ACTIVITY OF THE PREPARATION "INFLUCID" WITH RESPECT TO FLU VIRUSES IN MODEL SYSTEMS

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Flu viruses are responsible for a number of incidents of ORVI [expansion unknown] exceeding 60% during a seasonal epidemic especially in children. In periods between epidemics, they cause 5-15% of ORVI incidents. Flu is a special threat to the elderly (older than 65) and plays an important role in the structure of mortality statistics due to various complications of this infection.

IN recent years, a worldwide epidemic of bird flu caused by the highly pathogenic strain A (H5N1) has been spreading intensively. Bird flu viruses of the H5N1 type present a great danger for both birds and humans. At present, we know that this virus can be passed on by close contact from birds to people without intermediate hosts. The group mainly at risk are workers in poultry factories, veterinary workers, village residents living the traditional village life with domestic foul at their homesteads. Regardless of the fact that possibility of transmitting this virus from person to person has not been definitively demonstrated, according to data of the World Health Organization, of 335 known cases of the disease in people worldwide, 206 died (data from November 12, 2007). Everything cited above makes constant search for new anti-flu measures urgent, especially in view of the high rate of evolution of flu viruses and development of new strains resistant to widely distributed anti-flu preparations [4, 5].

The homeopathic preparation, "Influcid" has the following composition: 100 g of a solution contains: Aconitum D3, 10 g; Gelsernium D3, 10 g; Ipecacuanha D3, 10 g; Phosphorus D5, 10 g; Bryonia D2, 10 g; Eupatorum perfoliatum D1, 10 g. Other ingredients: Eucalyptus globules, 96% ethanol, and purified water. According to data in the literature, Influcid ameliorates symptoms present in all ORZ such as hyperthermia, joint pain, cough, mucosal hyperemia in the mucosa and inflammatory processes in the throat and larynx [1].

The purpose of this work was to investigate the possibility of anti-viral activity of Influcid with respect to a series of actual strains of human and bird flu virus in cell culture and in mice.

MATERIALS AND METHODS

Anti-virus activity of "Influcid" was evaluated in vitro on embryonic dog kidney cells (MDCK) in 96-well plates for cell cultures. From the original virus-containing allantoic fluid, a series of tenfold dilutions from 10-1 to 10-7 were prepared and introduced into corresponding wells with cells in single layers. The results were read after 48 hours for hemagglutination (HA) with a 0.5% suspension of chicken erythrocytes. For a virus titer in the control and the experiment, we took the value inverse to the decimal logarithm of the maximum dilution of the original virus capable of eliciting a positive hemagglutination reaction in a well and termed it a 50% infectious dose (ID50). The virus-inhibiting activity of the preparation was evaluated according to the decrease in virus titer in the experiment in comparison with the control (ΔlgID50).

In the work, we studied actual common strains of human flu viruses: A/New Caledonia 20/99 (H1N1); A/Victoria/35/72 (H5N2); A/Wisconsin/67/05 (H3N2); and B/Malaysia/2506/04. Of bird flu viruses, we studied: A/NIBRG-14 (H5N1) (vaccination strain derived from A/Vietnam/1194/04, A/duck/Potsdam/1402/6/86 (H5N2), A/mallard duck/NT/12/02 (H7N3) and A/Hong Kong/1073/99 (H9N2).

Anti-virus activity of the preparation was evaluated on white, four-week-old, common mice weighing 15-20 g obtained from the breeding farm, "Rappolovo." In our work, we used the flu virus strain A/Puerto Rico/8/34 (H1N1). The selection of the strain for the work was based on the fact that it adapts well to mice and can elicit specific infection in them. The research was done according to proven recommendations of the Ministry of Health and Social Development

[4]. We chose the liquid (drop) form of the preparation as the best model for the study.

RESULTS AND DISCUSSION

Determination of possible cytotoxicity of Influcid

An indispensable preliminary step in studying any preparations in vitro is determining their possible toxicity for test-culture cells [2]. The plan for testing is presented in Fig. 1.

