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ВЫЯВЛЕНИЕ ВОЗБУДИТЕЛЯ ВИРУСНОГО ЗАБОЛЕВАНИЯ У РАСТЕНИЙ БУЗИНЫ ЧЕРНОЙ

Впервые в Украине выявлено вирусное заболевание растений бузины черной (Sambucus nigra L.). Исследована симптоматика болезни и морфология вируса. Базируясь на данных научной литературы, проведен скрининг вирусов, которые могут поражать бузину в Украине. Антигенов этих вирусов в растениях бузины с симптомами вирусного заболевания не выявлено.

Ключевые слова: бузина, Sambucus spp., вирусные болезни.

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ANTIVIRAL AND IMMUNOSTIMULATORY POTENTIAL OF FLUORINE CONTAINING TRIAZOLES

The problem of finding effective antiviral drugs caused high morbidity and wide spread of viral infections. The purpose of this study was to investigate of antyherpetic activity fluorinated nucleoside G8 and G9 compounds (2-N-substituted-4-tosyl-5-polyfluoroalkyl-1,2,3-triazole) in in vivo models and determine their immunomodulatory potential. Shown significant inhibition of virus reproduction under the influence of the compounds at concentrations of 0.4 and 0.5 mg/kg, which was more effective of acyclovir. Protection ratio amounted to 80%. Increasing level of IFN-γ and IL-2 in serum of animals, indicated available immunomodulatory effect fluorinated nucleoside compounds. Our studies indicated that there is antiherpetic, immunomodulatory activity of fluorine containing triazole and there is need to in-depth study of the mechanisms of this process.

Ключовые слова: HSV-1, fluorinated nucleoside, antiherpetic activity.

Introduction. Herpes simplex virus type 1 (HSV-1) is member of the *Alphaherpesvirinae* subfamily within the *Herpesviridae* virus family [1]. HSV-1 is a common infection in developed countries where rates of seropositivity usually exceed 50%. In both humans and experimental animals, primary infection of skin or mucosa results in the local replication of virus, infection of sensory nerve ending, and spread via retrograde axonal transport to the ganglia of the peripheral nervous system (PNS) where a productive infection of neurons ensues. Although infectious virus is eventually cleared, a latent infection is established in neurons of the PNS ganglia [1,2]. In humans, HSV-1 is a common cause of sporadic viral encephalitis with mortality rates reaching 20-30% despite treatment [2]. Also the virus plays an important role in human infectious pathology, causing diseases such as keratoconjunctivitis, stomato gingivitis, congenital herpes and others [2].

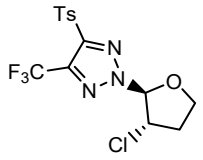
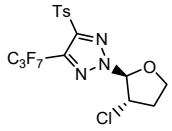
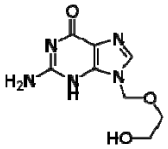
The problem of finding effective antiviral drugs caused high morbidity and wide spread of viral infections accompanied by the development of protracted and chronic forms of severe consequences. In clinical practice for treating these diseases most frequently use nucleosides, modified in heterocyclic, phosphate or carbohydrate fragment of the molecule. Today discovered many anti-herpetic drugs. However, acyclovir and other acyclic nucleosides in it is purpose and mechanism of action inhibit only those herpesvirus actively replicate, so the virus will prevent a latent state, is one of the problems of treatment of HSV-1. Another issue that complicates treatment herpesvirus is the development of viral resistance to abnormal nucleosides. There are many compounds are promising system *in vitro*, but only a few remain active *in vivo*.

