VIROLOGY

Antiviral Activity of Anaferon (Pediatric Formulation) in Mice Infected with Pandemic Influenza Virus A(H1N1/09)

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Anaferon (pediatric formulation) administered in the therapeutic-and-prophylactic regimen to mice receiving intranasally 100% infecting dose of A/California/07/2009(H1N1)v influenza virus exhibited an antiviral effect and 10-fold reduced the production of influenza virus in the lungs of infected mice on days 4, 6, and 8 after infection compared to the control (distilled water). The efficiency of Anaferon (pediatric formulation) administered before and after infection with A/California/07/2009(H1N1)v influenza virus was not inferior to the use of Tamiflu after infection.

Key Words: *influenza virus; Anaferon (pediatric formulation); Tamiflu; oseltamivir; antiviral drugs*

In 2009, epidemic situation in the world was complicated by influenza outbreaks caused by a new A(H1N1/09) variant of influenza virus appearing as a result of genome reassortment of Euro-Asian and North American swine influenza virus strains emerging in the late 1990s after triple assortment of H1N1, H3N2, and H1N2 subtypes within the classical swine influenza strains [5]. International experience in the treatment of pandemic influenza A(H1N1/09) shows that serious and lethal cases were related to late visit to the doctor, concomitant diseases, and limited access to symptomatic therapy. Moreover, this virus induces rapidly progressing and hard-to-treat general lung disease [2]. Four antiviral preparations are recommended by WHO for the treatment of influenza: inhibitors of viral M2 amantadine and remantadine and viral neuraminidase inhibitors oseltamivir and zanamivir. Recent studies showed that influenza virus A(H1N1/09) v isolated from humans is resistant to amantadine and remantadine, but sensitive to oseltamivir and zanamivir [1]. However, these etiotropic anti-flu drugs have a number of limitations and should be administered within the first 48 h of the disease.

This dictates the need in the search of new effective domestic anti-influenza drugs. Anaferon (pediatric formulation) based on affinity-purified antibodies to human IFN-γ is an innovation domestic preparation

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with antiviral activity. This preparation is successfully used for the therapy of various infectious diseases (influenza, acute respiratory viral disease, herpe-virus infections, acute intestinal viral infections, *etc.*) and is approved to the use in children and adults.

Here we evaluated antiviral activity of Anaferon (pediatric formulation) on mouse model of influenza A(H1N1/09)v.

MATERIALS AND METHODS

The study was performed on 96 Balb/c mice weighing 15-17 g (Nursery of Vector Research Center of Virology and Biotechnology). The animals were kept in accordance with Regulations for Conducting Animal Experiments and Guide for the Care and Use of Laboratory Animals [4]. The mice were infected with A/California/07/2009(H1N1)v influenza virus obtained from Center for Disease Control (USA) and passaged twice on developing chick embryos. Biological concentration of the virus in the virus-allantois fluid was determined by titration on developing chick embryos, calculated, and expressed in $lgEID_{50}$/ml (decimal logarithm of 50% embryonic infecting dose per ml). The mice were infected intranasally under light ether narcosis. The administered dose was 2.3 $lgEID_{50}$/mouse, which corresponded to ID_{100} causing influenza virus infection in 100% animals.

The animals were divided into 3 groups. Group1 mice (n=32) received Anaferon (pediatric formulation; Materia Medica Holding) in a solution by the therapeutic-and-preventive scheme: 0.2 ml per mouse 2 times a day for 5 days before and 8 days after influenza virus infection. Group 2 animals (n=32) received Tamiflu (oseltamivir, F. Hoffmann-La Roche Ltd) in a dose of 30 mg/kg/day *per os* according to the therapeutic scheme: 0.2 ml per mouse 2 times a day for 5 days after influenza virus infection; these animals also received 0.2 ml distilled water 2 times a day (0.4 ml/day) for 5 days before infection and for 3 days after the end of Tamiflu treatment. Group 3 animals (control; n=32) received distilled water by the scheme of Anaferon treatment (0.4 ml/day).

