

The action of the drug Influcid in vitro against a pandemic strain 2009 A (H1N1)
"Swine" ("Mexican") flu

M.Yu. Eropkin, N.I. Konovalova, V.A. Grigorieva, D.M. Baybus, T.M. Gudkova
(Research Institute of Influenza SZO RAMS, St. Petersburg)

SUMMARY

The global development of a pandemic of influenza of a new type of subtype A (H1N1) - the so-called "swine" or "Mexican" flu, as well as the lack of reliable anti-influenza etiotropic drugs effective against the whole variety of circulating strains, determine the search for less narrowly targeted homeopathic and herbal remedies against influenza. This work demonstrates the effectiveness of the Influcid preparation against the reference strain of pandemic influenza A (H1N1) in a model in vitro system. The activity of the drug is expressed, mainly, not in a direct antiviral, but rather in an adaptogenic effect, leading to an increase in the resistance of cells to a viral cytopathogenic effect.

Key words: influenza pandemic, A (H1N1), "swine flu", Influcid.

Introduction

Homeopathic medicine Influcid ("German Homeopathic Union" - DHU) has the following composition: 100 g of solution contains - Aconitum D3 10g, Gelsemium D3 10g, Ipecacuanha D3 10g, Phosphorus D5 10g, Bryonia D2 10g, Eupatorium perfoliatum D1 10g. Other ingredients: Eucalyptus globules, 96% ethanol, purified water. The alcohol content is 45% vol. According to the literature, Influcid significantly reduces the symptoms inherent in all acute respiratory infections, such as: hyperthermia, pain in the extremities, cough, mucosal hyperemia, inflammatory processes in the pharynx and larynx [1].

We have previously investigated the antiviral effect of the drug Influcid in vitro against panels of avian influenza A viruses (H5N1; H5N2; H7N3; H9N2), as well as reference strains of human influenza A (H3N2), A (H1N1) and B. in vivo in mice, infected with a lethal dose of the A / PR8 / 34 (H1N1) strain adapted to this model [2].

At the moment we are witnessing the deployment of the first pandemic of the XXI century - a pandemic of the so-called "swine" or "Mexican" influenza caused by a qualitatively new strain of influenza A subtype (H1N1) - a triple reassortant that combines RNA segments derived from the North American line of swine flu (segments HA, NP and NS), the Eurasian lineage of swine influenza (segments NA and M), the North American line of avian origin (segments RA and PB2) and segment PB1 from seasonal influenza H3N2 subtype [5]. At the time of this writing (07.07.2009), according to WHO, there are 94,512 laboratory-confirmed cases of "swine flu" registered in the world, of which 429 were fatal. The pandemic continues mainly in the countries of the Southern Hemisphere, where the midst of a climatic winter is now (Argentina, Australia, Chile, New Zealand).

According to laboratory studies, the pandemic strain A (H1N1) is resistant to adamantanes (rimantadine and amantadine), but sensitive to neuraminidase inhibitors (oseltamivir and zanamivir). However, there has already been a report on the isolation of the first virus of this variety, resistant to oseltamivir (Tamiflu) [6]. The experience of recent years shows that it is practically impossible to find an etiotropic antiviral drug that is effective against the whole variety of circulating seasonal viruses. Thus, rimantadine, in addition to being ineffective against influenza B, did not act on a significant part of viruses of the A (H3N2) subtype in recent epidemiological seasons. In contrast, viruses of the A (H1N1) subtype were mostly sensitive to rimantadine, but rapidly acquired resistance to oseltamivir. All this is the basis for the search for drugs, aimed not at the virus as such or its interaction with the cell, but at stimulating cell resistance, the production of interferons, and immune defense. While the prospects for the timely appearance of a pandemic vaccine against "swine flu" are unclear, the role of such drugs in

the initial phase of a pandemic is particularly critical. This prompted us to investigate a possible action in vitro drug Influcid against the reference strain of "swine flu" A / California / 07/09 (H1N1) swl.

Materials and research methods

The study of the effect of Influcid was carried out on a culture of MDCK cells recommended by the WHO reference centers for influenza and the Ministry of Health and Social Development of the Russian Federation for the isolation and study of influenza viruses [3]. The pandemic "swine flu" strain A / California / 07/09 (H1N1) swl was kindly provided to us for scientific research by Prof. A.I. Klimov (Center for Disease Control and Prevention (CDC), Atlanta, Georgia, USA).

For our purposes, the liquid (drop) form of Influcid turned out to be more convenient. As shown earlier, the drug was characterized by a complete absence of toxicity when diluted in 1 / 50-1 / 400 and contact with cells for 3 days.