In response to a viral infection in the body is activation of cytokines that modulate the overall immune response. In this regard, one of the methods of treatment of viral infections is the use of various drugs – interferon inducers that stimulate the production of interferon in the body, providing thus strengthening antiviral response [3, 4].Interferon-gamma (IFN-γ) is a cytokine that plays physiologically important roles in promoting innate and adaptive immune responses. The absence of IFN-γ production or cellular responsiveness in humans and experimental animals significantly predisposes the host to viral infection, a result that validates the physiologic importance of this cytokine in preventing infectious disease [4]. Recently, an additional role for IFN-γ in preventing development of primary and transplanted tumors has been identified. Focusing on the data implicating IFN gamma as a critical immune system component that regulates antiviral immune response is important question for research [4, 6, 7]. Interleukin (IL)-4 and IL-2 are lymphokines synthesized primarily by activated T helper lymphocytes, and both are important regulators for development of T helper subsets (Th1-like vs. Th2-like) [8, 9]. Th1 cells are involved in cellular immunity (delayed type hypersensitivity and cellular cytotoxicity) and produce IL-2, tumor necrosis factor (TNF)-β, and IFN-γ. Th2 cells are involved in humoral (antibody-mediated) immunity and produce IL-4, IL-5 and IL-10 [10]. Il-4 is an important regulator of isotype switching, inducing IgE production in B lymphocytes and can exhibit anti-inflammatory effects [10, 11, 12]. Il-2 is important for *invitro* growth of cytotoxic T cell (CTL) lines and can enhance NK cell and B cell responses [13, 14]. The IFN-γ production is the most rapid reaction in response to a virus infecting cells, as determined immunomodulatory potential nucleoside compounds at the level of IFN-γ and two pro- and anti-inflammatory cytokine IL-2 and IL-4 [15].

The purpose of this study was to investigate of antyherpetic activity fluorinated nucleoside G8 and G9 compounds (2-N-substituted-4-tosyl-5-polyfluoroalkyl-1,2,3-triazole) in *in vivo* models and determine their immunomodulatory potential.

Materials and methods. Herpes simplex virus type 1 (HSV-1, strain US1), obtained from the Institute of antiviral chemotherapy, The Center for Clinical and Theoretical Medicine (Germany). The compounds under study were G8 and G9 (they are the 2-N-substituted-4-tosyl-5-polyftoralkyl-1,2,3-triazoles). They were provided by the Institute of Organic Chemistry of Ukraine. The substance of acyclovir was used as a reference compound. Their structural formulas are given on table 1.

***Table* 1. Structure of studied compounds**

		
G8	G9	Acyclovir

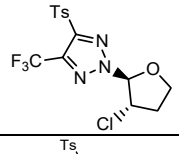
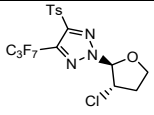
Animals. Inbread mice (3–4 weeks old) were obtained from vivarium of D.K. Zabolotny institute of microbiology and virology NAS of Ukraine. Animals were maintained under protocols approved by the Institutional Animal Use and Care Committee. Mice were inoculated with HSV by intracerebral inoculationwith 1,5-10^3 PFU HSV-1, which is a 50% lethal dose for mice. Acyclovir (ACV) at 0,1 μg/kg of body weight and G8 and G9 at 1 μg/kg of body weight as a control were administered by intraperitoneal injection.

Cytokines. Levels of cytokines were determined in blood serum and by isolating splenocytes using the "Pro immuno" protocol for preparation of murine splenocyte (BD Biosciences). The level of IFN- γ, IL-2 and IL-4 was investigated. The levels of cytokines were detected by using "Mouse INF-γ ELISA kit", "Mouse IL-2 ELISA kit", "Mouse IL-4 ELISA kit" (Thermo Scientific, USA).

Statistical analysis. Protective parameters and levels of cytokines were analyzed by Microsoft Excel. Results were considered statistically significant at $p < 0.05$.

Results and discussion. Previously at the system *in vitro* was determined cytotoxicity level and antiviral activity of the compounds. Cytotoxic concentration (CC_{50}), which was 887 and 990, effective concentration (EC_{50}) 50 and 7,6 μg/ml, was shown, respectively (table 2). Selective index of compounds G8 and G9 is 18 and 130.

***Table* 2. Cytotoxicity and antiviral activity of fluorinated nucleoside in *in vitro* system**

Code	The structural formula	Mol. mass of compounds	The cytotoxicity concentration (CC_{50}), μg/ml	The effective concentration (EC_{50}) μg/ml	IS
G8		395.78	887	50	18
G9		495.81	990	7,6	130

In vivo studies conducted fluorinated compounds on white outbred mice weighing 16-18 gram. The paper had 12 groups of 10 mice each. Animals were injected 30 ml intracerebral of virus, LD_{50} which was 1.5 * 10^3 PFU. The compounds were administered intraperitoneally at 200 ml, 3 concentrations, G8 – 40, 100 and 500 μg/ml, and G9 for 50, 100 and 500 μg/ml. As a reviewer of the drug was used acyclovir in concentrations of 10 μg/ml.

