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Antiviral potential of combinations of etiotropic antiherpetic agents including LAS-131, a novel terminase complex inhibitor, to affect the reproduction of herpes simplex virus type 1 (*Orthoherpesviridae: Simplexvirus: Simplexvirus humanalpha1*)

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Abstract

Introduction. Herpes simplex virus type 1 (HSV-1) causes various diseases in humans that can lead to disability and death. Current treatments are effective and relatively safe. However, the high prevalence of HSV, the necessity for long-term therapy associated with the development of drug resistance in the virus (mainly in immunocompromised patients) and severe side effects of second-line drugs complicate treatment. Obviously, there is a necessity to develop therapeutic agents with a new mechanism of action and new ways of influencing herpes infection to improve the effectiveness of therapy. The combined use of drugs with different mechanisms of action is one such approach.

The aim of the study is to evaluate the antiviral activity of combinations of LAS-131 ((3S)-4-[6-(purin-6-yl)aminohexanoyl]-3,4-dihydro-3-methyl-7,8-difluoro-2H-[1,4]-benzoxazine) with basic antiherpetic drugs and with two new compounds.

Materials and methods. The effect of LAS-131 combinations against HSV-1 was studied by constructing an isobologram and calculating the fractional inhibitory concentration index.

Results. LAS-131 selectively inhibits the reproduction of acyclovir-sensitive and -resistant HSV-1 variants (IC_{50} is 1.95 $\mu\text{g}/\text{mL}$, selectivity index is 63). Its target protein is the large subunit of the terminase complex (*pUL15*). When LAS-131 is used in combination with viral DNA polymerase inhibitors (acyclovir and related compounds) or with the minor-groove inhibitor 15Lys-bis-netropsin, a potentiating effect is observed, which allows decreasing the concentrations of the combined compounds by 4 times or more while maintaining antiviral activity. LAS-131 interacted additively with foscarnet, ribavirin, and α -interferon.

Conclusion. Combinations of LAS-131 with known antiviral agents have been established, providing synergistic and additive effects of interaction against HSV-1.

Keywords: *herpes simplex virus type 1; antiviral activity; combined effect; drug resistance; in vitro*

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¹ Patent RU 2644351 C1; 2018.

ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ

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Антивирусный потенциал комбинаций этиотропных противогерпетических агентов, включающих новый ингибитор терминазного комплекса LAS-131, для воздействия на репродукцию вируса простого герпеса 1-го типа (*Orthoherpesviridae: Simplexvirus: Simplexvirus humanalpha1*)

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Резюме

Введение. Вирус простого герпеса 1-го типа (ВПГ-1) является причиной различных инфекций у людей, которые могут привести к инвалидности и даже к смерти. Современные методы лечения эффективны и относительно безопасны. Однако чрезвычайно высокий уровень распространенности этого вируса, а также необходимость проведения длительной терапии, связанной с развитием лекарственной резистентности у вируса (главным образом у пациентов со сниженным иммунитетом), и тяжелые побочные эффекты препаратов второго ряда осложняют лечение. Очевидно, что существует необходимость не только в разработке терапевтических средств с новым механизмом действия, но и новых путей воздействия на герпетическую инфекцию для повышения эффективности проводимой терапии. Комбинированное использование лекарств с различным механизмом действия является одним из таких подходов.

Цель работы – оценка противовирусной активности LAS-131 ((3S)-4-[6-(пурин-6-ил)аминогексаноил]-3,4-дигидро-3-метил-7,8-дифтор-2H-[1,4]-бензоксазин) при сочетанном использовании с базовыми противогерпетическими препаратами, а также с двумя оригинальными соединениями.

Материалы и методы. Комбинированный противовирусный эффект LAS-131 исследовали на модели ВПГ-1 методами построения изоболограммы и вычисления индекса фракционной ингибирующей концентрации.

Результаты. LAS-131 высокоселективно ингибирует репродукцию вируса (ИД₅₀ составляет 1,95 мкг/мл, индекс селективности – 63) и сохраняет активность против варианта ВПГ-1, резистентного к ацикловиру. Белком-мишенью этого соединения является большая субъединица терминального комплекса (*pUL15*). При сочетанном использовании LAS-131 с ингибиторами вирусной ДНК-полимеразы (ацикловиrom и родственными соединениями) и с малобороздчатым ингибитором 15Lys-bis-нетропсином наблюдается потенцирующий эффект, что позволяет снизить концентрации комбинируемых соединений в 4 раза и более при сохранении противовирусной активности. Концентрации LAS-131, фоскарнета, рибавирина и α-интерферона при использовании в комбинации можно снизить в 2 раза, что соответствует аддитивному эффекту.

Заключение. Установлены комбинации LAS-131 с известными антивирусными агентами, обеспечивающие синергический и аддитивный эффекты взаимодействия против ВПГ-1.

Ключевые слова: вирус простого герпеса 1-го типа; антивирусная активность; комбинированный эффект; лекарственная резистентность; *in vitro*

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¹ Патент РФ № 2644351 С1; 2018.

Introduction

Herpes simplex virus type 1 (HSV-1), an extremely widespread pathogen in the human population, remains in the body throughout life and periodically reactivates, leading either to asymptomatic shedding or to overt infection. The clinical manifestations of herpes infection vary depending on the degree of immune competence of the host, ranging from mild localized forms (labial herpes) to rare but severe and life-threatening infections (blindness, herpes encephalitis, generalized infections). However, even labial or genital herpes, which is not life-threatening, is painful and can significantly reduce the quality of life of patients².

