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ANTIVIRAL AND OTHER PHARMACOLOGICAL PROPERTIES OF THE DRUG INFLUCID: A CRITICAL REVIEW OF CURRENT DATA

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[*QR-code*] Considering the wide spread of known and new pathogens of acute respiratory viral infections (ARVI), it is important to search for drugs that have a wide spectrum of antiviral activity and additionally the ability to act pathogenetically. Equally important is the confirmation of therapeutic activity in drugs known for their effectiveness and safety. In order to monitor the susceptibility of ARVI pathogens to the antiviral drug Influcid, a series of in vitro studies was performed. Materials and methods: classical virological controlled studies were performed using current strains of human influenza virus (A/Moscow/225/2019 (H1N1)pdm09 and B/Moscow/17/2019) and MDCK cell culture. Antiviral, cytoprotective (against viruses, antiviral and antipyretic drugs) properties of the Inflicid were evaluated by changing the logarithm of the tissue cytopathic infecting dose, which causes 50% of cells to be damaged (lgTCID50), as well as by changing the optical density when using tetrazolium dyes. Results and discussion: Influcid dose-dependently suppressed the reproduction of epidemic strains A/Moscow/225/2019(H1N1)pdm09 and B/Moscow/17/2019 by 50% at concentrations of 0,34 and 0,69–0,86% vol., respectively. Also, the Influcid reduced the reproduction of human influenza virus strains: A/New Caledonia/20/99(H1N1), A/Victoria/35/72, Wisconsin/67/05(H3N2) and B/Malaysia/2506/04, reducing the titers by 0,5–2,5 lgTCID50 and Dengue virus DENV-2/RUS/TH-Novosibirsk02/2012. Influcid exerted a cytoprotective effect on cells infected with A/IIV-Moscow/01/2009 (H1N1)pdm09, increasing the proportion of surviving cells by 4–52,0%. For cells infected with the pandemic strain A/California/07/09(H1N1)pdm09, there was a significant decrease in the cytopathic effect of the virus up to full protection with the «preventive» scheme of the drug application. In addition, the Influcid protected cells from the action of herpes viruses types I and II, type III adenovirus, parainfluenza virus, seasonal coronavirus and respiratory syncytial virus, toxic doses of umifenovir and rimantadine, and did not increase the cytotoxicity of acetaminophen and ibuprofen. Conclusion: having a combination of confirmed antiviral activity against current epidemic virus strains, cytoprotective action, the ability to exert a pathogenetic (immunomodulatory and anti-inflammatory) effect, as well as a favourable safety profile, Influcid may be a relevant and promising drug in the arsenal of medical practitioners.

Keywords: Influcid, specific antiviral activity, respiratory viruses, cytoprotection, virucidal activity, the human influenza viruses.

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According to data of the Ministry of Health of the Russian Federation as of 2018 in the country there were registered almost 3.5 mln cases of ARVI and influenza representing more than 92% of all cases of infectious diseases and 27% of the overall incidence rate (diseases of all classes diagnosed for the first time). The number of patients with ARVI and influenza in 2018 equaled to 20% of the total population of the Russia. Data for the pediatric population are more impressive: almost 20.5 mln cases of ARVI and influenza represented 45% of all cases of diseases with newly proven diagnosis and 79% of the number of all children (henceforward at the age of up to 14) [1]. According to rough estimates of the Rospotrebnadzor the economic burden of influenza and ARVI in 2018 exceeded RUB 520 bln [2]. In 2019, the incidence decreased by 3%, the number of ARVI and influenza cases among the total population of Russia reached approximately 29.9 million cases, and in the pediatric population - approximately 19.8 million [3].

Taking into account the pandemic associated with the SARS-CoV-2 virus, not without interest are the data on the number of ARVI cases in Russia and Moscow for the first 4 months of 2020 as compared to 2019. During the specified period the number of registered cases of ARVI and influenza constituted 13.5 million among total population and 8.3 million among children, which were even less than in the previous year by 3 and 7%, respectively. For the largest and most complicated from the perspective of epidemic city of Russia, the sickness levels were determined as 1.2 million for the total population ("-2%" compared to the previous year) and 699 thousand cases among children up to 14 years old ("-8%" compared to 2019).