The dynamics of animal deaths were recorded daily for 21 days. In the control group, virus death of the animals took place on 4, 6, 10 and 14 days.

After analyzing the results, were identified 50% of the death of animals in the control group of HSV-1. In version 8 compounds at concentrations of 500 and 100 μg/ml recorded 10% and 20% of animals deaths (table 2).

***Table* 2. Analysis of animal deaths in the experimental group**

Groups of experimental animals	The dose, ml	Amount of mice	Animals death		Protection factor	Effectiveness Index
			Amount	%		
Controlof HSV-1	0,2	10	5	50	-	-
ControlG8, 100μg/ml	0,2	10	0	0	-	-
G8, 40μg/ml	0,2	10	0	0	-	-
G8, 100μg/ml	0,2	10	2	20	2,5	60
G8, 500μg/ml	0,2	10	1	10	-	-
ControlG9, 100μg/ml	0,2	10	0	0	-	-
G9, 50μg/ml	0,2	10	0	0	-	-
G9, 100μg/ml	0,2	10	1	10	5	80
G9, 500μg/ml	0,2	10	0	0	-	
Acyclovir, 10μg/ml	0,2	10	0	0		

The percentage of deaths of animals in the group G9 at a concentration of 100 μg/ml indicates high efficiency protection compound. Based on experimental data was determined protection ratio and the index of efficiency of the studied compounds. Effectiveness index amounted to 60% and 80% for G8 and G9 compouds, respectively. Our studies indicated that there is antiherpetic activity of fluorine containing triazole and there is need to in-depth study of the mechanisms of this process.

Previous studies had shown triazole derivatives of antiviral properties, but the impact of these compounds on the launch of major cytokine synthesis is still unknown. Therefore, was conducted a comparative study of production of

proinflammatory cytokines, activators of cellular immunity: IL-2 and IFN-γ and their antagonist IL-4.

The level of IFN and IL-2 was investigated in the blood serum of animals 14 days. In all experimental groups observed a significant increase in the level of IFN compared with the control virus. By adding the compound G9 indicators of interferon were increased with increasing concentration (fig.1).

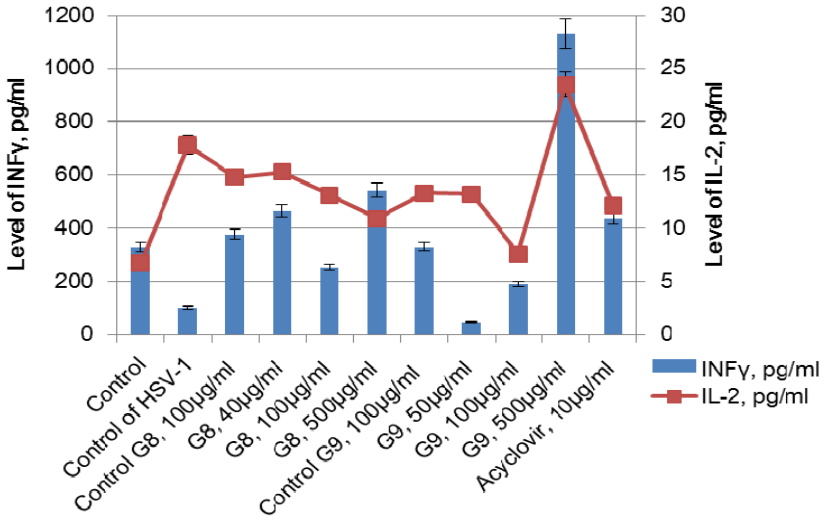


Fig. 1. The levels of interferon γ and interleukin-2 in the blood serum of experimental animals were determined

Interferon gamma suppresses viral replication in cells, my immunomodulatory properties. High levels INF compounds in samples from G8 and G9, may indicate immunostimulatory properties of the compound.

In the study of experimental data on levels of IL-2 was set pretty low. In the control group, HSV-1 levels of IL-2 was 17,8 pg/ml, while in other groups (G8 /1-3, G9/1-2, acyclovir) index were lower than control.Such data can be explained by one of the functions of IL-2 is to stimulate immune cells such as cytotoxic lymphocytes (for example, fast action in the early days of infection). Since the samples were selected on day 14, the level of IL-2 decreased in the groups of compounds. As a control virus observed high levels of IL-2, indicating that the active development of viral infection of inflammation (fig. 2).

