**ANTIVIRAL AND IMMUNOSTIMULATORY POTENTIAL OF FLUORINE CONTAINING TRIAZOLES**

**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. There are many compounds that regulate 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 in vitro 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 antiherpetic activity fluorinated nucleoside G8 and G9 compounds (2-N-substituted-4-tosyl-5-polyfluoralkyl-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 fluoroine containing triazole and there is need to in-depth study of the mechanisms of this process.

**KEY WORDS:** HSV-1, fluorinated nucleoside, antiherpetic activity.

Animals. Inbred 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 inoculation with 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 (CC50), which was 887 and 990, effective concentration (EC50) 50 and 7.6 µg/ml, was shown, respectively (table 2). Selective index of compounds G8 and G9 is 18 and 130.

Table 1. Structure of studied compounds

<table>
<thead>
<tr>
<th>Code</th>
<th>The structural formula</th>
<th>Mol. mass of compounds</th>
<th>The cytotoxicity concentration (CC50), µg/ml</th>
<th>The effective concentration (EC50) µg/ml</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>G8</td>
<td>F_C=N=N_F3C-Ts-Cl</td>
<td>395.78</td>
<td>887</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>G9</td>
<td>C3F7=O=N=C-Ts-Cl</td>
<td>495.81</td>
<td>990</td>
<td>7.6</td>
<td>130</td>
</tr>
</tbody>
</table>

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, LD50 which was 1.5 * 10^3 PFU. The compounds were administered intraperitoneally at 200 ml.

Groups of experimental animals

<table>
<thead>
<tr>
<th>Groups of experimental animals</th>
<th>The dose, ml</th>
<th>Amount of mice</th>
<th>Animals death Amount</th>
<th>Protection factor</th>
<th>Effectiveness Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control of HSV-1</td>
<td>0.2</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>Control G8, 100µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>G8, 40µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>G8, 100µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>2.5</td>
</tr>
<tr>
<td>G8, 500µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Control G9, 100µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>G9, 50µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>G9, 100µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>G9, 500µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Acyclovir, 10µg/ml</td>
<td>0.2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

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 compounds, respectively. Our studies indicated that there is antitherpetic 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](image)

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](image)

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).
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 antivirals HSV-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 antitherpetic, immunomodulatory activity of fluorine containing triazole and the need for in-depth study of the mechanisms of this process.

References
АНТИВІРУСНИЙ ТА ІМУНОСТИМУЛЮЮЧИЙ ПОТЕНЦІАЛ ФТОРВМІСНИХ ТРИАЗОЛІВ

Проблема пошуку ефективних противовірусних препаратів зумовлена високою захворюваністю і широким розповсюдженням вірусних інфекцій. Метою представленої роботи є дослідження антивірусних та імуномодулюючих властивостей триазолів, заміщеніх фтором у 2-й, 3-й та 4-й положеннях, що можуть бути використані як антивірусні агенти.

Ключові слова: HSV-1, фторований нуклеозид та нуклеозидні сполуки, антигіперетична та імуномодулююча активність.

АВТОРСТВО

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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 study Ukrainian IBDVs represented two genetic lines: very virulent (vv) IBDVs and classical IBDV closely related to attenuated vaccine stains. The nucleotide identity among Ukrainian vvIBDVs ranged between 87.2% and 98.8%. Ukrainian vvIBDVs strains clustered together with very virulent strains from other countries 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 are pathogenic to chickens, whereas serotype 2 strains are non-pathogenic [2, 5]. Serotype 1 IBDV isolates comprise the variant, classical virulent (vvIBDV) and very virulent (vVIBDV) strains, which greatly differ 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.