Currently, treatment of infections caused by herpes simplex virus (HSV) is based on the use of synthetic nucleoside analogues. Acyclovir (ACV) was the first drug in this class, characterized, unlike iodo-deoxyuridine, triflortimidine and adenine arabinoside, by low toxicity to the macroorganism and high selectivity of antiviral action against HSV-1, HSV type 2, and varicella-zoster virus. ACV is specifically phosphorylated by viral thymidine kinase (TK) to monophosphate, so this conversion is only possible in an infected cell. ACV monophosphate is then converted by cellular kinases into the biologically active form ACV triphosphate, which is incorporated into the growing DNA chain by viral polymerase (DNA-pol), inhibiting elongation [1, 2].

Unfortunately, ACV and related compounds have a similar mode of action and, therefore, common disadvantages, including minimal effect in acute episodes [2]. Furthermore, their widespread use may lead to the emergence of strains resistant to this group of drugs, mainly in patients with immunodeficiency conditions of various etiologies who require long-term drug therapy [3]. Since vaccines for the effective prevention and treatment of HSV infections are currently in the clinical development stage and none of them have been released on the market, the creation of new antiviral drugs, primarily with mechanisms of action not related to viral DNA polymerase, as well as the search for new ways to treat herpes infections that reduce not only the likelihood of adverse effects of etiotropic drugs but also drug resistance in the virus, has attracted considerable attention in recent decades. One area of such research is the development of combination therapy regimens that include both traditional anti-herpes drugs and new compounds. Many years of practical experience with highly effective combination antiretroviral therapy for HIV infection [4] and combination therapy for hepatitis C virus infection [5] convincingly confirm the rationality of this strategy.

We were the first to discover the activity of a number of purine and benzoxazine conjugates against HSV-1, including strains resistant to the effect of ACV [6]. LAS-131, which highly selectively inhibits HSV-1 replication (**Fig. 1**, **Table 1**), was selected for study to establish the biological target and mechanism of action of this group of compounds.

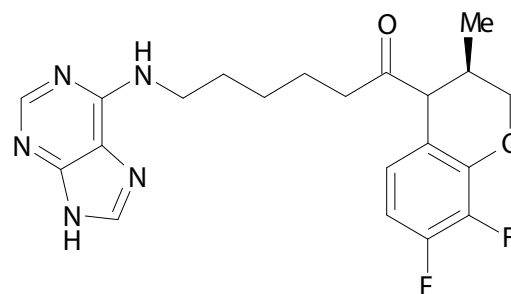


Fig. 1. LAS-131 ((3S)-4-[6-(purin-6-yl)amino]hexanoyl]-3,4-dihydro-3-methyl-7,8-difluoro-2H-[1,4]-benzoxazine).

Рис. 1. LAS-131 ((3S)-4-[6-(пурин-6-ил)аминогексаноил]-3,4-дигидро-3-метил-7,8-дифтор-2H-[1,4]-бензоксазин).

According to our proposed model of the molecular mechanism of LAS-131's antiviral action, the most likely target protein with which this compound binds is the large subunit of the HSV-1 terminase complex, pUL15. The binding of LAS-131 to pUL15 prevents the packaging of viral DNA into the capsid [7].

The aim of this study is to evaluate the combined effect of LAS-131 with known anti-herpes agents, primarily those of practical significance, *in vitro*, as well as to study the possibility of affecting the reproduction of the ACP-resistant variant of HSV-1 with combinations of LAS-131 and compounds that do not require activation of viral TK.

Materials and methods

Drugs. LAS-131 ((3S)-4-[6-(purin-6-yl)amino]hexanoyl]-3,4-dihydro-3-methyl-7,8-difluoro-2H-[1,4]-benzoxazine, C₂₀H₂₂F₂N₆O₂, Mw 416.42) was synthesized at the I.Y. Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences (Yekaterinburg) [6].

The study also used ACV, penciclovir (PCV), ganciclovir (GCV), bromovinyl deoxyuridine (BVDU), brivudine, 5-iodo-2'-deoxyuridine (IDU), adenine arabinoside (Ara-A), cidofovir (CDV), foscarnet (FOS) manufactured by Sigma-Aldrich (USA), ribavirin (RIB) manufactured by ICN Switzerland AG (Switzerland), and monosubstituted ammonium glycyrrhizinate (GLN) manufactured by Khimfarm OAO, Kazakhstan). The structural formulas and rational names of the above compounds are given in the table in the appendix³. α -Interferon (α -IFN, Reaferon-EC – lyophilized α -IFN preparation for injection (Vector-Medica CJSC, Koltsovo, Novosibirsk Region). Phosphate ACV (F-ACV) was generously provided by Yu.S. Skoblov (Institute of Bioorganic Chemistry named after Academicians M.M. Shemyakin and Yu.A. Ovchinnikov, Russian Academy of Sciences), the structure is given in [8] and the **table in the appendix**³. 15-Lys-bis-netropin (15Lys-bis-Nt) was synthesized at the V.A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, and kindly provided by G.V. Gursky (the structure

²Herpes simplex virus. Fact sheet. December 11, 2024. Available at: <https://www.who.int/ru/news-room/fact-sheets/detail/herpes-simplex-virus>

³<https://doi.org/10.36233/0507-4088-336-1>

Table 1. Antiherpetic activity of some compounds in the HSV-1 model with different drug sensitivities in Vero E6 cell culture (multiplicity of infection 0.1 PFU/cell)

Таблица 1. Противогерпетическая активность ряда соединений на модели ВПГ-1 с различной лекарственной чувствительностью в культуре клеток Vero E6 (множественность инфицирования 0,1 БОЕ/кл)