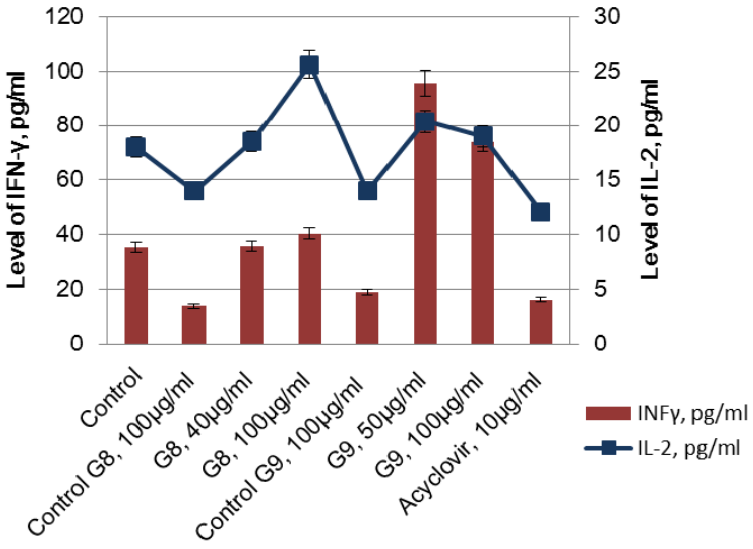


Fig. 2. Studying the levels of interferon γ and interleukin 2 secreted by isolated splenocytes

It was also determined activity of interferon producing by isolated splenocytes of mice under the influence of the studied compounds *in vivo*. Compared with controls, the compound G9 caused increased production INFγ, indicating that the interferon-inducing potential.

In the study of IL-2 secreted isolated splenocytes observed a significant increase in both compounds (G8, G9), as the level of IL-2 significantly higher than the control.

The data point to a slight activation of IL-4 isolated splenocytes, but this activation was not significant compared to the control. However, when examined serum of infected and control animals was detected slightly lower rates of IL-4 (fig. 3).

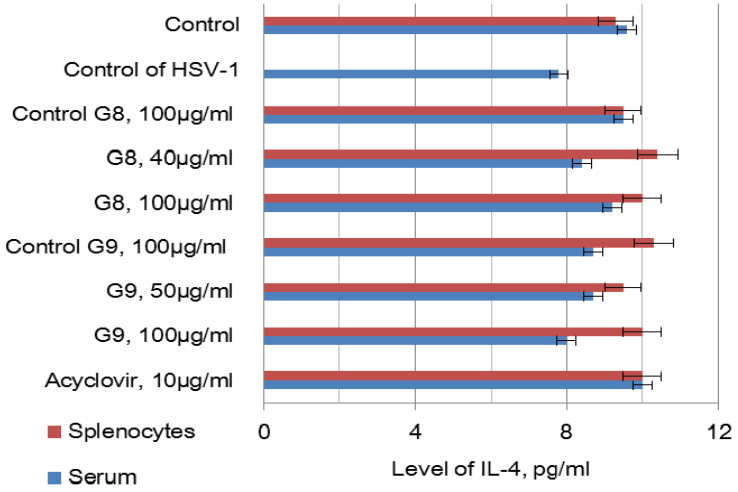


Fig. 3. Determining the level of interleukin-4 in the blood serum of animals and secreted by isolated splenocytes.

Thus, the compounds do not activate the production of IL-4. In turn, this cytokine enhances the proliferation and differentiation of B-lymphocytes, that contributes to the development of humoral immune response. Thus, the effect of these compounds is not directed at the development of humoral immune response.

In general, control of infection against viruses is linked to the induction of a Th1 response, while protection against extracellular pathogens correlates with a Th2 response [13]. IL-2 is a cytokine that exhibits an impressive number of different functions largely dictated by the biological context in which it operates. It is pivotal for cellular activation, important for primary T-cell responses and essential for secondary T-cell responses. Although, IL-2 specifically promotes T-cell activation and proliferation of only those cells that have been stimulated by cognate antigenic interaction, downregulation of T-cell responses occurs nonspecifically by facilitating a separate population T cell [10]. IL-4 induces the expression of class II major histocompatibility complex (MHC) molecules on macrophages and dendritic cells. IL-4 is a well-documented mediator of Th2 cell commitment, and induces Ig class switching to the Th2-associated isotypes IgG and IgE. However, IL-4 can exhibit anti-inflammatory effects, including suppression of macrophage function such as IL-1 and TNF production [12].