The preparation was determined to be completely free of toxicity when diluted 1/25-1/400 in three-day contact with cells.

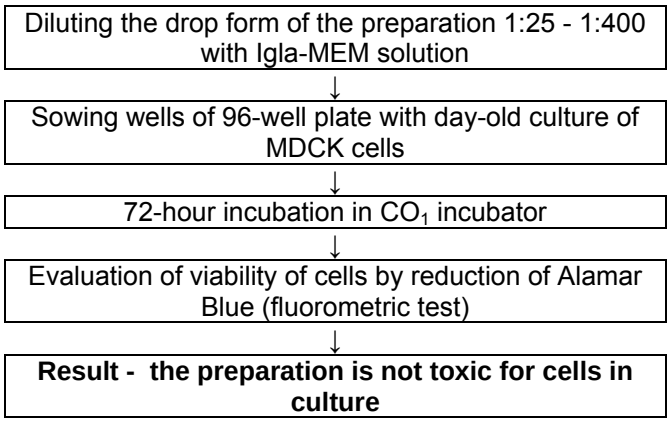

Fig. 1. Plan for evaluating safety of Influcid *in vitro*.

The plan for studying anti-virus activity of Influcid is presented in Fig. 2.

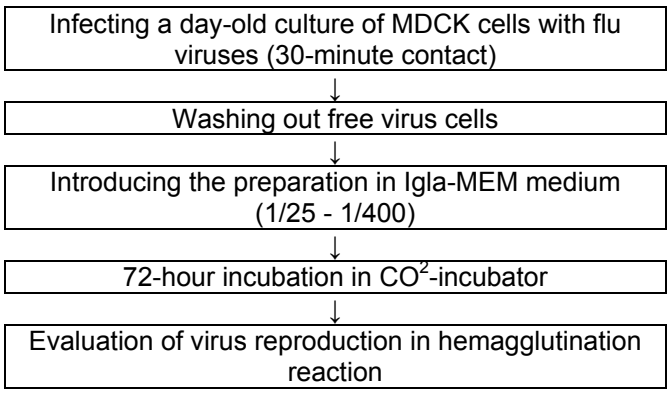

Fig. 2. Plan of experiment evaluating anti-virus activity of Influcid *in vitro*.

The results of testing anti-virus activity of the preparation are presented in Table 1.

Table 1. Results of testing anti-virus activity of Influcid *in vitro*

[column headings obliterated]	
A/N Caledonia/20/99 (H1N1)	1.5
A/Victoria/35/72 (H3N2)	2.5
A/Wisconsin/67/05	1.0
B/Malaysia/2506/04	2.0
Strain of bird flu virus	
A/N18RG-14 (H5N1)	1
A/Duck/Potsdam/1402/6/86 (H5N2)	0.5
A/Mallard//NT/12/02 (H7N3)	0
A/Hong Kong/1073/99 (H9N2)	0

As is apparent from Table 1, the preparation has a good anti-virus effect with respect to strains A/Victoria/35/72 (H3N2) and B/Malaysia/2506/04 (an anti-virus effect is considered good if ΔlgID50 ≥ 2.0), a moderate effect with respect to the other strains of human flu virus of the subtypes A(H3N2) and A(H1N1) and bird flu A(H5N1), and was inactive with respect to other common strains of bird flu.

It is our opinion that in evaluating the results of the study in vitro, attention should be given to the fact that homeopathic preparations of a complex composition probably act systemically and the model of virus infection of cell cultures cannot reflect all aspects of their protective effect on organisms. In addition, strictly speaking, the concentrations of the preparation we tested in vitro were not, homeopathic, that is, in this case, it is more precise to speak of Influcid as a phytopreparation. For this reason, the logical next stage is testing Influcid on animals.

