an optimum both for the cell lines growth dynamics and for the EBV reproductive cycle. The levels of inhibition of viral DNA accumulation in infected cells treated with various concentrations of the substances were determined and compared with the infected untreated cells, in which viral DNA accumulation was taken for 100%.

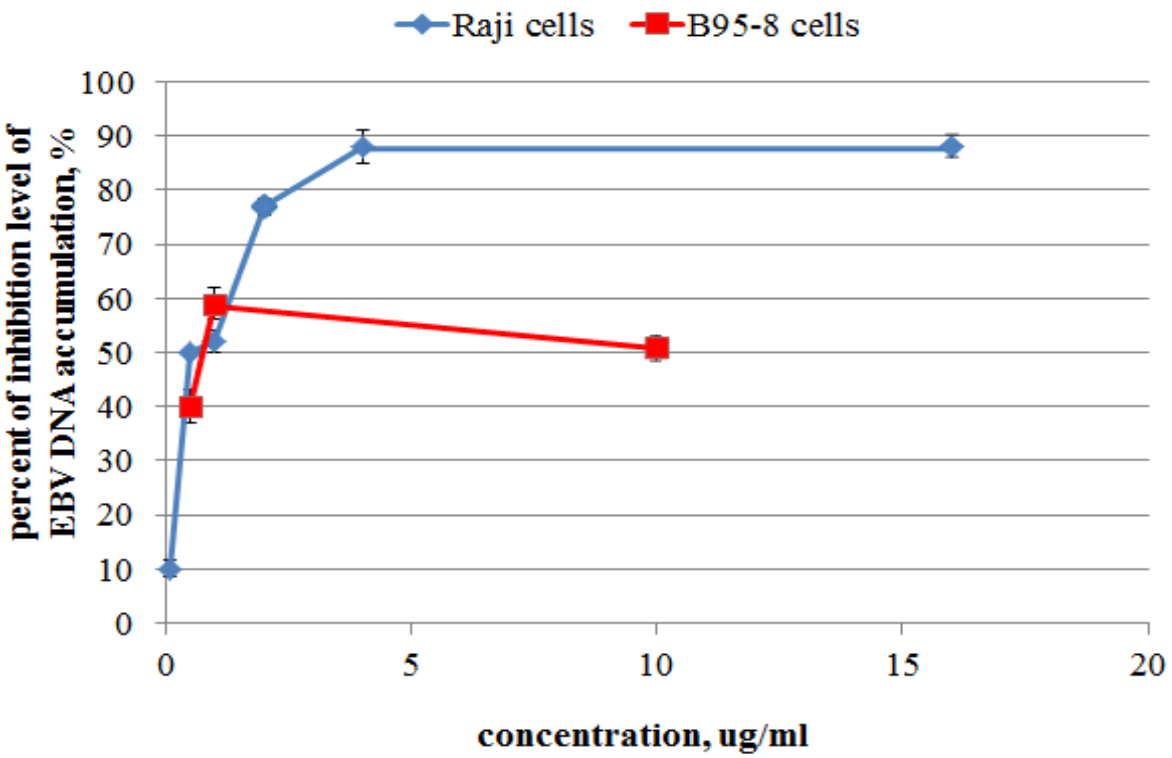


Figure 1. Antiviral effect of 2-(*beta*-D-ribofuranosyl)-5-amino-1,2,4-triazine-3(2H)-one in acute (Raji cells) and chronic (B95-8 cells) EBV infection models.

Study of an antiviral action of RFAT against EBV in infected Raji cells (Fig. 1) showed that this compound at concentration of 0.5 ug/ml is effectively inhibited the viral DNA accumulation by 50%. Maximum inhibition of virus by 88% was observed at 4.0 ug/ml and at higher concentrations the dose-response curve achieved a plateau. This indicates the achievement of the "load amount" of the compound for maximum inhibition of virus replication at 88% level. Thus, the IC_{50} for Raji cells was 0.5 ug/ml. The same calculations for B95-8 cells let to determine the IC_{50} equal to 0.76 ug/ml.

A slightly different response to increasing concentrations of the substance was observed in the model of chronic EBV infection (Fig. 1). The dose-response curve increased up to the concentration of the compound of 1.0 ug/ml achieving a threshold at this point, after which the decline was observed at increasing concentrations of the compound up to 10.0 ug/ml. Effective viral DNA inhibition at low concentrations of the compound and decrease of its effectiveness at higher concentrations could be caused by the overloading of the homeodynamic system that resulted in the counter inhibition and therefore cause neutralization of the inhibitory action [1]. Increased concentrations of the compound may lead to an activation of the cell protection systems, such as DNA repair, for example.