Compound Соединение	CC ₅₀ , µg/mL ЦД ₅₀ , мкг/мл	IC ₅₀ , µg/mL ИД ₅₀ , мкг/мл	IC ₉₅ , µg/mL ИД ₉₅ , мкг/мл	SI ИС
LAS-131	122.22 ± 6.48	1.95 ^{1,2}	7.8 ^{1,2}	63 ^{1,2}
ACV / АЦВ	> 250	0.39 ¹ / > 400 ²	1.56 ¹ / > 400 ²	> 1026 ¹ / 0 ²
GCV / ГЦВ	> 100	0.56 ¹ / > 250 ²	11.25 ¹ / > 250 ²	> 179 ¹ / 0 ²
PCV / ПЦВ	> 100	1.95 ¹ / > 100 ²	7.8 ¹ / > 100 ²	> 51 ¹ / 0 ²
BVDU / БВДУ	> 250	0.096 ¹ / > 24.5 ²	0.19 ¹ / > 24.5 ²	> 4167 ¹ / 0 ²
IDU / ИДУ	> 250	3.9 ¹ / 62.5 ²	31.25 ¹ / 125 ²	> 64 ¹ / > 4 ²
Нр-ACV / Ф-АЦВ	> 1000	15.6 ¹ / 31.2 ²	31.25 ¹ / 62.5 ²	> 64 ¹ / > 32 ²
FOS / ФОС	> 62.5	15.6 ¹ / 31.2 ²	62.5 ^{1,2}	> 4 ¹ / > 2 ²
Ага-А / Ара-А	80.0 ± 4.12	15.6 ¹ / 31.2 ²	62.5 ^{1,2}	5.12 ¹ / 2.56 ²
CDV / ЦДВ	> 62.5	3.9 ^{1,2}	15.6 ^{1,2}	> 52 ^{1,2}
RIB / РИБ	> 500	250 ^{1,2}	500 ^{1,2}	> 2 ^{1,2}
15Lys-bis-NT	173.12 ± 4.37	3.9 ¹ / 3.9 ²	15.6 ^{1,2}	44.39 ^{1,2}
GLN / ГЛН	> 1000	500 ^{1,2}	> 1000 ^{1,2}	> 2 ^{1,2}
α-IFN / α-ИФН /	> 1000	250 ^{1,2}	> 1000 ^{1,2}	> 4 ^{1,2}

Note. CC₅₀ is the concentration of the compound in the presence of which 50% of the cells survive; «>» – effect is not achieved; IC₅₀ and IC₉₅ are the concentrations of the compounds in the presence of which the development of the virus-induced cytopathic effect (CPE) is inhibited at 95–100% CPE in the virus control after 48 h of incubation. The selectivity index (SI) was calculated as the ratio of CC₅₀ to IC₅₀¹ – antiviral activity of the compounds was studied on the model of the reference strain of the HSV-1/L₂ virus; ² – antiviral activity of the compounds was studied on the model of the TK-negative variant of the HSV-1/L₂/ACV^R virus, deeply resistant to ACV. The results of two independent experiments are presented.

Примечание. ЦД₅₀ – концентрация соединения, в присутствии которой выживает 50% клеток; «>» – эффект не достигается; ИД₅₀ и ИД₉₅ – концентрации соединений, ингибирующие развитие вирусиндуцированного ЦПЭ при 95–100% ЦПЭ в контроле вируса через 48 ч инкубации. Величину индекса селективности (ИС) вычисляли как отношение ЦД₅₀ к ИД₅₀¹ – исследование противовирусной активности соединений проводили на модели эталонного штамма вируса ВПГ-1/L₂; ² – исследование противовирусной активности соединений проводили на модели ТК-негативного варианта вируса ВПГ-1/L₂/АЦВ^Р, глубоко резистентного к АЦВ. Приведены результаты двух независимых опытов.

of the compound is given in [9] and in the table in the appendix³).

Viruses and cells. HSV-1, strain L₂ (HSV-1/L₂) was obtained from the State Virus Collection of the N.F. Gamaleya Federal Research Center for Epidemiology and Microbiology of the Russian Ministry of Health (its division D.I. Ivanovsky Institute of Virology). A deeply resistant to ACV variant of HSV-1 (HSV-1/L₂/ACV^R) was obtained by serial passage in the presence of increasing concentrations of ACV, followed by cloning, and was previously characterized phenotypically and genotypically as TK-negative [8, 10, 11].

The Vero E6 cell culture was kindly provided by Professor A.M. Butenko (N.F. Gamaleya Research Center for Epidemiology and Microbiology of the Russian Ministry of Health). The Igla medium with the addition of 5% (v/v) fetal calf serum (PanEco, Moscow) was used as the growth medium for cell cultivation.

The cytotoxic and antiviral properties of the compounds were evaluated using a microassay in 96-well plates (Linbro, Flow Laboratories, UK) with a formed cell monolayer. Serial dilutions of the preparations or their combinations were prepared with a 2-fold dilution and added to the support medium (to determine cytotoxic properties) or virus-containing fluid in a dilution ensuring a multiplicity of infection of 0.1 PFU/cell (to determine antiviral activity) and incubated at 37 °C in a 5% CO₂ atmosphere.

The cytotoxicity of the compounds and their combinations was assessed after 72 hours of incubation using the

trypan blue exclusion method [12], as described previously [6]. The number of dead (stained) cells was counted and the CC₅₀ value (the compound concentration at which cell survival is 50%) was determined.

The antiviral activity of compounds and their combinations was evaluated *in vitro* using the method of inhibiting the development of virus-induced cytopathic effect (CPE) [6, 8]. The IC₅₀ and IC₉₅ concentrations of compounds or their combinations, providing 50% and complete inhibition of viral CPE development, were determined after 48 hours, when 100% CPE developed in the control infected cultures.