STUDY OF ANTI-VIRUS ACTIVITY OF INFLUCID ON MICE

An indispensable stage of the work was determining the average lethal dose of the virus strain for mice, which required preliminary studies. Under light ether anesthetic, mice were injected nasally with three dilutions of the virus, each consisting of 0.5 ml of the virus-containing fluid. Ten mice were used for each dilution. Then, for eight days, the mice were kept under vivarium conditions and the number of dead mice was recorded each day.

After that, mice of the experimental and control groups were infected with two intranasal doses of the virus – LD50 and 0.1 LD50. There were 10 mice in each group. A total of 40 mice was used in the experiment. The preparation was administered to the experimental group perorally with a probe once a day in a dose of

0.05 ml according to the standard therapeutic/prophylactic plan that included a five-time dosage (in 24 hours and 1 hour before infection, for 24, 48 and 72 hours after infection). This plan anticipates a one-time trial of the prophylactic and therapeutic activity of the compound. A one-time dose of the preparation consisted of 50 µl Influcid (drop form) diluted 25-fold by physiological solution, which, taking into account the ratio of average weight of man and mouse, corresponds to the daily dose of the preparation in intensive therapy (according to instructions for using the preparation, it corresponds to 10 drops every 12 hours a day, that is, 120 drops). Unfortunately, administering the preparation to mice entailed significant stress and for this reason, the dose was administered once and not twice.

The first death of an animal was recorded 5 days after infection. Lethality was calculated for 9 days after infection. In all, 50% of the control group died as against 30% in the experimental group. With 0.1LD50 in the control group, mortality was 40%, and in the experimental group, 10%. The results were processed by regressive analysis, and specially devised linearized dependence of number of surviving animals on time after infection. The analysis was done with a Statistica-60 program. The results are presented graphically in Fig. 3-5. As is apparent from the figures, the slope of the curve along the abscissa (regression coefficient) reflecting the rate of animal mortality is substantially higher for the control group (particularly convincingly for an infection dose of 0.1LD50) – Fig. 5.

Statistical processing of the data was also done using the nonparametrical method (Wald-Wolowitz Runs Test, Statistica program for Windows 6.0). For combined data of both infecting doses of the virus, the criteria value was $Z = -2.297$, $p = 0.022$; for the 0.1LD50 dose, $Z = 3.676$, $p = 0.000237$. Thus, in both cases, $p < 0.05$, therefore the difference between the experimental and control groups can be considered proved. Criteria of effectiveness of the preparation are the coefficients of protection (CP) and index of protection (IP) [3, 6]. The preparation is considered active if IP is ≥ 40%.