Peak levels of the monthly incidence rate were registered in February 2020 for all discussed population groups in Moscow and across the country. After adoption of tough anti-epidemic measures both in Russia and Moscow there was recorded a significant decrease in the number of reported cases of ARVI. In Moscow the restrictions were adopted earlier, whereby as early as March 2020 it was possible to achieve a decrease in the incidence rate among the total population and children by 31 and 40%, respectively, compared to the peak rates. In April 2020, against the background of compliance with sanitary and anti-epidemic regime there was noted the further decrease in the number of ARVI cases by 59% of total and by 82% in children compared with the peak rates, which exceeds the incidence rate in April 2019 by a factor of 1.6 and 2.2. Generally, in Russia the effect was noted also, however, significant reduction was achieved in April 2020 only (by 55% of total and by 71% among children as compared to February rates, which exceeds the natural reduction in the burden of disease in April 2019 by a factor of 1.2 and 1.7 (calculated by authors according to [3] data).

In accordance with the data given above, even very tough and closely followed anti-epidemic measures can't prevent ARVI spreading completely, so the matter of its treatment continues to be relevant. The situation with COVID-19 showed that even at the modern level of science, under condition of coordinate efforts of the state and leading research groups, development of a new drug requires a lot of time and sources. With that, need for an adequate treatment, not only etiotropic but also pathogenetic, rises exponentially. For that reason, all related parties pay attention to already registered drugs having confirmed specific antiviral activity and known spectrum of pharmacological effects. A very important positive point in this case is that with respect to such drugs there is a great experience of use, proper evaluation of the drug-drug interaction data and other aspects of pharmacological safety in the total population and in specific categories including in children. One of such drugs is Influcid.

The drug Influcid is developed and manufactured in Germany by the pharmaceutical company Deutsche Homöopathie-Union DHU-Arzneimittel GmbH & Co. KG. In 1978 the drug underwent international registration and is currently registered and widely used in 25 countries of Europe, Asia, the Middle East and South America (according to the manufacturing company's data). In Russia the drug was registered in 1996 with indications for use such as prevention and treatment of influenza and other ARVIs (quoted after the instruction for medical use [4]).

As active pharmaceutical ingredients Influcid contains complex of pharmacologically active substances of herbal and mineral origin* within the range close to the lower limit of pharmacological concentrations (10-1-10-5 mass fractions). From literature data it is known that the drug has a strong anti-inflammatory effect and contributes to the proven decrease in the intoxication syndrome manifestation (increase in body temperature, adynamia, appetite and sleep disorders, irritation, sweating, headache and muscle pain) and catarrhal syndrome (sore throat, cough, rhinitis, hoarseness) attributable to ARVI of different etiology [5-9].

It is known that on the background of wide use of antiviral as well as antibacterial drugs a resistance to them may form [10]. This fact supports a high importance of the regular monitoring of susceptibility of ARVI pathogens to leading anti-viral preparations.

Materials and methods of the study

Evaluation of the susceptibility of influenza viruses to the drug Influcid was conducted by the Federal State Budgetary Institution “National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya” of the Ministry of Health of the Russian Federation during the period from December 2019 to January 2020. In the course of this work employees of the Laboratory of Etiology and Epidemiology of Influenza performed the non-clinical controlled prospective trial “Study of antiviral properties of the drug Influcid against current strains of human influenza A and B viruses as well as its cytoprotective properties in vitro”. All experiments performed within with study were conducted using control (negative and positive). In view of the fact that in vitro studies use limited number of observations, pre-calculation of the sample size was not performed.

Statistical processing and analysis of the data obtained was carried out using Statistical Package Statistica 10.0. Due to small volume of the sampling during analysis of the findings the non-parametric tests were used. To evaluate the differences between continuous variables obtained in two different (independent) groups the Mann-Whitney U test was used; while for the frequency analysis of 2x2 contingency tables – the $\chi 2$ test (if expected values are greater than 5) or the Fisher’s exact test (if one of expected value is less than 5). Differences at $p<0.05$ were regarded as statistically significant.

* Aconitum napellus, Gelsemium sempervirens, Cephaelis ipecacuanha, Phosphorus, Bryonia, Eupatorium perfoliatum.