The nature of the interaction of binary combinations of compounds was evaluated using the S. Loewe method by constructing isobolograms and calculating the fractional inhibitory concentration (FIC) index according to the formula:

$$FIC = \frac{ID_{50} \text{ of compound A combination}}{ID_{50} \text{ of compound A}} + \frac{ID_{50} \text{ of compound B combination}}{ID_{50} \text{ of compound B}} \quad [8, 13].$$

Synergistic interaction (potentiation of effects) corresponded to an FIC ≤ 0.5, and sub-synergistic interaction corresponded to 0.5 < FIC < 1.0. At FIC = 1.0, the observed nature of the interaction between compounds was defined as additive (simple summation).

Results

This study investigated the combined effect of LAS-131 with etiotropic antiherpes drugs against HSV-1/L₂. In addition, it was of interest to determine whether LAS-131

could enhance the antiviral activity of drugs that do not require viral TK activation against the TK-negative HSV-1/L₂/ACV^R strain. When studying the combined effect of the compound, non-cytotoxic concentrations were used. The IC₅₀ values of the tested compounds when used individually and in combination, necessary for calculating the FIC, as well as the FIC values of the combinations, characterizing the interaction of the compounds when used in combination, are given in **Tables 1 and 2**.

LAS-131, when used individually, exhibits pronounced selective antiviral activity with a selectivity index (SI) of 63 in models of the reference strain HSV-1/L₂ and the ACV-resistant strain HSV-1/L₂/ACV^R (Table 1).

When analyzing the results obtained using the FIC calculation method on the model of the reference strain HSV-1/L₂ (data in Tables 1 and 2), it was found that the interaction of LAS-131 with nucleoside analogues was determined as synergy, since the effect of each of the compounds in combination increased by more than the

sum of their responses when used individually, which allows the dose of individual agents to be reduced by more than 2 times while maintaining the antiviral effect corresponding to IC₅₀. Thus, the combined use of LAS-131 with ACV and GCV causes a sub-synergistic effect (0.5 < FIC < 1). Combinations of LAS-131 with PCV, BVDU, IDU, Ara-A, and F-ACV showed a synergistic effect (FIC ≤ 0.5), i.e., the concentrations of the compounds when used in combination were reduced by at least four times compared to the IC₅₀ of each drug when used individually.

According to the method of constructing isobolograms, if the effects of two compounds are summed (additive interaction effect), the isobologram lies on a straight line connecting the IC₅₀ value of one combined compound, plotted on the y-axis, with the IC₅₀ value of the other compound in this pair, plotted on the x-axis. The position of the isobologram below this theoretical line (concave shape of the isobologram) indicates synergy, while the position above (convex

Table 2. Efficacy of LAS-131 in combination with known antiherpetic agents in the model of HSV-1 in Vero E6 cell culture (multiplicity of infection 0.1 PFU/cell)

Таблица 2. Эффективность LAS-131 в комбинации с известными противогерпетическими агентами на модели ВПГ-1 в культуре клеток Vero E6 (множественность инфицирования 0,1 БОЕ/кл)

Compound Соединение	CC ₅₀ , µg/mL ЦД ₅₀ , мкг/мл	IC ₅₀ , µg/mL ИД ₅₀ , мкг/мл	IC ₉₅ , µg/mL ИД ₉₅ , мкг/мл	FIC	Effect Эффект
LAS-131 + ACV LAS-131 + АЦВ	> 15.6 + 0.05	0.39 + 0.15 ¹	3.9 + 0.05 ¹	0.59 ¹	Subsynergistic Субсинергический
LAS-131 + GCV LAS-131 + ГЦВ	> 6.25 + 0.35	0.78 + 0.18 ¹	0.78 + 0.35 ¹	0.72 ¹	Subsynergistic Субсинергический
LAS-131 + PCV LAS-131 + ПЦВ	> 3.9 + 3.9	0.49 + 0.49 ¹	0.24 + 0.98 ¹	0.50 ¹	Synergistic Синергический
LAS-131 + BVDU LAS-131 + БВДУ	> 15.6 + 0.018	0.49 + 0.023 ¹	1.95 + 0.094 ¹	0.50 ¹	Synergistic Синергический
LAS-131 + IDU LAS-131 + ИДУ	> 3.9 + 1.95	0.49 + 0.49 ¹ / 0.49 + 12.5 ²	0.98 + 1.95 ¹ / 0.98 + 31.25 ²	0.38 ¹ / 0.45 ²	Synergistic Синергический
LAS-131 + FOS LAS-131 + ФОС	> 62.5 + 7.8	0.98 + 7.8 ¹ / 0.97 + 15.6 ²	1.56 + 12.5 ^{1,2} / 3.12 + 62.5	1.0 ^{1,2}	Additive Аддитивный
LAS-131 + Ara-A LAS-131 + АраА	> 3.9 + 62.5	0.49 + 3.9 ¹ / 0.75 + 3.9 ²	0.49 + 15.6 ¹ / 0.75 + 31.25 ²	0.5 ¹ / 0.51 ²	Synergistic / subsynergistic Синергический / субсинергический
LAS-131 + CDV LAS-131 + ЦДВ	> 15.6 + 31.25	0.25 + 1.95 ¹ / 0.49 + 1.50 ²	0.25 + 7.8 ¹ / 0.75 + 1.95 ²	0.63 ¹ / 0.64 ²	Synergistic Синергический
LAS-131 + Hp-ACV LAS-131 + Ф-АЦВ	> 7.8 + 62.5	0.49 + 3.9 ¹ / 0.49 + 15.6 ²	0.97 + 3.9 ¹ / 1.95 + 31.25 ²	0.50 ¹ / 0.75 ²	Synergistic / subsynergistic Синергический / субсинергический
LAS-131 + 15Lys-bis-Nt LAS-131 + 15Lys-bis-Nt	> 15.6 + 31.25	0.49 + 0.78 ¹ / 0.49 + 0.78	0.39 + 3.12 ¹ / 0.78 + 1.95	0.45 ^{1,2}	Synergistic Синергический
LAS-131 + RIB LAS-131 + РИБ	> 3.9 + 500	0.97 + 125 ^{1,2}	3.9 + 500 ^{1,2}	1.0 ^{1,2}	Additive Аддитивный
LAS-131 + GLN LAS-131 + ГЛН	> 3.9 + 1000	0.49 + 125 ^{1,2}	0.97 + 250 ^{1,2}	0.50 ^{1,2}	Synergistic Синергический
LAS-131 + α-IFN LAS-131 + α-ИФН	> 7.8 + 1000	0.97 + 125 ^{1,2}	1.95 + 250 ^{1,2}	1.0 ^{1,2}	Additive Аддитивный

