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Antiviral Activity of a Substance from Yeast RNA

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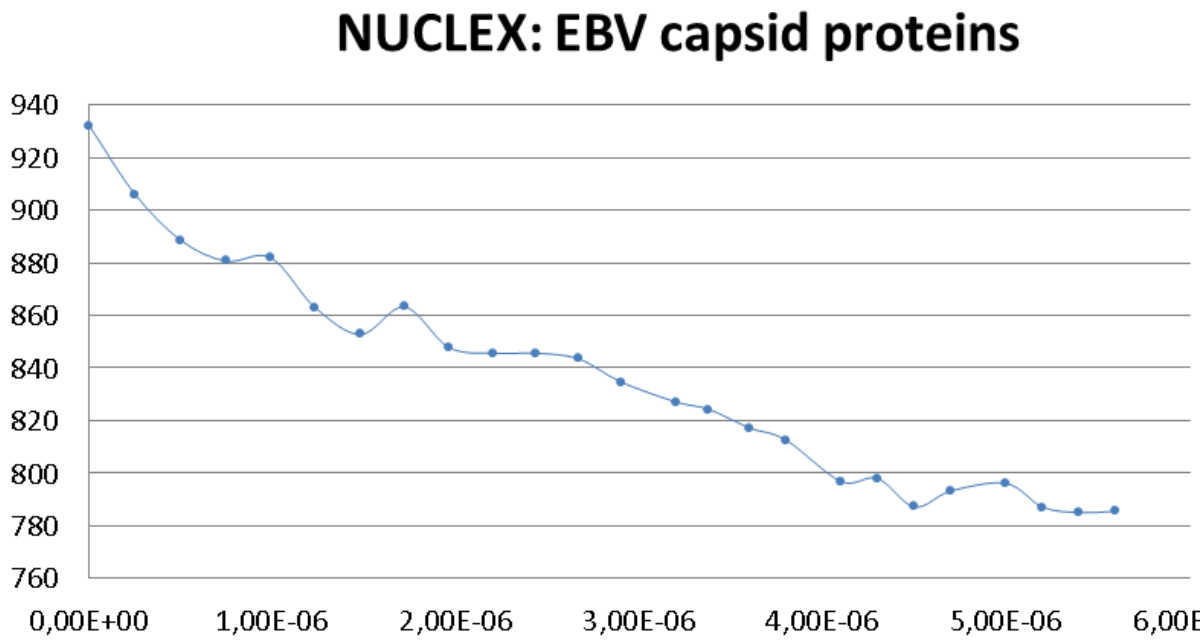
Nuclex - Oligoribonucleotides-D-Mannitol Complexes (total RNA of Saccharomyces cerevisiae with the dominant fraction of 3-8 nucleotides modified by D-Mannitol)



Our results showed that the complex of ORNs-D-mannitol binds to EBV capsid proteins and in this manner reduces its activity. These results indicate that one of mechanisms of ORNs-D-mannitol antiviral activity may be the changes of EBV proteins conformation.

Abstract:

RNA based drugs are used in clinic for treatment of infectious diseases as immunomodulators and antiviral agents. Nuclex is low molecular weight exogenous yeast RNA that consists of a pure homogeneous 25-membered oligonucleotide. The preventive and therapeutic effects of Nuclex were demonstrated against pandemic strains of influenza virus, Hepatitis C, Herpes simplex virus types 1 and 2, cytomegalovirus, and HIV. An antiviral effect of Nuclex against Epstein-Barr virus (EBV) an etiological agent of infectious mononucleosis associated with a number of lymphoproliferative and autoimmune diseases was studied in this work.

The EBV-positive human B-cells (Raji) and EBV-producing cells B95-8 were used in the study. The drug showed low toxicity and 50% inhibition of cell viability (IC_{50}) was achieved at 5 mg/ml of the drug for both cell lines. An antiviral activity of Nuclex was examined by PCR validation of the viral DNA accumulation after 48 h of treatment. An effective concentration (EC_{90}) of Nuclex that caused 90% decrease of the accumulation of viral DNA was 50 µg/ml for both cell lines. A virucidal activity of the drug was studied too, considering the ability of Nuclex to influence the conformation of the viral surface proteins. EC_{50} was achieved at 200 µg/ml of the drug after 30 min of treatment. Thus, Nuclex showed a strong antiviral activity against EBV.