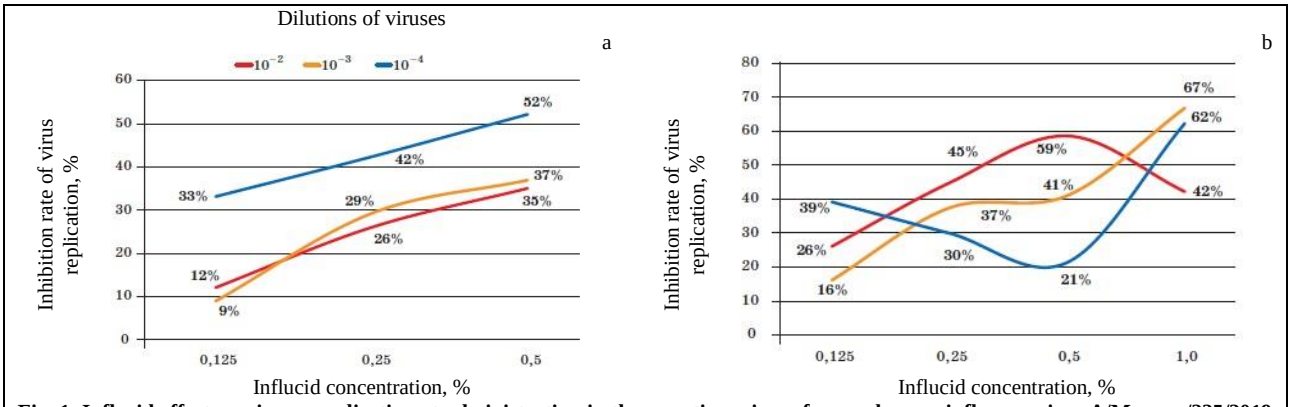


Fig. 1. Influcid effect on viruses replication at administration in therapeutic regimen for: a - human influenza virus A/Moscow/225/2019 (H1N1)pdm09 and human influenza virus B/Moscow/17/2019.

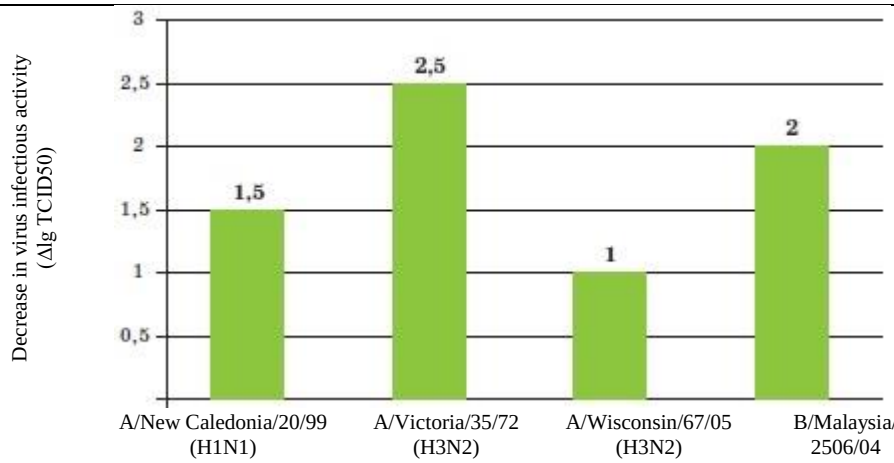


Fig. 2. Suppression the reproduction of human influenza A and B virus strains and in MDCK cell culture according to data of M.Yu. Eropkin et al. [12].

Results

Using control in inverted microscope and colorimetric method it was previously confirmed that Influcid in the wide range of concentrations (0.125-1.0%) doesn't have cytotoxicity at long-term exposure (48 hrs). This fact is completely consistent with studies performed by the Federal State Budgetary Institution "Smorodintsev A.A. Research Institute of Influenza" of the Ministry of Health of the Russian Federation in 2006-2010 within the framework of which was confirmed the Influcid safety at even longer exposure (up to 72 hrs) [11-13].