Note. See note to Table 1. ¹ – the study of the antiviral activity of the combinations of compounds was carried out on the model of the reference strain of the HSV-1/L₂ virus; ² – the study of the antiviral activity of the combinations of compounds was carried out on the model of the TK-negative variant of the HSV-1/L₂/ACV^R virus, highly resistant to ACV. FIC – fractional inhibitory concentration. The results of two independent experiments are presented.

Примечание. См. примечание к таблице 1. ¹ – исследование противовирусной активности комбинаций соединений проводили на модели эталонного штамма вируса ВПГ-1/L₂; ² – исследование противовирусной активности комбинаций соединений проводили на модели ТК-негативного варианта вируса ВПГ-1/L₂/АЦВ^Р, глубокорезистентного к АЦВ. FIC – фракционная ингибирующая концентрация. Приведены результаты двух независимых опытов.

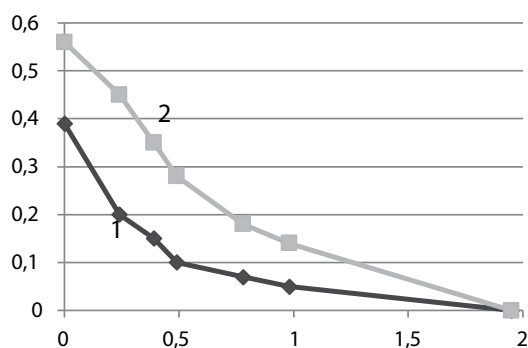


Fig. 2. Isobolograms for combinations of LAS-131 with ACV (curve 1) and GCV (curve 2).

The X-axis shows the IC_{50} values of LAS-131 for individual use and in combination ($\mu\text{g/mL}$), the Y-axis shows the IC_{50} values of ACV and GCV for individual use and in combination ($\mu\text{g/mL}$).

Рис. 2. Изоболограммы для комбинаций LAS-131 с АЦВ (кривая 1) и ГЦВ (кривая 2).

По оси абсцисс отложены величины ID_{50} LAS-131 при индивидуальном использовании и в комбинации (мкг/мл), по оси ординат – величины ID_{50} АЦВ и ГЦВ при индивидуальном использовании и в комбинации (мкг/мл).

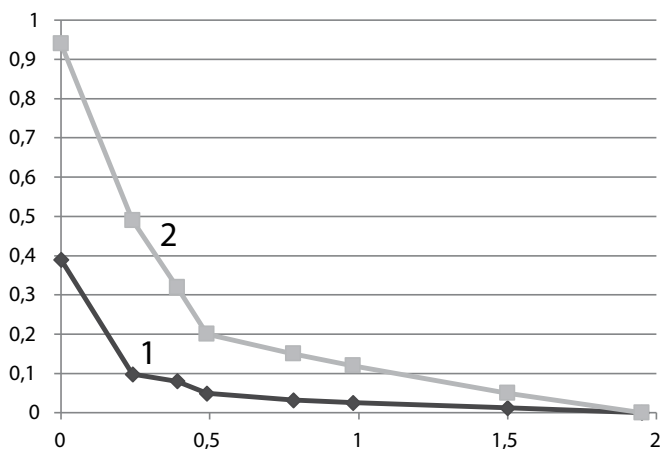


Fig. 3. Isobolograms for combinations of LAS-131 with PCV (curve 1), CDV (curve 2) and 15Lys-bis-Nt (curve 3).

The X-axis shows the IC_{50} values of LAS-131 for individual use and in combination ($\mu\text{g/mL}$), the Y-axis shows the IC_{50} values of PCV, CDV and 15Lys-bis-Nt for individual use and in combination ($\mu\text{g/mL}$).

Рис. 3. Изоболограммы для комбинаций LAS-131 с ПЦВ (кривая 1), ЦДВ (кривая 2) и 15Lys-bis-Nt (кривая 3).

По оси абсцисс отложены величины ID_{50} LAS-131 при индивидуальном использовании и в комбинации (мкг/мл), по оси ординат – величины ID_{50} ПЦВ, ЦДВ и 15Lys-bis-Nt при индивидуальном использовании и в комбинации (мкг/мл).

shape) indicates antagonism between the drugs [13].

The nature of the isobolograms constructed for combinations of LAS-131 with nucleoside analogues (ACV, PCV, GCV, BVDU, IDU, Ara-A, F-ACV) indicates the synergistic nature of the interaction between these pairs of compounds (Figs. 2–5).

ACV, PCV, GCV and BVDU were not included in the next stage of research because they are not active against TK-negative strains of HSV-1, as they require phosphorylation involving viral TK to exhibit biological activity.