Also, the IFN-γ antiviral defense mechanism that occurs very early during the course of infection interferes both with the early steps of virus invasion and replication, and with the control of persistent infection. IFN- γ has immunomodulatory effects on T cells, macrophages, NK and B cells [5].

Analyzed data of the levels of cytokines indicate that significant immunostimulatory potential of the investigated compounds were determined. It is shown that the G8 and G9 affect at IFN-γ and IL-2, ie on the cellular immunity. Investigated that the compounds did not affect IL-4, ie on the humoral immunity. Our studies include compounds G8 and G9 to a relatively perspective antiviralsHSV-1 with immunomodulatory potential and can be used in further research.

Conclusions. The research activity anti-herpetic fluorinated nucleoside compounds in model *in vivo* were established. The models of HSV-1 herpes meningoencephalitis stimulated mice show antiviral activity of the compounds in minimally investigated concentrations of 0,4 and 0,5 mg/kg, they significantly inhibited the reproduction of the virus. Showing raising INFγ in the blood serum of animals when administered the compounds HSV-1 infected mice, which causes additional antiviral protection of animals. Increasing level of IFN-γ and IL-2 in serum of animals, indicated available immunomodulatory effect fluorinated nucleoside compounds. The results suggest the presence antiherpetic, immunomodulatory activity of fluorine containing triazole and the need for in-depth study of the mechanisms of this process.

References

1. Kollias C. Animal models of herpes simplex virus immunity and pathogenesis. J.Neurovirol. 2015;21:8-23.
2. Kastrukoff L. The effect of mouse strain on herpes simplex virus type 1 infection of the central nervous system. Herpesviridae.2012;3:1-14.
3. Young H.A.Role of interferon-ɣ in immune cell regulation./Young HA, Hardy KJ.// Journal of Leukocyte biology. 1996;58:373-8.
4. Ikeda H., Old L. The role of INF gamma in protection against tumor development and cancer immunoediting./ Ikeda H, Old L, Schreiber R. // Cytokine Growth Factor Rev. 2002;13(2):95-109.
5. Ghiasi H.Infection of BALB/c mice with a herpes simplex type 1 recombinant virus expressing IFN-ɣ driven by the LAT promoter./ Ghiasi H, Osorio Y, Hedvat Y, Perng G-C, Nesburn AB, Wechsler SL// Virology. 2002;45:144-54.
6. Schroder K.Interferon-ɣ: an overview of signals, mechanisms and functions./ Schroder K, Hertzog PJ, Ravasi T, Hume DA. // Journal of Leukocyte biology. 200;75:163-76.
7. Cantin E. Role of gamma interferon in control of herpes simplex virus type 1 reactivation./ Cantin E, Tanamachi B, Openshaw H. // Journal of Virology. 1999;73(4):3418-32.
8. Ghiasi H. Overexpression of interleukin-2 by a recombinant herpes simplex virus type 1 attenuates pathogenicity and enhances antiviral immunity. / Ghiasi H, Osorio Y, Hedvat Y, Perng G-C, Nesburn AB, Wechsler SL.// Journal of Virology. 2002;58:9069-78.
9. Geng X. Interleukin-2 and autoimmune disease occurrence and therapy./Geng X, Zhang R, Yang G, Jiang W, Xu C.// Europian review for medical and pharmacological sciences. 2012;16:1462-67.
10. Bachmann M. Interleukin 2: from immunostimulation to immunoregulation and back again./Bachmann M, Oxenius A.// EMBO report. 2007;8(12):1142-48.
11. Luzina I. Regulation of inflammation by interleukin-4: a review of 'alternatives'./ Luzina I, Keegan AD, Heller NM, Rook GW, Shea-Donohue T, Atamas SP. // Journal of Leukocyte biology. 2012;92:1-8.
12. Ohmura K. Interleukin-4 can be a key positive regulator of inflammatory anthritis / Ohmura K, Nguyen LT, Lochsley M, Mathis D, Benoist C.// Arthritis and Rheumatism. 2005;52:1866-75.
13. Ghiasi H.The role of interleukin(IL)-2 and IL-4 in herpes virus type 1 ocular replication and eye disease / Ghiasi H, Cai S, Slanina S, Perng G-C, Nesburn AB, Wechsler SL.// Journal of infectious diseases. 1999;179:1086-93.
14. Mott KR. Role of interleukin-2 and herpes simplex virus 1 in central nervous system demyelination in mice / Mott KR, Zandian M, Allen SJ, Ghiasi H.// Journal of Virology. 2013;87:12102-109.
15. Conventry B.J. The 20-th anniversary of interleukin-2 therapy: bimodal role explaining longstanding random induction of complete clinical responses./Conventry BJ, Ashdown ML.// Cancer Management and Research. 2012;4:215-21.