Evaluation of the antiviral effect of Influcid was carried out on cells seeded at a standard dose in 96-well cell culture plates. A series of tenfold dilutions from 10^{-1} to 10^{-7} and added to the appropriate wells with a cell monolayer. The results were taken into account after 48 hours by the hemagglutination reaction (RHA). For the titer of the virus in the control and experiment, the reciprocal of the decimal logarithm of the highest dilution of the original virus capable of causing a positive hemagglutination reaction in the well was taken, and was expressed in the amount of 50% of tissue infectious doses (TID₅₀). The virus-inhibiting effect of the drug was assessed by the decrease in the titer of the virus in the experiment as compared with the control ($\Delta \lg \text{TID}_{50}$). The second important indicator was the cell reduction reaction in the culture of the tetrazolium dye MTT (Thiazolil blue), the intensity of which reflects the degree of cell viability as a result of the reduction of the dye by mitochondrial and partially cytoplasmic dehydrogenases. The test is often used in virology to assess the cytopathogenic effect of viruses on a cell [8]. Its results can be interpreted as the degree of cell resistance to the action of viruses. Microtetrazolium test is also widely used in assessing the effect on cells of toxicants, pharmacological drugs, unfavorable environmental factors [4], therefore, simultaneously with the antiviral effect, the toxicity of the studied drugs can be assessed in vitro. The drug was injected into the cell culture medium for 1 hour before virus infection ("prophylactic" administration regimen) and 1 hour after virus infection ("therapeutic" administration regimen) diluted in 0.5% and 1% PBS buffer (concentration in the medium of the original liquid preparation).

Research results

With the introduction of Influcid samples according to the treatment scheme (1 hour after cell infection), the drug moderately suppressed the production of viral particles (detected in the hemagglutination reaction) - at a concentration of 1%, a decrease in hemagglutinating activity ($\Delta \lg \text{TID}_{50}$) was 1.0; and at a dose of 0.5% - 0.5, i.e. the infectious activity of the virus dropped by 10 and 6.7 times, respectively. The results of a comparative study of the effect of the drug according to this criterion in relation to a panel of human and avian influenza viruses are presented in table. 1. The greatest activity of Influcid in this study was manifested in relation to the reference strains of human influenza A and B viruses in different years of isolation and was relatively low or absent in relation to a number of varieties of avian influenza.

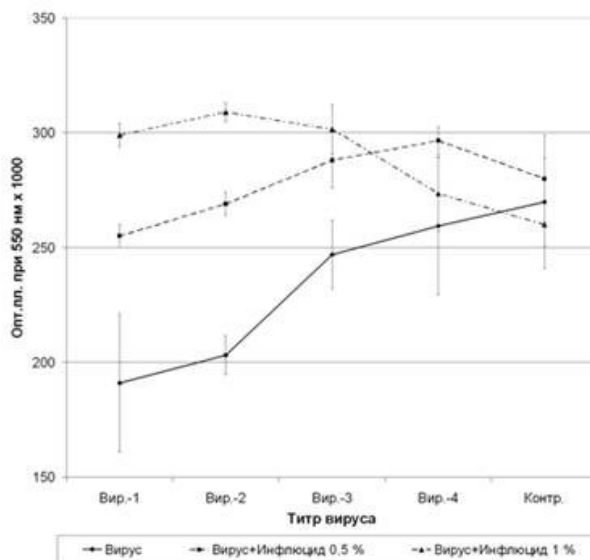
Table 1

Comparative antiviral effect of the drug Influcid in vitro for strains of various influenza virus subtypes assessed by the RHA method

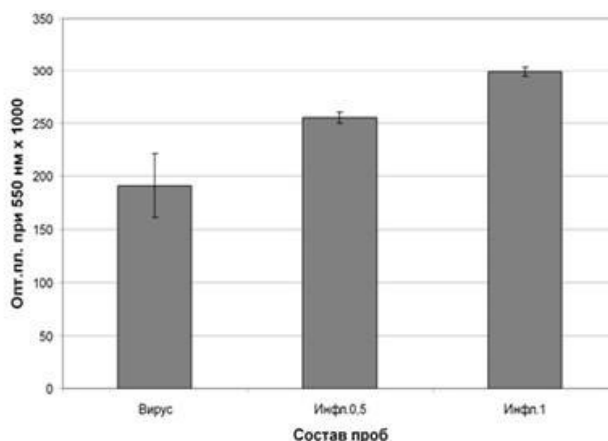
№	Штамм вируса	Противовирусный эффект в $\Delta \lg \text{TID}_{50}$
1	А/Н.Каледония/20/99 (H1N1) *	1,5
2	А/Виктория/35/72 (H3N2) *	2,0
3	А/Висконсин/67/05 (H3N2) *	1,0
4	В/Малайзия/2506/04 *	2,0
5	А/NIBRG-14 (H5N1) *#	1,0
6	А/утка/Потсдам/1402/6/86 (H5N2) +	0,5
7	А/кряква/NT/12/02 (H7N3) +	0
8	А/Гонконг/1073/99 (H9N2) +	0
9	А/Калифорния/07/09 (H1N1)sw1 *##	0,75

Note: * - human influenza viruses, * # - vaccine strain A (H5N1), * ## - pandemic virus A (H1N1) ("swine" or "Mexican" influenza virus), which has caused a pandemic that has been developing since April 2009 influenza, + - avian influenza viruses.