Antiviral activity of the test preparation was evaluated by the titer of influenza A virus in mouse lungs 2, 4, 6, and 8 days after infection. The concentration of influenza virus in the lungs of each animal in all groups was measured by titration of lung homogenates on cultured MDCK cells, calculated by the method of Spearman–Karber [3], and expressed in $lgTCD_{50}$/ml (decimal logarithms of 50% tissue cytopathic dose per ml).

The data were processed by routine statistic tests for biological studies [1] and by Student t test using Microsoft Excel software.

RESULTS

The study revealed high antiviral activity of Anaferon (pediatric formulation) and Tamiflu against pandemic influenza virus strain A/California/07/2009(H1N1)v. On day 2 after infection, the mean influenza virus titers in the lungs in all mouse groups did not exceed the sensitivity threshold of the method of titration on MDCK cells and were 0.50±0.17 $lgTCD_{50}$/ml. However, on day 4 postinfection the mean influenza virus titers in the control group was considerably higher than on day 2 and significantly surpassed the corresponding parameters in mice receiving Anaferon (pediatric formulation) and Tamiflu (Table 1).

Analysis of the dynamics of virus production in mouse lungs showed that the test preparation inhibited virus reproduction throughout the observation period. The influenza virus titers in the lungs of mice receiving Anaferon (pediatric formulation) and Tamiflu on days 4, 6, and 8 were significantly lower than in control group animals (Table 1). It should be noted that influenza virus titers in the lungs of mice receiving

TABLE 1. Dynamics of Influenza Virus Titers in the Lungs of Mice Receiving Anaferon (Pediatric Formulation) and Tamiflu after Infection with ID100 Influenza Virus Strain A/California/07/2009(H1N1)v

Group	Mean titers ($lgTCD_{50}$/ml±S_m, ±I_{95}) of influenza virus A(H1N1)v in homogenates of the lungs from infected animals			
	day 2	day 4	day 6	day 8
Control	0.56±0.03, ±0.07	1.96±0.14, ±0.33	2.86±0.11, ±0.26	2.65±0.23, ±0.54
Anaferon (pediatric formulation)	0.54±0.03, ±0.07	0.59±0.03, ±0.08*	1.63±0.19, ±0.44*	0.98±0.17, ±0.39*
Tamiflu	0.52±0.02, ±0.05	0.54±0.03, ±0.07*	1.77±0.09, ±0.22*	1.10±0.16, ±0.39*

Note. S_m (error of the mean) and I_{95} (95% confidence interval) were calculated and compared using Student t test. Each sample included 8 animals. *p=0.05 compared to the control.

Anaferon (pediatric formulation) did not differ from the corresponding parameters in mice receiving Tamiflu at all terms of the observation period. Thus, out findings suggest that Anaferon (pediatric formulation) effectively suppressed replication of influenza virus A/California/07/2009(H1N1)v. Anaferon (pediatric formulation) was administered to mice *per os* for 5 days before and 8 days after infection, while Tamiflu (30 mg/kg per day) was given *per os* for 5 days after infection. Anaferon (pediatric formulation) administered according to this scheme was not less effective than Tamiflu, which confirms the possibility of using Anaferon (pediatric formulation) as a therapeutic and preventive means in influenza infection.

Thus, therapeutic and preventive administration of Anaferon (pediatric formulation) was comparable by its efficiency to therapeutic administration of Tamiflu after infection with pandemic influenza virus strain A/California/07/2009(H1N1)v and significantly (by more than 10-fold) suppressed virus reproduction in mouse lungs 4, 6, and 8 days postinfection. These results suggest that the anti-influenza activity of Anaferon (pediatric formulation) should be studied in further experiments, *e.g.* its activity against avian influenza virus A(H5N1).

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