When LAS-131 was used in combination with known

antiherpetic agents whose mechanism of action does not depend on herpes TK activity, the following results were obtained in the HSV-1/ L_2 /ACV^R model (Tables 1 and 2).

The synergistic effect of LAS-131 and Ara-A was reduced to a sub-synergistic effect. The combined use of LAS-131 and CDV, a nucleotide (nucleoside phosphonate analogue) phosphorylated only by cellular kinases to biologically active diphosphate, also resulted in a sub-synergistic interaction in both models.

The study included two more compounds, F-ACV and 15Lys-bis-Nt, which effectively suppress the reproduction of both sensitive and ACV-resistant variants of HSV-1. Their mechanisms of action have been studied and described in detail by us in a number of publications [14–16]. When combined with LAS-131, a marked increase in the antiviral activity of 15Lys-bis-Nt or F-ACV against HSV-1/ L_2 was observed, with FIC values of 0.45 and 0.50, respectively. However, unlike 15Lys-bis-Nt, the potentiating effect of LAS-131 on the activity of F-ACV against the TK-negative strain was less pronounced and corresponded to a sub-synergistic effect.

The interaction of LAS-131 with GLN also led to potentiation of the effects of these compounds in both the HSV-1/ L_2 model and the HSV-1/ L_2 /ACV^R model. An additive type of interaction was observed with the combined use of LAS-131 and FOS, which is also independent of viral TK activity (FIC = 1), in the HSV-1 model, regardless of the virus's sensitivity to ACV (Tables 1 and 2). Similar results were obtained for combinations of LAS-131 with RIB or with α -IFN.

Discussion

It is known that the HSV-1 terminase complex consists of six identical heterotrimeric subunits pUL15/pUL28/pUL33 and has a torus shape with an internal channel, the surface of which functions as a biomotor (recognition, binding, and transport of viral double-stranded DNA (dsDNA) into the capsid). pUL15 plays a key role in the complex's functions. It is this protein in each subunit that forms the nuclease and ATPase catalytic centers and the working surface of the biomotor [17]. pUL15 is also involved in the interaction of the terminase/DNA complex with the capsid portal complex [18]. The multifunctionality of pUL15 potentially makes it a promising target for small molecule inhibitors. However, the analysis of the scientific literature showed that LAS-131 is apparently the first anti-herpes agent whose target protein is pUL15 HSV-1. Our previous data indicate that LAS-131 binds to the site formed by pUL15, disrupting the packaging of viral dsDNA into the capsid [7].

It should be noted that the drug Letermovir Previmis (Previmis, MerckSharp & Dohme B.V., USA), introduced into clinical practice in the USA in 2017 and in Russia in 2022, is intended for the prevention of diseases caused by cytomegalovirus (CMV) after bone marrow transplantation in adults and is the first drug to inhibit the function of the CMV terminase complex. However, the spectrum of action of letermovir is limited to CMV; the drug does not affect the reproduction of other herpesviruses and targets the pUL56 protein, which is homolo-

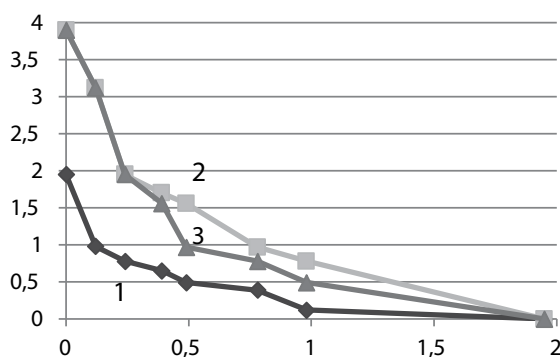


Fig. 4. Isobolograms for combinations of LAS-131 with IDU (curve 1) and BVDU (curve 2).

The X-axis shows the IC_{50} values of LAS-131 for individual use and in combination ($\mu\text{g/mL}$), the Y-axis shows the IC_{50} values of IDU ($\mu\text{g}/10^{-1} \text{ mL}$) and BVDU ($\mu\text{g}/10 \text{ mL}$) for individual use and in combination.

Рис. 4. Изоболограммы для комбинаций LAS-131 с ИДУ (кривая 1) и БВДУ (кривая 2).

По оси абсцисс отложены величины $ИД_{50}$ LAS-131 при индивидуальном использовании и в комбинации (мкг/мл), по оси ординат – величины $ИД_{50}$ ИДУ (мкг/ 10^{-1} мл) и БВДУ (мкг/10 мл) при индивидуальном использовании и в комбинации.

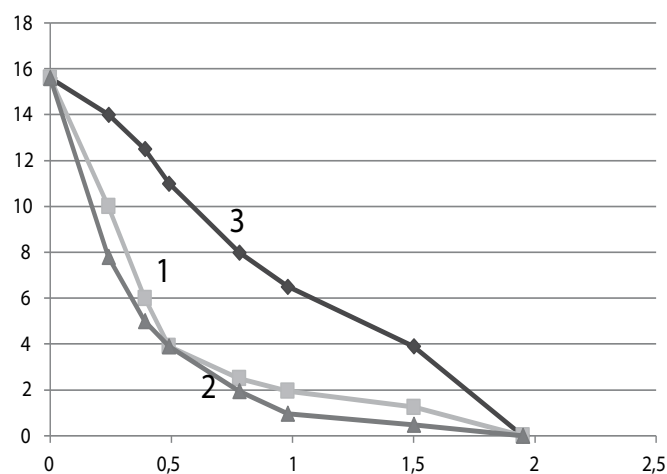


Fig. 5. Isobolograms for combinations of LAS-131 with Ara-A (curve 1), F-ACV (curve 2) and FOS (curve 3).