When evaluating the MTT recovery test, a significant decrease in the cytopathogenic effect of the virus was revealed both in the "prophylactic" (Fig. 1 and 2) and "therapeutic" (Fig. 3 and 4) regimens of drug administration. With a "prophylactic" scheme of administration, the effect of the drug was dose-dependent (Fig. 1) - Influcid at a concentration of 1% had a significantly stronger effect than 0.5%. The effect was most pronounced in the zone of high virus titers (-1 and -2 log or 10^4 and 10^3 TID₅₀), and the drug at a concentration of 1% almost completely blocked the cytopathic reaction of cells in culture even with the highest infectious dose of the virus - 10^4 TID₅₀ (fig. 2). Influcid significantly reduced the cytopathogenic effect of the virus also in the case of a "therapeutic" scheme of administration (Fig. 3 and 4), and in this case the effect of the drug at a dose of 0.5 and 1.0% was approximately the same, but significantly different from the control (Fig. 4).



Rice. 1. Antiviral effect of Influcid against the "swine flu" virus A / California /07/09 (H1N1) sw1 in MDCK cell culture. Pre-incubation with the drug for 1 hour, virus infection, registration results after 24 hours. Test method - MTT.

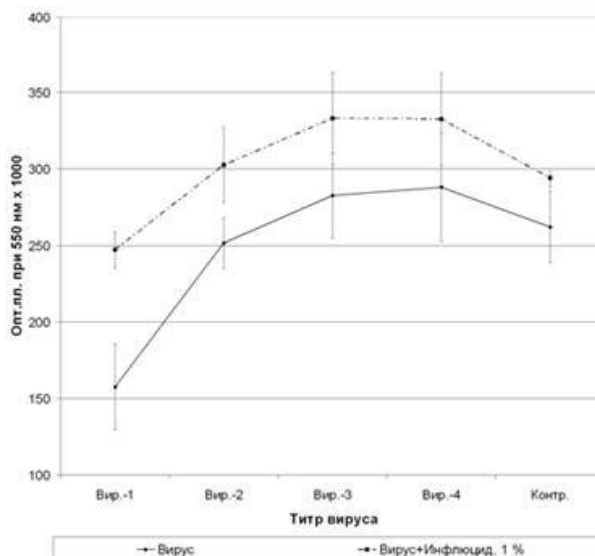


Rice. 2. Antiviral effect of Influcid against the "swine flu" virus A / California /07/09 (H1N1) swl. Pre-incubation with the drug for 1 hour. Test method - MTT. Data at a virus dose of 10^4 TID₅₀...

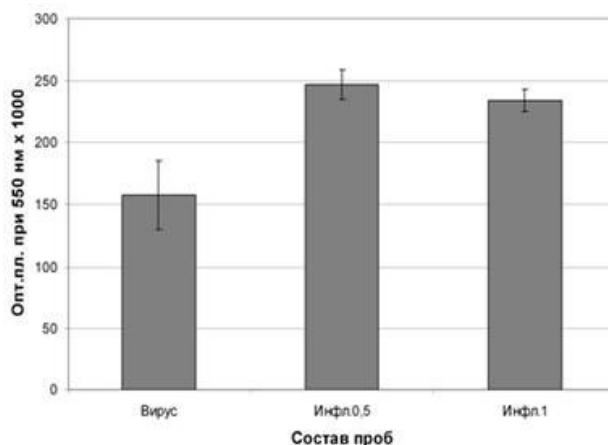
Discussion

When comparing the data obtained by the MTT method with the results of registration by the RHA method, it can be seen that the drug Influcid, in the case of infection with a highly pathogenic strain of "swine" influenza A / California / 07 / 09swl, has a positive effect, mainly by reducing the cytopathic response of cells to the virus, that is, resistance of the cells themselves. In this sense, its action can be considered, rather, not as a direct antiviral, but as adaptogenic, the result of which is an increase in the body's resistance to viral infections.

Comparison of the data on the activity of the drug against various reference strains of the influenza virus shows that its activity in terms of RHA against the pandemic strain A (H1N1) is relatively low - the greatest activity was shown against strains A (H3N2) and B. A small effect was also observed against the model vaccine strain of influenza A (H5N1) - NIBRG-14 (Table 1).