Keywords: antiviral activity; Epstein-Barr virus; yeast RNA

Introduction

Inhibition of virus-cell interactions, which are required for virus replication, is a subject of study of an exciting area of research aimed at discovering novel antiviral agents. RNA based drugs are used in clinics to treat various virus-induced infections because of their immunomodulatory, anti-inflammatory and antiviral activities. Yeast-derived RNA molecules have been long used as therapeutic agents. Nuclex is one of such drugs. It contains low molecular weight exogenous yeast RNA that is a pure homogeneous 25-membered oligonucleotide. The preventive and therapeutic effects of Nuclex were demonstrated against pandemic strains of influenza virus, parainfluenza virus, Hepatitis C, Herpes simplex virus types 1 and 2, cytomegalovirus, and HIV with a help of various experimental models *in vitro* and *in vivo*. The mechanism of the antiviral action of the drug is sought to lay on its ability to change the conformation of cell regulatory proteins and viral coating proteins. An antiviral effect of Nuclex against Epstein-Barr virus (EBV) is under study in the current work.

Epstein-Barr virus (EBV) is a human *γ*-herpesvirus that infects 90% of the world population and causes latent, acute and chronic infections. Primary infection occurs in childhood or adolescence and may be asymptomatic or manifests as an acute respiratory viral infection. However, in immunocompromised persons the acute infectious mononucleosis can develop. As a result of infection it can be asymptomatic virus carrier state or latent infection, chronic recurrent EBV infection with lesions, including CNS, myocardium, kidney; erased and atypical EBV infection to the development of secondary immunodeficiency; development of cancer (nasopharyngeal carcinoma, Burkitt's lymphoma, stomach cancer) and autoimmune diseases.

Results and discussion

The study was carried with EBV-positive human B-cells (Raji cells) that latently infected with the virus, and B95-8 cells (Tamarin monkey cells), the producers of EBV. The cytotoxicity of Nuclex was determined by MTT-method for the concentration range from 312 to 10000 µg/ml. It has been shown that the drug possessed low toxicity levels for both types of lymphoblastoid cells. The inhibitory concentrations that suppress 10% of viable cells (IC_{10}) were 2000 µg/ml, while IC_{50} was 5000 µg/ml for both cell lines (Table 1). Acyclovir was used as a reference drug. It was found to be less toxic for B95-8 cells (IC_{50} value was 2364 µg/ml), while toxicity for Raji cells was the same as Nuclex (IC_{50} = 5000 µg/ml).

Results and discussion

Table 1.

***In vitro* antiviral activity of Nuclex in Lymphoblastoid cell cultures**

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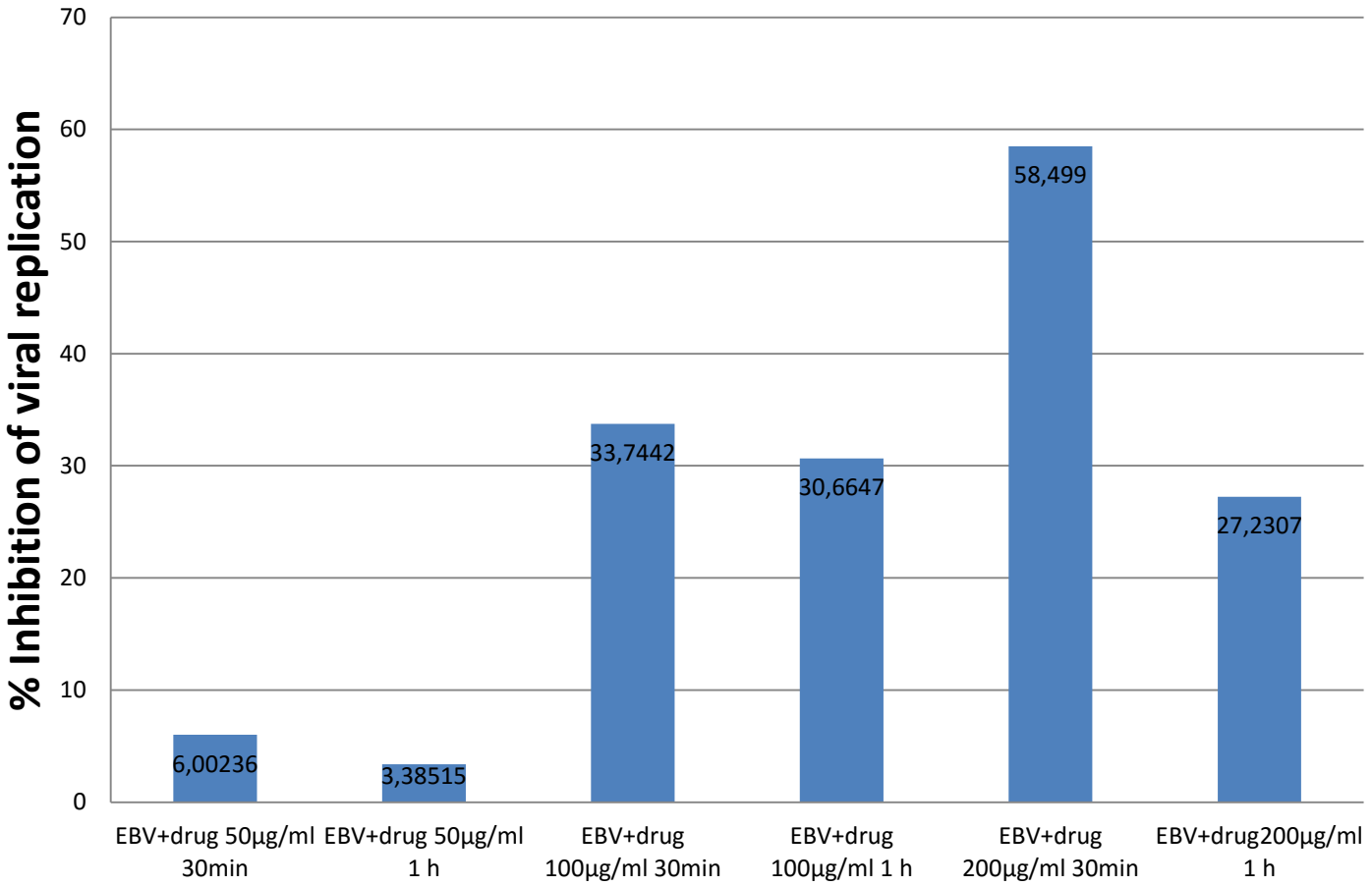
Cells/Indexes	IC_{10}, µg/ml	IC_{50}, µg/ml	EC_{90}, µg/ml	IC_{10}/ EC_{90}
Raji	2000	5000	50	40
B95-8	2500	5200	50	50