The X-axis shows the IC_{50} values of LAS-131 for individual use and in combination, the Y-axis shows the IC_{50} values of Ara-A, F-ACV and FOS for individual use and in combination ($\mu\text{g/mL}$).

Рис. 5. Изоболограммы для комбинаций LAS-131 с Ара-А (кривая 1), Ф-АЦВ (кривая 2) и ФОС (кривая 3).

По оси абсцисс отложены величины $ИД_{50}$ LAS-131 при индивидуальном использовании и в комбинации, по оси ординат – величины $ИД_{50}$ Ара-А, Ф-АЦВ и ФОС при индивидуальном использовании и в комбинации (мкг/мл).

gous to the small subunit of the HSV terminase complex pUL28, rather than the large pUL15 [19] (pUL15 is homologous to pUL89).

As shown in models of various viral infections, including herpesvirus, the synergy of antiviral agents results from the drugs acting on different biomolecules or on different target sites of the same protein. Since the target protein of all first- and second-line antiherpes drugs is the catalytic subunit of DNA pol, their use in combination with a compound that inhibits the activity of the

herpesvirus terminase complex allowed us to suggest the possibility of their potentiating interaction, despite the fact that when letermovir was combined with GCV, CDV, FOS and ACV (herpes DNA pol inhibitors) only additive effects against CMV were observed [20].

We investigated combinations of LAS-131 primarily with commercially available antiherpes drugs (see table in the appendix) – nucleoside analogues: ACV (Zovirax), GCV (Cytoven), PCV (Denavir, Vectavir), BVDU (Brivudine, Zovirax, Zerpex), Ara-A (VIRA-A, ARASENA-A), IDU (Oftan, Keretsid, Ridonex, Idoxuridine, etc.), an analogue of the acyclic nucleotide dCMP CDV (Cidofovir, Cidnavir) and an analogue of pyrophosphate FOS (Foscavir), as well as GLN (Epigen Intim), broad-spectrum antiviral drugs RIB (Ribavirin-Vertex, Ribavirin Canon, Ribavirin-Lipint, Ribavirin-SZ, Ribavirin-FPO, Ribamidil, etc.) and α -IFN (Altevir, Binnoferon alpha, Viferon, Interferal, Liferon, Reaferon-EC, Reaferon-EC-Lipint, Intron A, Realiron, etc.), as well as two original compounds, F-ACV and 15-Lys-bis-Nt.

The mechanism of action of all the above drugs is not related to pUL15 HSV. As described above, ACV, PCV, and GCV, as well as BVDU, IDU, and Ara-A, after activation with the formation of corresponding triphosphates and CDV with the formation of diphosphate, compete with natural deoxyribonucleoside triphosphates and selectively inhibit viral DNA pol. It should be noted that the first stage of IDU phosphorylation is catalyzed by both viral and cellular TK, and Ara-A triphosphorylation does not require the participation of viral enzymes at any stage [1, 21], thus preserving their activity against TK-negative virus strains that are resistant to TK-dependent modified nucleosides (ACV, PCV, GCV, BVDU). CDV, as an analogue of nucleoside phosphonate, bypasses the first stage of viral TK phosphorylation and is phosphorylated to a diphosphorylated active metabolite by cellular enzymes [1, 22].

Thus, all compounds discussed here, being inhibitors of herpes DNA pol, cause the cessation of concatenated viral DNA synthesis, which is a substrate for the terminase complex. It is quite possible that under conditions of combined use, the decrease in the concentration of modified nucleosides and, as a result, the decrease in the effectiveness of DNA synthesis inhibition is offset by the inhibition of LAS-131 packaging of dsDNA into the capsid and may lead to enhanced inhibition of virus reproduction.

F-ACV, an ACV derivative, selectively inhibits HSV-1 reproduction, including ACV-resistant strains that are mutant in the TK gene, which can be explained by the possibility of F-ACV conversion directly to ACV monophosphate, bypassing the stage of conversion to ACV, with subsequent formation of ACV triphosphate. Thus, F-ACV activation is likely to occur via an alternative pathway that does not depend on viral TK activity [1, 14]. Since the activated form of both F-ACV and ACV is ACV triphosphate, it is logical to assume that these compounds will interact with LAS-131 in combination in a similar manner. Indeed, the inhibitory effect of both compounds on the reproduction of the reference strain HSV-1/L₂ was potentiated in the presence of LAS-131 and was

characterized by similar FIC values.

As expected, IDU, Ara-A, F-ACV, and CDV, which are independent of HSV-1 TK activity, synergistically inhibit the replication of both wild-type HSV-1 and the ACV-resistant TK-negative variant HSV-1/L₂/ACV^R when combined with LAS-131.

The potentiating effect of LAS-131 on the antiviral activity of modified nucleosides is confirmed by both methods of assessing the nature of the interaction of compounds in combination—by calculating the FIC and by constructing isobolograms. For example, as shown on the isobolograms, the use of ACV and PCV in combination with LAS-131 significantly reduces the IC₅₀ of these compounds to concentrations that are one and two orders of magnitude lower than the maximum concentrations (C_{max}) in plasma after oral administration of valacyclovir and famciclovir (pro-drugs of ACV and PCV) at the recommended doses – 0.05–0.2 and 0.14–0.35 µg/ml versus C_{max} 6.1 [23] and 33.4 µg/ml [24], respectively. Given the neurotropism of HSV, it is important to emphasize that C_{max} for example, ACV in cerebrospinal fluid after oral administration of 1000 mg of valacyclovir after 2 and 8 hours is only 0.56 and 0.52 µg/ml [23], which is very close to the IC₅₀ value of ACV *in vitro*. It is evident that the combined use of ACV in combination has the potential to increase the effectiveness of therapy for herpes infections of the CNS.