Rice. 3. Antiviral effect of Influcid on the "swine flu" virus A / California /07/09 (H1N1) swl with preliminary infection of MDCK cells with a virus (1 hour before drug administration). Test Method - MTT.



Rice. 4. Antiviral effect of Influcid on the "swine flu" virus in cell culture MDCK at preliminary infection with a virus 1 hour before drug administration. Virus dose 10^4 TID₅₀...

However, it should be noted that this comparative study did not use a method for assessing the cytopathogenic effect of viruses on the recovery of infected MTT cells, so that all possible aspects of the drug's action could not appear. Nevertheless, the importance of even a relatively low positive effect of Influcid against A (H5N1) strains should be emphasized, since the threat of an epidemic of highly pathogenic "avian" flu cannot be removed from the agenda, despite the outbreak of a "swine" flu pandemic, since there are regular reports of cases diseases of influenza A (H5N1) in developing countries, the mortality rate from which exceeds 50% [7]. Finally, despite the simplicity and objectivity of the models in vitro, their limitations should be noted in the study of homeopathic medicines, which, of course, act mainly at the systemic level. Therefore, the antiviral effect of Influcid was also confirmed by us earlier on an animal model - white mice infected with lethal and sublethal doses of the widely known A / PR / 8/34 (H1N1) strain adapted to them [2]. The drug diluted with saline was administered to mice intragastrically once a day for 4 days from the moment of infection in a pharmacologically adequate dose. According to the criterion of the protection index (IZ) and taking into account the mortality of experimental animals for all periods and both used infectious doses of the virus 1LD₅₀ and 0.1LD₅₀, we received IZ = 41.2%, and with an infectious dose of 0.1LD₅₀ IZ = 75% (the drug is considered active if IZ ≥ 40%). Thus, the effectiveness of the drug has been proven as a whole, 2) when FROM >> 40%. Statistical processing by nonparametric methods (Wald-Wolfowitz Runs Test, Statistica for Windows 6.0 program) also showed a significant effect of Influcid in vivo (for the dose of the virus 0.1LD₅₀ Z = - 3.676; p = 0.000237). A convincing result was also obtained when processing by the method of regression analysis (constructing linearized dependences of the number of surviving animals on the period after infection).

Conclusions:

1. Influcid is effective as an adaptogenic anti-influenza drug in vitro in culture MDCK cells infected with swine flu virus (new pandemic strain A (H1N1)).
2. The activity of the drug is manifested in both "prophylactic" and "therapeutic" regimens introduction.
3. The positive effect of Influcid was revealed mainly in the recovery test MTT cells, which indicates the cellular mechanism of the antiviral effect, expressed in the increased resistance of cells to the cytopathogenic action of the virus.

Literature

1. Garashchenko T.I. Complex homeopathic medicines in the treatment of inflammatory diseases of ENT organs // Russian Medical Journal. - 2002. - T.20 - No. 10.
2. Eropkin M.Yu., Grigorieva V.A., Gudkova T.M., Konovalova N.I., T.G. Lobova, Baybus D.M., Yaglovskaya I.B. The activity of the drug "Influcid" against influenza viruses in model systems // MEDLINE-Express. - 2007. - No. 6. - P. 23–26.

3. Sominina A.A., Burtseva E.I., LoboVA T.G. and others. Isolation of influenza viruses in cellular cultures and chicken embryos and their identification: Methodical recommendations. - SPb., 2006. -- 24 p.
4. Borenfreund E, Babich H, Martin-Alguacil N. Comparison of two in vitro cytotoxicity assays - the neutral red (NR) and tetrazolium (MTT) tests // Toxicol. In Vitro. - 1988. - Vol. 2 - No. 1. - P. 1-6.
5. Dawood FS, Jain S., Finelli L. et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans // New Engl. J. Med. - 2009. - Vol. 361 - P. 1-10. 6.<http://www.who.int/csr/disease/swineflu/en/index.htm>
- 7.http://www.who.int/csr/disease/avian_influenza/en/index.html
8. Watanabe W., Konno K., Ijichi K., et al. MTT colorimetric assay system for the screening of anti-orthomyxoand anti-paramyxoviral agents // J. Virol. Methods. - 1994. - Vol. 48 - No. 2-3 - P. 257-265.

Author's address

Doctor of Biological Sciences Eropkin M.Yu.

Head of the Laboratory of Evolutionary Variability of Influenza Viruses, Research Institute of Influenza, SZO RAMS 198096, St. Petersburg, P.O. Box 52
eropkin@influenza.spb.ru

Effect of the drug Influcid in vitro against the pandemic strain of 2009 A (H1N1) "swine" ("Mexican") flu / M.Yu. Eropkin, N.I. Konovalova, V.A. Grigorieva, D.M. Baybus, T.M. Gudkova // Traditional medicine. - 2009. - No. 3 (18). - pp. 14-18.

[To favorites](#)