Evaluation of the specific anti-viral activity of Influcid against strains of human influenza A and B viruses (A/Moscow/225/2019 (H1N1)pdm09 and B/Moscow/17/2019) current in 2020 was conducted using enzyme-linked immunosorbent assay (ELISA). For the studies MDCK culture cells were used. There was studied the ability of Influcid to block replication of viruses at concentrations of 0.125%; 0.25%; 0.5%; 1.0% at its therapeutic application. For this purpose, simultaneously the Influcid and viruses (at working doses of 2-5 lg of the tissue cytopathic infecting dose, which causes 50% of cells to be damaged - TCID50*) were added to the special prepared cells. Anti-viral effect of Influcid was evaluated 24-48 hrs later by optical density (OD) at 450 nm using Biotek, Synergy HT spectrophotometer.

Influcid suppressed the replication of the studied viruses. Based on the set of experiments data a dependency graph of viral replication rate against drug dose (Fig.1) was made, which allowed to calculate the average values of the minimal drug concentration inhibiting viral activity by 50% (MIC50). The anti-viral activity of Influcid was dose-dependent. Suppression of reproduction of the pandemic strain of A/Moscow/225/2019(H1N1)pdm09 virus and the epidemic strain of B/Moscow/17/2019 virus by 50% at concentrations of 0,34 and 0,69–0,86% vol., respectively, was observed.

Presented results correspond to the data obtained from the studies performed by the Federal State Budgetary Institution "Smorodintsev A.A. Research Institute of Influenza" of the Ministry of Health of the Russian Federation in 2006-2010. According to M.Yu. Eropkin et al., at the evaluation of the drug anti-viral activity using hemagglutination assay (HA)** Influcid inhibited the reproduction of human influenza virus strains: A(H1N1) - A/New Caledonia/20/99(H1N1), A/Victoria/35/72 and Wisconsin/67/05(H3N2) as well as influenza B virus - Malaysia/2506/04, reducing the titers by 0,5–2,5 lg (Fig. 2) [12,13].

In 2015-2016, within research and development aimed to search of the candidate etiotropic formulations to treat patients with Dengue fever at the “Collection of Microorganisms” Department of the State Research Center of Virology and Biotechnology «Vector» of Rospotrebnadzor led by O.V. Pyankov there was performed the study of inhibitory activity of the drug Influcid in vitro. In the course of the work there was used the strain of Dengue virus DENV-2/RUS/TH-Novosibirsk02/2012 (subtype 2) obtained from the “State Collection of Microorganisms” of the State Research Center of Virology and Biotechnology «Vector» of Rospotrebnadzor.

* Virus dose that upon contamination can cause a cytopathic effect in 50% of the studied preparations of cell culture/tissue.

** Due to one of the surface proteins (hemagglutinin) influenza virus can induce agglutination of erythrocytes (hemagglutination) and its rate depends on the virus concentration.