References (Scopus)

1. *Kollias C.* Animal models of herpes simplex virus immunity and pathogenesis. J.Neurovirol. 2015;21:8-23.
2. *Kastrukoff L.* The effect of mouse strain on herpes simplex virus type 1 infection of the central nervous system. Herpesviridae.2012;3:1-14.

3. *Young HA, Hardy KJ.* Role of interferon-ɣ in immune cell regulation. Journal of Leukocyte biology. 1996;58:373-8.

4. *Ikeda H, Old L, Schreiber R,* The role of INF gamma in protection against tumor development and cancer immunoediting. Cytokine Growth Factor Rev. 2002;13(2):95-109.

5. *Ghiasi H, Osorio Y, Hedvat Y, Perng G-C, Nesburn AB, Wechsler SL.* Infection of BALB/c mice with a herpes simplex type 1 recombinant virus expressing IFN-ɣ driven by the LAT promoter. Virology. 2002;45:144-54.

6. *Schroder K, Hertzog PJ, Ravasi T, Hume DA.* Interferon-ɣ: an overview of signals, mechanisms and functions. Journal of Leukocyte biology. 200;75:163-76.

7. *Cantin E, Tanamachi B, Openshaw H.* Role of gamma interferon in control of herpes simplex virus type 1 reactivation. Journal of Virology. 1999;73(4):3418-32.

8. *Ghiasi H, Osorio Y, Hedvat Y, Perng G-C, Nesburn AB, Wechsler SL.* Overexpression of interleukin-2 by a recombinant herpes simplex virus type 1 attenuates pathogenicity and enhances antiviral immunity. Journal of Virology. 2002;58:9069-78.

9. *Geng X, Zhang R, Yang G, Jiang W, Xu C.* Interleukin-2 and autoimmune disease occurrence and therapy. Europian review for medical and pharmacological sciences. 2012;16:1462-67.

10. *Bachmann M, Oxenius A.* Interleukin 2: from immunostimulation to immunoregulation and back again. EMBO report. 2007;8(12):1142-48.

11. *Luzina I, Keegan AD, Heller NM, Rook GW, Shea-Donohue T, Atamas SP.* Regulation of inflammation by interleukin-4: a review of 'alternatives'. Journal of Leukocyte biology. 2012;92:1-8.

12. *Ohmura K, Nguyen LT, Lochsley M, Mathis D, Benoist C.* Interleukin-4 can be a key positive regulator of inflammatory anthritis // Arthritis and Rheumatism. 2005;52:1866-75.

13. *Ghiasi H, Cai S, Slanina S, Perng G-C, Nesburn AB, Wechsler SL.* The role of interleukin(IL)-2 and IL-4 in herpes virus type 1 ocular replication and eye disease // Journal of infectious diseases. 1999;179:1086-93.

14. *Mott KR, Zandian M, Allen SJ, Ghiasi H.* Role of interleukin-2 and herpes simplex virus 1 in central nervous system demyelination in mice // Journal of Virology. 2013;87:12102-109.

15. *Conventry BJ, Ashdown ML.* The20th anniversary of interleukin-2 therapy: bimodal role explaining longstanding random induction of complete clinical responses. Cancer Management and Research. 2012;4:215-21.