The anti-EBV action of Ganciclovir (the official anti-herpetic drug) was studied for comparison (Fig. 2).

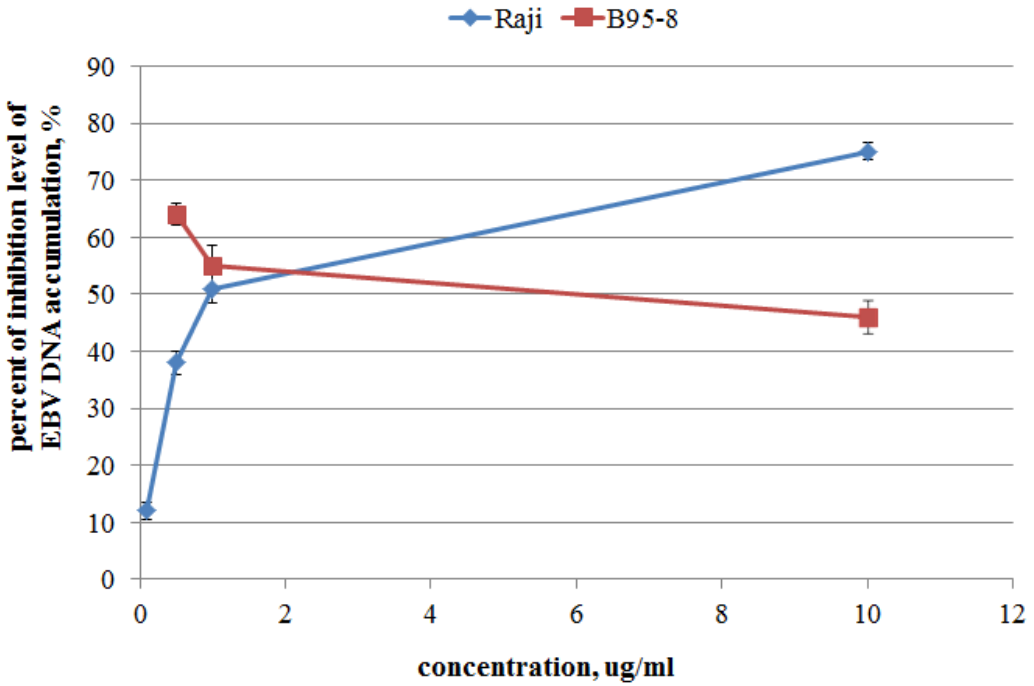


Figure 2. Antiviral effect of Ganciclovir in acute (Raji cells) and chronic (B95-8 cells) EBV infection models

The nonlinear positive dose-dependent inhibition of EBV replication with increasing concentrations of Ganciclovir was marked. Exponential curve has a slight slope and the IC_{50} of 1.3 ug/ml was determined for Raji cells.

In model of chronic EBV infection the minimal studied concentration 0.5 ug/ml of Ganciclovir led to 64% inhibition of viral DNA accumulation. At higher concentrations the level of inhibition decreased (Fig. 2). With some assumptions it can be considered as a phenomenon of hormesis: the low doses of substances caused the opposite effect to higher ones [1-3].

Thus IC_{50} value for Ganciclovir was below 0.5 ug/ml in B95-8 cell culture.

Conclusions

In the work antiviral activity of the nucleoside analogue 2-(*beta*-D-ribofuranosyl)-5-amino-1,2,4-triazine-3(2H)-one was studied by real – time quantitative PCR on EBV DNA replication in two EBV–infection models. IC_{50} were 0.5 ug/ml and 0.76 mg/ml for the test compound respectively on the acute EBV infection in infected Raji cells and chronic EBV infection in B95-8 cell culture models. Simultaneous study was carried out of the official anti-herpetic drug Ganciclovir. IC_{50} value for Ganciclovir was 1.3 ug/ml in infected cells Raji. Higher level of viral inhibition $IC_{50} < 0.5$ ug/ml was observed in B95-8 cells.

The marked differences in the values of inhibitory concentrations of the compounds in acute and chronic EBV infections can be due to the individual differences of the studied cell lines: in Raji cells an acute infection cause an active synthesis of viral nucleic acids, proteins, enzymes and other components in large quantities while in the B95-8 cells only 10% of the population chronically produce viral particles. B95-8 cells chronically produce virus and thus its support system viability and reparation adapted to viral cytolysis.

The studied antiviral compound inhibited the virus at low concentrations, which caused no detectable host cells reaction. The rise of the compound concentrations led to decrease of its inhibition efficiency, which can be associated with the counter responses of the host cells to the chemical agent. On the other hand, the target of this antiviral compound is a viral DNA polymerase and at low concentrations the compound may has an optimal solution, the availability of conformation or other physical indicators effectively bind to the target action.

References:

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