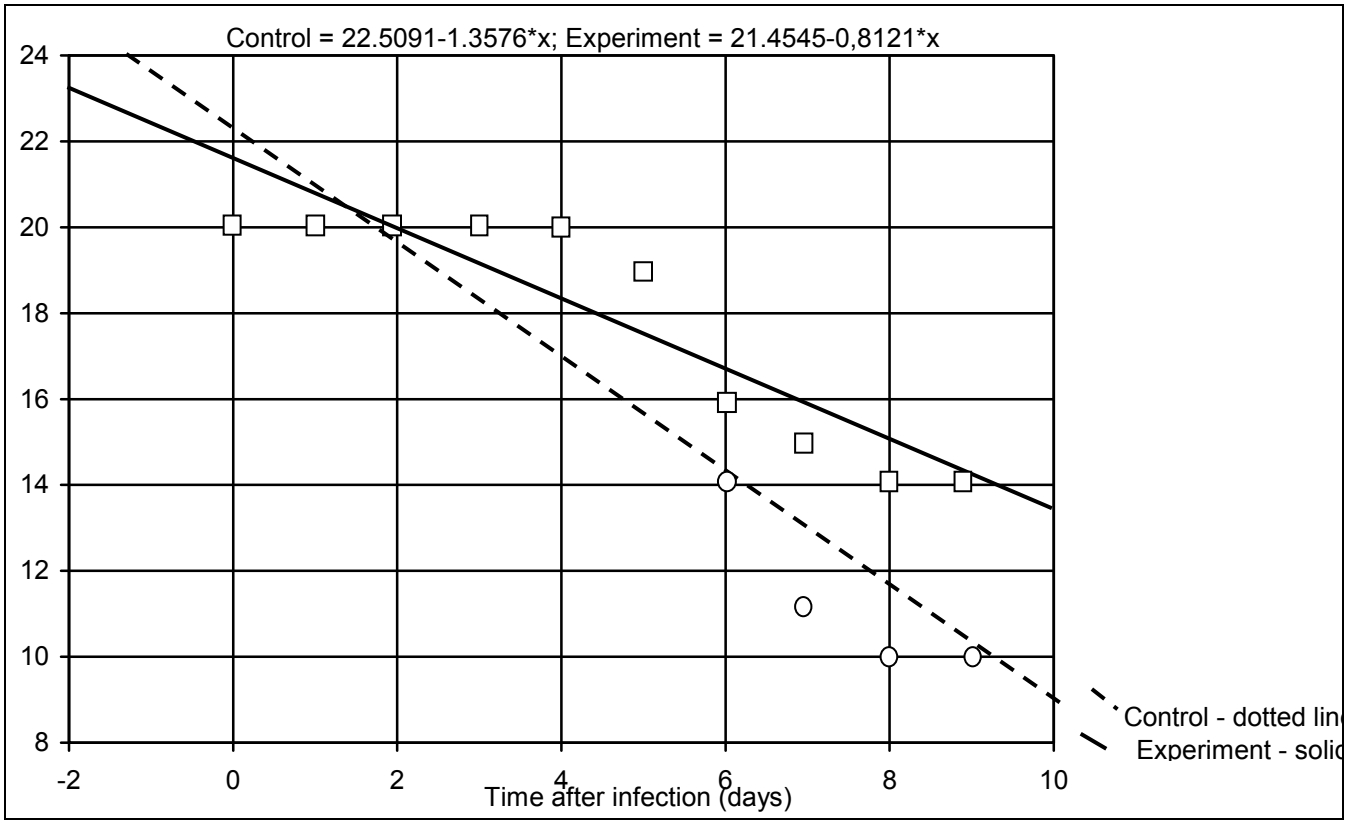

Fig. 3. Regressive analysis of survival of control mice and experiment mice (those receiving Influcid) after being infected with flu virus A/PR8/34. Ordinate: number of surviving animals (n = 20). Data for all infectious doses (LD50 and 0.1 LD50).

In our case, we have the following: in computing mortality for all the times with a 1.0 LD50 infectious virus dose, CP = 1.2, and IP, 16.7%; with a 0.1 LD50 virus dose, CP = 4 and IP = 75%; considering mortality for all times in both doses of the virus, CP = 1.7 and IP = 41.2%, Thus, the effectiveness of the preparation may be considered proved as a whole, and particularly in infection with an 0.1 LD50 virus dose where IP >> 40%.

CONCLUSIONS:

Anti-virus activity of Influcid in vitro in cultures of MDCK cells infected with flu virus has been proved. Its activity manifests itself with respect to subtypes of human flu viruses: A (H3N2) and B and to a lesser degree with respect to A (H1N1) and to one of the strains of bird flu: A (H5N1). Studies of the protective effect of Influcid in mice with respect to the common strain of flu A (H1N1) disclosed a reliable positive therapeutic/ prophylactic effect of the preparation.