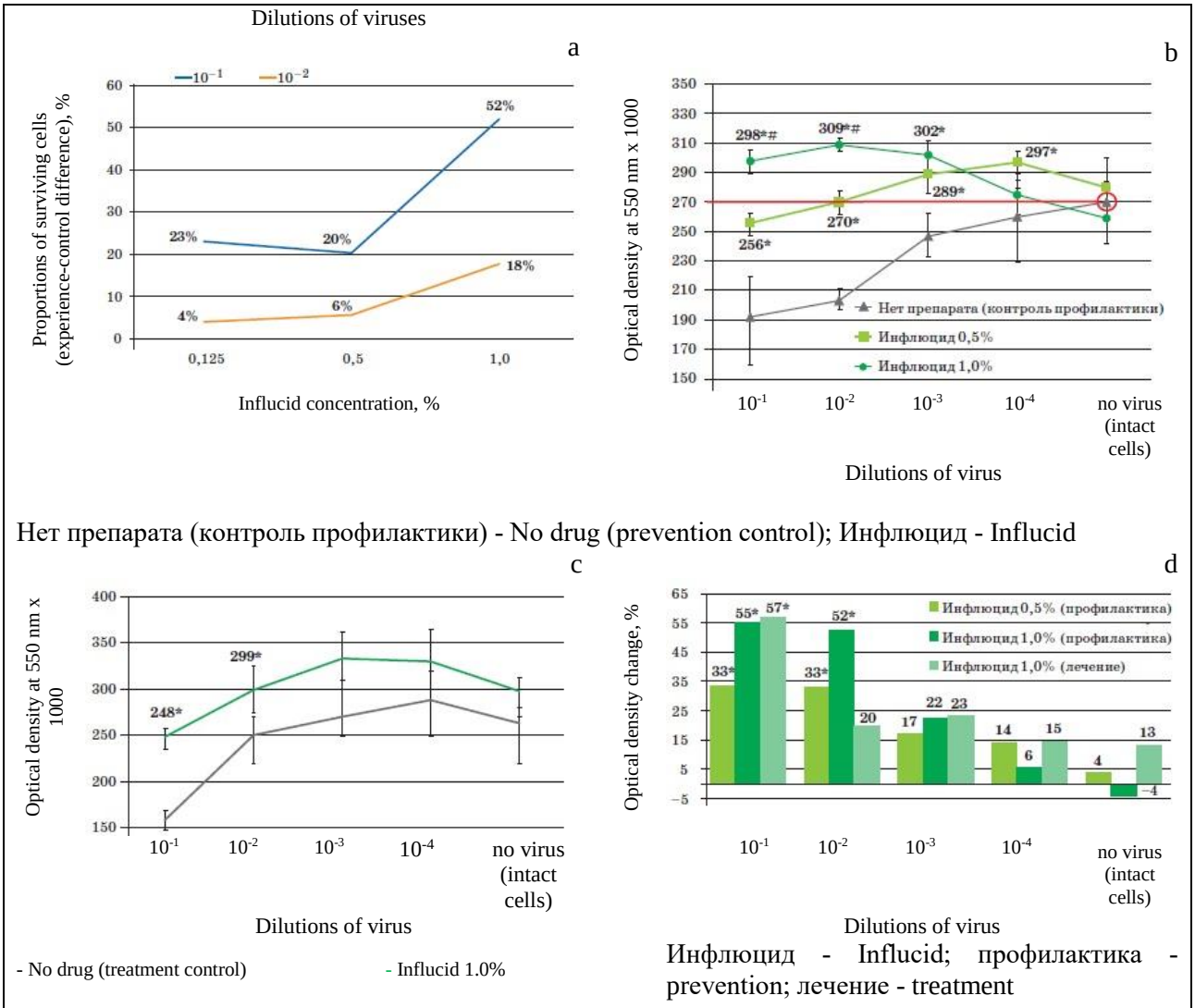


Fig. 3. Influcid cytoprotective activity manifestation: f - rates of the survived cells infected with A/IIV-Moscow/01/2009 (H1N1)pdm09 compared to the control without treatment in XTT-test (data of E.I. Burtseva); b and c - optical density in cell samples when Influcid is used for prevention (b) and treatment (c); d - optical density change vs. control (b-d according to data of M.Yu. Eropkin et al.); in Fig. 3b and 3c values are presented as M±SD (where M is point value, whiskers' range - SD meaning); *differences are statistically significant compared to the control group (U-test, $p<0.05$); # differences are statistically significant in the Influcid 1.0% group compared to the Influcid 0.5% group (U-test, $p<0.05$).

To determine effect of Influcid on infective ability of the virus the drug at concentrations ranging (0.12-3.75%) was added to Vero cells an hour after infection with Dengue virus. Within 10 days Influcid has influenced the life cycle of viruses in infected cells, after which the liquid containing the virus was used for virological analysis.

After Influcid impact there was evaluated the ability of Dengue virus to cause cytopathic effects (CPE)* in newly infected Vero cell cultures and the titre was presented as (TCID50/ml) [14].

According the data obtained, Influcid reduced CPE beginning from the concentration of 4.69 mg/ml (0.5%) and decreased by 1.61 lg CPE at 18.75 mg/ml (1.9%). It was determined the Minimum 50% Inhibitory Concentration (MIC50) of Influcid which was equal to 2.1 mg/ml (0.21%). Values of the IC90 (inhibitory concentration reducing CPE by 1 logarithm i.e. by a factor of 10) and IC99 (reducing of CPE by 2 logarithms i.e. by a factor of 100) were also determined and were equal to 9.3 mg/ml (0.93%) and 30.9 mg/ml (3.1%), respectively [12].

