phenotype. To better characterize the activity of ODE-MPMPA replicon rebound and compound uptake studies were performed.

Methods: Huh-7.5.1 cells stably expressing the BM4-5 FEO replicon (1b) were seeded in 96 well plates (2500 cells/well) and exposed to ODE-MPMPA at the EC50 (1.5 μM) and EC90 (8 μM). After 48 h compound was removed and replaced with complete media. Luciferase expression was determined and replication levels expressed as a percentage of control wells. Cellular uptake studies were performed in Huh7 cells with 8 μM ^{14}C-labeled MPMPA and ODE-MPMPA.

Results: Replications levels at 48 h were 52% and 8% at the EC50 and EC90, respectively. At 96 h after compound removal replication levels had declined further (23% and 2%). At one week replication remained suppressed (57% and 2%). After exposure to 8 μM drug, intracellular concentrations of radiolabeled MPMPA at 2, 4 and 24 h were 43, 46 and 54 pmol/10^6 cells compared with 330, 970 and 3340 pmol/10^6 cells for ODE-MPMPA. At 2, 4 and 24 h, cellular levels of ODE-MPMPA were 8-, 21- and 62-fold higher than those observed with 8 μM MPMPA.

Conclusions: ODE-MPMPA displays potent and prolonged suppression of HCV replicon replication after a single exposure. Prolonged elevated intracellular concentrations of drug and metabolites lead to the delayed rebound on removal. High intracellular levels combined with the high fitness cost and/or low fold-change of resistant mutants contribute to the high resistance barrier. Studies to determine the intracellular concentrations of the active metabolite (MPMPApp) are underway.

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Anti-EBV Activity of Hemocyanin Isolated from Helix Lucorum

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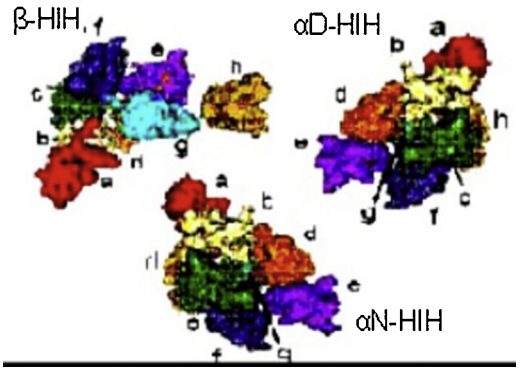
The Epstein-Barr virus (EBV) is the representative of the family of *Herpesviridae*. EBV can be the agent that causes miscellaneous lymphomas as well as nasopharyngeal carcinoma, carcinoma of parotid glands, stomach adenocarcinoma and other diseases. EBV, as other herpesviruses, affects on central and peripheral nervous system. The object of the present investigation was to study the activity of hemocyanin from *Helix lucorum* (HlH) against Epstein-Barr virus. Hemocyanin was isolated from the hemolymph of Bulgarian garden snails. In contrast with other molluscan hemocyanins, three isoforms (β-HlH, α_N-HlH and α_D-HlH) (figure) with molecular mass about 450 kDa were isolated.

Cytotoxicity and antiviral activity of the isoforms of HlH were investigated in cell culture Raji. Cytotoxic concentration (300 μg/ml) was determined using the trypan blue. Samples for analysis of antiviral activity were collected in 48 h after infecting. AntiEBV activity of the samples was determined according to the level of inhibition of EBV DNA using PCR and primers to the capsid antigen. Clear dose-response effect was observed in a concentration range from 1 to 100 μg/ml, when analysis of the preparations was carried out immediately after infecting. 50% inhibition of the level of accumulation of viral DNA was observed at the lowest concentration. Proceeding from the index of selectivity, that is 300 for hemocyanins isolated from *Helix lucorum*, it is possible to make a conclusion about their availability for the further researches as of drugs, active against an Epstein-Barr virus.



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Investigation of Anti-EBV Activity of Ganciclovir in Combination with Antiflogistics

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Contamporary approaches to the treatment of herpes infections, especially Epstein-Barr virus, include the use of etiotropic medicines, as well as the holding of sensitizing therapy. The spectrum of drugs active against EBV remains quite limited and ganciclovir and acyclovir are used in medical practice. Both drugs are nucleoside analogues and consequently affect at the stages of viral DNA synthesis. Anti-inflammatory drugs are used in combination with antiviral drugs. The question of their joint effect both at the cellular level and at the level of a microorganism remains open. The goal of this investigation was to study the antiviral activity of ganciclovir against EBV in combination with anti-inflammatory drugs *in vitro*. As an anti-inflammatory drugs indomethacin, brufen and amizon were used. Indomethacin is the derivative of indol acetic acid and one of the most active non-steroidal anti-inflammatory drugs. It is strong inhibitor of prostaglandin biosynthesis also. Ibuprofen is nonsteroidal anti-inflammatory drug that has anti-inflammatory, analgesic and moderate antipyretic effect. The essential role in its mechanism of action play inhibition of the biosynthesis of prostaglandins E and F, both at central and at peripheral level. Amizon – non-opioide analgesic with a pronounced anti-inflammatory, antipyretic, interferonogenic and immunomodulatory properties. We tried to investigate how will affect at level of anti-EBV activity of ganciclovir the adding of strong antiflogistics such as, ibuprofen, indometacin and amizon. Tested compounds were added to culture cell Raji in non-toxic concentration and various proportional ratios between ganciclovir and every antiflogistics (mM) 1:3, 1:1, 3:1. Antiviral activity was estimated by PCR according to the level of inhibition of accumulation of viral DNA. We found that ibuprofen has no antiviral activity per se and inhibit ganciclovir effect. Indometacin is not active against EBV but and does not change ganciclovir activity. We confirmed our previous data that anti-EBV activity of amizon is the same as ganciclovir one. Combined action of amizon and ganciclovir did not altered effect of ganciclovir.

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