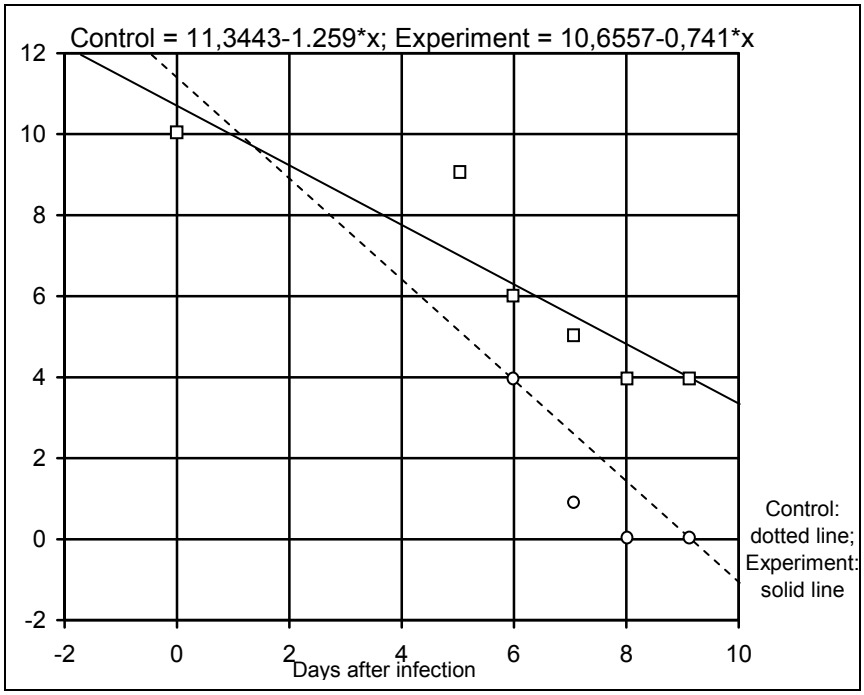

Fig. 4. Regressive analysis of survival of control mice and experiment mice (those receiving Influcid) after being infected with flu virus A/PR8/34. Ordinate: number of surviving animals (n = 10). Data for infectious virus dose LD50).

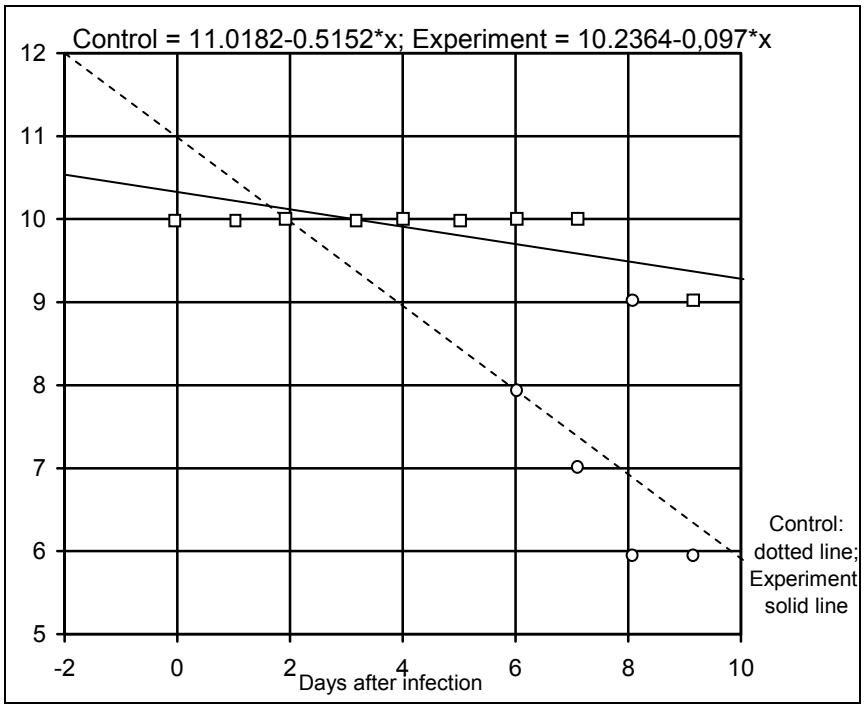

Fig. 5. Regressive analysis of survival of control mice and experiment mice (those receiving Influcid) after being infected with flu virus A/PR8/34. Infectious virus dose 0.1 LD50.

LITERATURE

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