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АНТИВІРУСНИЙ ТА ІМУНОСТИМУЛЮЮЧИЙ ПОТЕНЦІАЛ ФТОРВМІСНИХ ТРИАЗОЛІВ

Проблема пошуку ефективних противірусних препаратів зумовлена високою захворюваністю і широким розповсюдження вірусних інфекцій. Метою представленої роботи було дослідити антигерпетичну активність фторованих нуклеозидних сполук G8 та G9 (2-N-заміщені-4-тозил-5-поліфторалкіл-1,2,3-триазоли) на моделі invivo тавизначити їх імуномодулюючий потенціал.Показано значне інгібування репродукції вірусу під дією досліджуваних сполук в концентраціях 0,4 та 0,5 мг/кг, що було в рази ефективніше дії ацикловіру. Коефіцієнт захисту становив 80%. Встановлено збільшення рівня ІНФγ та ІЛ-2 в сироватці крові, що вказує на наявний імуномодулюючий ефект фторованих нуклеозидних сполук. Проведені дослідження дозволяють стверджувати про наявність антигерпетичної, імностимулюючої дії фторвмістних триазолів та необхідність поглибленого вивчення механізмів даного процесу.

Ключові слова: HSV-1, фторовані нуклеозидні сполуки, антигерпетична активність.

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АНТИВИРУСНЫЙ И ИММУНОСТИМУЛИРУЮЩЕЕ ПОТЕНЦИАЛ ФТОРСОДЕРЖАЩИХ ТРИАЗОЛОВ

Проблема поиска эффективных противовирусных препаратов обусловлена высокой заболеваемостью и широким распространениэм вирусных инфекций. Целью представленной работы было исследовать антигерпетические активности фторированных нуклеозидных соединений G8 и G9 (2-N-замещены-4-тозил-5-полифторалкил-1,2,3-триазола) на модели invivo и определить их иммуномодулирующий потенциал. Показано значительное ингибирование репродукции вируса под действием исследуемых соединений в концентрациях 0,4 и 0,5 мг/кг, что было в разы эффективнее действия ацикловира. Коэффициент защиты составлял 80%. Установлено увеличение уровня ИФНγ и ИЛ-2 в сыворотке крови, что указывает на имеющийся иммуномодулирующий эффект фторированных нуклеозидных соединений. Проведенные исследования позволяют утверждать о наличии антигерпетические, имностимулюючои действия фторвмистних триазолов и необходимость углубленного изучения механизмов данного процесса.

Ключевые слова: HSV-1, фторированные нуклеозидные соединения, антигерпетическая активность.

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GENETIC CHARACTERIZATION OF INFECTIOUS BURSAL DISEASE VIRUS ISOLATES IN UKRAINE

The objective of the investigation was to characterize infectious bursal disease viruses (IBDV) circulating in commercial poultry farms in Ukraine between 2014 and 2016. IBDV genetic material was amplified directly from bursa. The nucleotide sequence for VP2 hypervariable region of 16 IBDVs were determined by RT-PCR method, sequenced and compared to well characterised IBDV isolates worldwide. Neighbor-joining method was used for phylogenetic analyses. In result of the studyUkrainian IBDVs represented two genetic lineages: very virulent (vv) IBDVs and classical IBDV closely related to attenuated vaccine stains. The nucleotide identity among UkrainianvvIBDVs ranged between 87.2% and 99,8%. Ukrainian vvIBDV strains clustered together with very virulent strains from other counties like: United Kingdom, Egypt, China, Netherlands and Spain. In conclusion this report demonstrates the circulation of vvIBDV in commercial poultry farms in Ukraine.

Keywords: Infectious bursal disease virus, vvIBDV, VP2, RT-PCR, sequencing, phylogenetic analyses.

Introduction

Infectious bursal disease virus (IBDV) belongs to the Birnaviridae family Avibirnavirus genus. It has a non-enveloped, icosahedral capsid. Viral genome consists of two segments of double-stranded RNA. Virus replicates in immature IgM+ B-cells residing in the bursa of Fabricius of young chickens and causes infectious bursal disease or Gumboro disease. Two serotypes of the virus have been described. Serotype 1 IBDV strains arepathogenic to chickens, whereasserotype 2 strains are non-pathogenic [2, 5]. Serotype 1 IBDVisolates comprise the variant, classical virulent (cvIBDV) and very virulent (vvIBDV) strains, which greatlydiffer in their pathogenicity to chickens.VvIBDV strains were detected in Europe in 1986 and caused 70% mortality in susceptible chickens. These strains still cause great economical impact in poultry industry worldwide [3]. VvIBDV strains have been characterized in many countries, but there were no publications about these strains in Ukraine.