Results and discussion

An antiviral activity of Nuclex was measured by PCR after 48 h of treatment. The Raji cells infected with Epstein-Barr virus were used as a model of acute EBV infection, while the B95-8 cells, without virus induction, were used as a model of chronic infection to study *in vitro* the antiviral activity of compounds. The drug effectively inhibited viral replication at concentrations 10-500 µg/ml in both cell lines. An effective concentration of Nuclex that inhibited an accumulation of viral DNA by 90% (EC_{90}) was 50 µg/ml (Table 1). Same time the EC_{50} value of Acyclovir was 220,3 µg/ml in EBV infected Raji cells, while, in the model of chronic EBV infection, Acyclovir was able to inhibit the replication of EBV DNA only by 42% at the concentration 500 µg/ml. The ratio of maximum tolerated concentration to the maximum inhibitory dose (IC_{10}/EC_{90}) of Nuclex was 2-2.5 fold higher (Table 1) than the therapeutic index (IC_{50}/EC_{50}=23) of reference-drug Acyclovir. This indicates a high antiviral efficacy of Nuclex relatively EBV.

A virucidal activity of the drug was studied too, considering the ability of Nuclex to influence the conformation of the viral surface proteins. The accumulation of the EBV DNA was inhibited by 30% after the EBV was pretreated with the drug at concentration 100 µg/ml for 30 and 60 min and subsequently added to Raji cells and incubated for 48 h. 50% inhibition of the viral DNA accumulation was observed at higher concentration of the drug (200 µg/ml) after 30 min of exposure, while 60 min exposure resulted in 30% inhibition only.



Virucidal effect of the study drug

Conclusions

An antiviral activity of Nuclex that is a complex preparation of highly purified yeast RNA was studied. The Nuclex is a broad spectrum antiviral agent that possesses the antiviral properties of the proteins analogs of 2'-5'-oligoadenylate synthetase/endoribonuclease L system. It is active against various RNA- and DNA-containing viruses. Nuclex showed a high antiviral activity against EBV. Considering the very low EC_{90} values of the drug (50 µg/ml) and its high virucidal activity (EC_{50} = 200 µg/ml), the determination of the mechanism of action of the drug will be the next stage of our research.

Based on the preliminary results of study of binding of purified viral capsid proteins to tested drug by mass spectroscopy, we can conclude that its structure influences surface epitopes of the virus and thus inhibits cell infection process. Considering the RNA content of the Nuclex we can assume that the mechanism of its action is related to the biological functions of siRNA.