FOS, which belongs to another class of broad-spectrum antiherpes drugs targeting herpes DNA polymerase, is a pyrophosphate analogue that exists in an active form, binds directly to the active site of DNA polymerase, and does not require further modification to exhibit antiviral activity. Since FOS does not require phosphorylation by viral TK, most HSV strains resistant to ACV are sensitive to FOS [1, 25]. It is the only second-line drug officially recommended for the treatment of cutaneous and mucosal herpesvirus infections that do not respond to ACV therapy. The low selectivity of FOS can lead to the development of severe neurological or cardiovascular disorders, acute nephrotoxicity, etc.

Despite fundamental differences in the mechanisms of action of FOS and LAS-131, and the same target protein pUL30 for FOS as for the triphosphates ACV, PCV, GCV, BVDU, IDU, Ara-A, or the diphosphate CDV, the combined use of FOS and LAS-131 provided only an additive effect. Nevertheless, even when the effects of the compounds are combined, the possibility of reducing their concentrations without losing their antiviral effect may be crucial for reducing the likelihood of side effects, as well as for inhibiting the reproduction of virus variants with low-level drug resistance to one of them. Thus, peak plasma concentrations of FOS (C_{max}) after intravenous administration at doses recommended for clinical use average 113 µg/ml [26], which is significantly higher than the IC₅₀ against HSV-1 (7.8–31.25 µg/mL). However, with a decrease in plasma concentrations of compounds over time (C_{min} 21.88 µg/mL) [26] or with the development of low-level resistance of the virus to FOS, when the IC₅₀ of the compound when used individually will be close to or even slightly exceed the C_{max} value, the combined use of FOS will reduce the IC₅₀ value to a level com-

parable to the plasma levels of the drug.

The mechanism of action of 15-Lys-bis-Nt is not related to the inhibition of DNA polymerase activity and does not depend on TK. When 15-Lys-bis-Nt binds to extended clusters of AT pairs in the narrow groove of DNA at ori, the function of the viral initiator protein pUL9 is blocked, which in turn leads to disruption of the processes of initiation of replication/transcription of the viral genome [15, 16]. As we reported earlier, 15-Lys-bis-Nt effectively potentiates the activity against HSV-1 of all modified nucleosides of practical importance (ACV, GCV, BVDU, Ara-A, synergistic effect) and pyrophosphate analogues (FOS, phosphonoacetic acid, sub-synergistic effect) [9]. When 15-Lys-bis-Nt is combined with LAS-131, a synergistic effect of inhibiting HSV/L₂ reproduction is also observed.

The molecular mechanism of GLN antiviral action is not fully understood. It has been shown that the compound exhibits both virucidal (inactivates extracellular HSV-1) and virustatic (irreversibly disrupts the synthesis of viral glycoproteins in the range of active non-toxic concentrations *in vitro*) activity against HSV [27, 28]. Inhibition of HSV-1 replication using a combination of LAS-131 and GLN in *in vitro* assays demonstrated an obvious synergistic effect, which indirectly confirms the difference in the mechanisms of action of the combined compounds in this viral model.

RIB is easily phosphorylated intracellularly by adenosine kinase to mono-, di-, and triphosphate metabolites. In the form of triphosphate, RIB inhibits the cellular enzymes inosine monophosphate dehydrogenase and guanylyl transferase, which leads to a deficiency of intracellular GTP and, as a result, suppression of viral nucleic acid and protein synthesis⁴.

α-IFN has a complex effect on viral infections, including antiviral, immunostimulatory, and antiproliferative actions. α-IFN does not have a direct antiviral effect. Suppression of viral reproduction is due to the expression of interferon-stimulated genes (ISG) and the synthesis of cellular proteins, including 2'-5'-oligoadenylate synthetase/RNase L, cAMP-dependent protein kinase, tetherin, ISG15, and others that block the penetration of viral DNA into the nucleus, ensuring the suppression of transcription, genome replication, translation and transport of the viral capsid, assembly and release of viral particles [29]. Since HSV suppresses the synthesis of α-IFN by immune cells [29], it is advisable to include α-IFN or its inducers in the composition of combined anti-herpes therapy. The Ministry of Health of the Russian Federation has approved the inclusion of drugs containing IFN-α2b as an adjunct to acyclic nucleosides in clinical guidelines for the treatment of HSV infections [30].

When LAS-131 was combined with RIB or α-IFN, only a cumulative effect on HSV-1 reproduction was observed, but it did not depend on the activity of the virus TK. Therefore, combinations of this kind may be useful in cases where the virus develops drug resistance to mod-

⁴Ribavirin-Vertex. Available at: https://www.vidal.ru/drugs/ribavirin_9015

ified nucleoside-based drugs.

Conclusion

Thus, it has been established that the combined effect of LAS-131 with modified nucleoside drugs and a number of compounds with a different antiviral mechanism provides a significant enhancement of antagonistic activity against HSV-1 *in vitro*.

Given that the development of HSV resistance to traditional antiherpes drugs renders them clinically useless, since the IC_{50} values of each of them when used individually exceed the C_{max} values of the drug in plasma, it is important to explore the potential for increasing the efficacy of these drugs by selecting suitable binary combinations with LAS-131 that have a synergistic effect, since the IC_{50} of individual drugs under such conditions can be reduced to the C_{max} level.

The results presented in this article are consistent with our conclusion that LAS-131 has a mechanism of action and a biological target that are different from those of modified nucleosides. LAS-131 and modified nucleosides, by directly interacting with various biological targets (viral proteins pUL15 and pUL30), potentiate the effect on the functions of vital terminase and replicative complexes in combination, providing significantly more effective inhibition of virus reproduction.

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