VIROLOGY

Study of Efficiency of Therapeutic and Preventive Anaferon (Pediatric Formulation) in Mice with Influenza Infection

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Therapeutic and preventive treatment of mice intranasally infected with a lethal dose of A/Aichi/2/68 (H3N2) influenza virus with anaferon (pediatric formulation) demonstrated an antiviral effect of the drug (increased percent of survivors and prolonged lifespan).

Key Words: *influenza; ultralow doses; anaferon for children; antiviral activity; antibodies to γ-interferon*

Preparations whose activity is realized through natural mechanisms of antiviral resistance, primarily IFN system, are the most perspective drugs for the treatment of influenza and acute respiratory viral infections. Adequate induction of endogenous IFN provides a benign course of viral infection, and hence, IFN inductors now play an important role in the treatment of influenza and acute respiratory viral infections.

Influenza virus (IV) is the best known and the most prevalent of more than a hundred viruses causing infections of the upper airways. Influenza is a respiratory disease, often with a severe course, sometimes causing serious complications, particularly in children and elderly patients. New epidemic strains of IV appear every 1-2 years as a result of point mutations in two surface glycoproteins (hemagglutinin and neuraminidase). Due to variety and changeability of virion antigenic structure, IV can escape the defense mechanisms of human immunity, and no long-lasting resistance develops even after natural infection or vaccination. Despite intense studies aimed at the creation of anti-influenza vaccines, influenza infection remains one of the most acute medical problems. That is why the search and testing of new effective drugs for IV stimulating nonspecific antiviral resistance primarily inductors of endogenous IFN is a pressing problem [3-5].

We compared the efficiencies of therapeutic and preventive treatment with anaferon (pediatric formulation) and tamiflu in mice intranasally infected with A/Aichi/2/68 (H3N2) IV in a 90% LD (LD_{90}).

MATERIALS AND METHODS

The study was carried out on 150 female BALB/c mice (17-18 g) from breeding center of Vector Center of Virology and Biotechnology. The animals

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TABLE 1. Survival of Mice Treated with Anaferon (Pediatric Formulation), Distilled Water, and Tamiflu after IV Infection in a Dose of LD_{90}

Group	Number of mice dead 24 h after IV infection														Number of survivors
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
1		2	14	10	2	4		2							6
2				4	3	2	7	1	3		2	1			17*+
3					1	2	1								36*

Note. $p<0.05$ compared to: *group 1; +group 3.

were kept at natural light on standard rations with free access to water.

The mice were divided into 3 groups, 40 per group:

1) control; intranasal distilled water for 5 days before infection and 16 days after infection (0.2 ml twice daily; 0.4 ml/day per animal);
2) intragastric anaferon (pediatric formulation, Materia Medica Holding) for 5 days before infection and 16 days after infection (0.2 ml twice daily; 0.4 ml/day per animal);
3) intragastric distilled water for 5 days before infection, tamiflu (10 mg/kg/day; 0.2 ml twice daily, or 0.4 ml/day/animal) for 5 days after infection, and distilled water during up to 16 days after infection.

Influenza virus strain A/Aichi/2/68 (H3N2) was obtained from D. I. Ivanovsky Institute of Virology. The virus was isolated after 12 passages in mice and 2 passages on developing chicken embryos (0.1 ml material was injected into the allantoic cavity) [2]. Biological concentration of the virus in the virus-allantoic fluid (VAF) was evaluated by titration in developing chicken embryos [2], calculated, and expressed in EID_{50}/ml (50% embryonal infective doses/ml) as described previously [1]. The virus concentration in VAF was 8.7±0.2 lg EID_{50}/ml (70 ml).

The mice were intranasally infected with IV under light ether narcosis at 50-70% humidity and 24°C. A total of 40 µl appropriate dilution of VAF was daily pipetted into both nostrils of a mouse with an automated pipette.

In order to determine the LD_{90}, the mice were infected with 5 doses of IV: $10^{2.3}$, $10^{3.3}$, $10^{4.3}$, $10^{5.3}$, and $10^{6.3}$ EID_{50}/mouse (6 mice per dose). Mortality in each group was recorded during the subsequent 14 days. The LD_{90} was calculated as described previously [1] from the IV doses used and animal mortality values. The LD_{90} in our study corresponded to 5.81 lg EID_{50}/animal.

The mice were observed for 17 days after IV infection. Drug efficiency was evaluated by the mean lifespan (MLS) and percentage of survivors. The MLS in each experimental group was evaluated by the number of animals living during a certain number of days until death and the number of survivors. The maximum lifespan of survivors was assumed to be 16 days (the next day after cessation of infected animals' deaths).

The data were statistically processed by common methods using Student's *t* test, Fisher's test, Yates' correction for small samples (Statistica 6.0 software). The differences were considered significant at $p<0.05$.

RESULTS

The highest mortality after infection (85%) was observed in the control group receiving distilled water (Tables 1, 2).

Tamiflu and anaferon increased the number of survivors in comparison with the control group (Table 1). The highest percentage of survivors was observed in group 3 (Table 2). In group 2, the survival rate was significantly higher than in the control ($p=0.0431$; with Yates' correction $p=0.0726$; Table 2).

Tamiflu and anaferon significantly improved survival of mice infected with IV LD_{90}. The longest MLS was recorded in group 3 (Table 2). In group 2, the MLS was also significantly longer than in the control (Table 2).

Hence, therapeutic and preventive treatment of mice intranasally infected by IV LD_{90} with ana-

TABLE 2. Percentage of Survivors and MLS in Groups of Mice Treated with Anaferon (Pediatric Formulation), Distilled Water, and Tamiflu after IV Infection in LD_{90} ($M\pm m$)

Group	% of survivors	MLS, days
1	15.0	9.30±0.51
2	42.5*+	12.55±0.55*+
3	90.0*	15.30±0.34*

Note. $p<0.05$ compared to: *group 1, +group 3.

feron (pediatric formulation) and tamiflu demonstrated antiviral effects of both drugs: they increased the percent of survivors and MLS in comparison with the control group. It seems that due to its immunomodulating effect and stimulation of humoral and cellular immune reactions [3-5], anaferon (pediatric formulation) promotes alleviation of the major manifestations of experimental influenza infection in mice and their duration.

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