The additional method to confirm the anti-viral activity of Influcid was the evaluation of the drug ability to decrease manifestation of the cytopathic action of influenza viruses. Principle of the method consists in use of the special tetrazolium dyes (MTT and XTT) which can be metabolized by the fully functioning living cells in the process of their vital life. As the result of reduction reaction the cells become colored. In the event of a damage by viruses (cytopathogenic effect, CPGE) or due to toxic effect of other substances such as pharmaceutical products, the color intensity decreases, which can be assessed by the calorimetric method. This text allows to

evaluate both CPGE of viruses on the cell and ability of drugs to protect the cells against pathogenic effect of viruses or toxicant agents (cytoprotective potential) [15, 16].

Cytoprotective properties of Influcid against MDCK culture cells were studied in two independent experiments using toxicity test with different dyes: MTT and XTT (Sigma). An hour after infection with influenza virus A/IIVMoscow/01/2009 (H1N1)pdm09 (within working dose of 1-7 lgTCID50) the studied drug was added to the cells at concentrations of 0.125%; 0.5%; 1.0%, a placebo equivalent (the Igla MEM medium without Influcid) was added to the control wells. 48 hrs after incubation there was performed a setup of colorimetric toxicity test according to standard practices.

In the comparison of control and test wells with the infected cells it was noted that with the «preventive» scheme of Influcid application the dose-dependent increase in the proportion of surviving cells was observed (Fig. 3a).

* The cytopathic effect manifests as morphological changes in the cell culture, occurring in the process of viral replication in the cells (cell fusion, cell degeneration, focal destruction of cell culture, necrosis etc.).

Antiviral and cytoprotective effect of the "preventive" scheme of Influcid application at concentrations ranges in vitro by the MTT test

Influcid concentration in culture medium, %	Difference lgTCID50 (ΔlgTCID50) at viral pathogens cultivation in the presence of Influcid vs. the control				
	Herpes virus type I HSV1/SPb/248/88	Herpes virus type II HSV2/etalon/2000	Adenovirus type III Ad3/etalon/4120	Pandemic influenza virus A/ California/07/09 (H1N1)pdm09	Pandemic influenza virus A/C - Petersburg/05/09 (H1N1)pdm09
0.5	0.5*	2.55	0.6*	1.15	0.9
0.25	2.7	3.45	2.15	1.0	0.53
0.125	2.8	3.58	3.16	No data	No data

* Pre-incubation with the drug 1 hr, in other cases - 24 hrs.

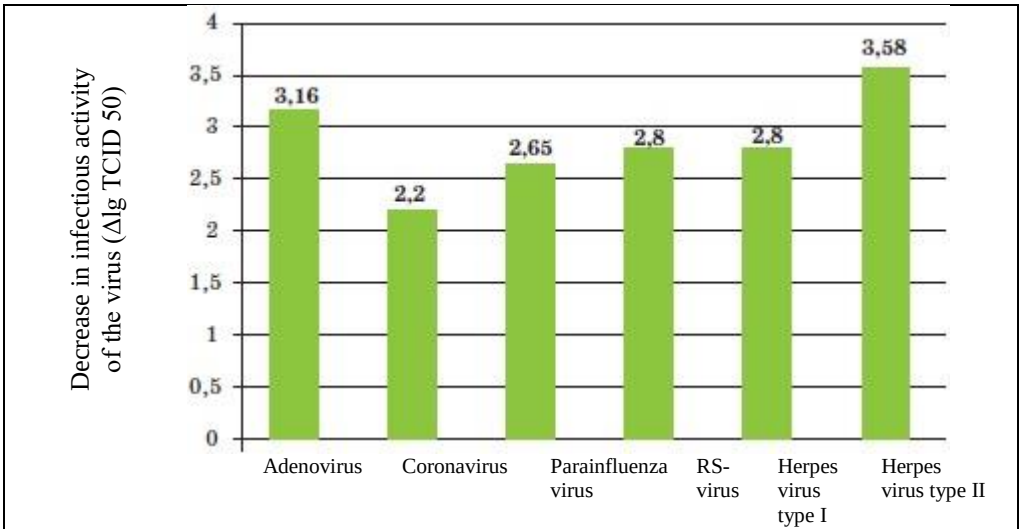


Fig. 4. Antiviral and cytoprotective effect of the «preventive» scheme of Influcid application at the concentration of 0.125% against DNA- and RNA-containing viruses.

In the experiment with the MTT dye, the moderate cytoprotective effect of Influcid was manifested upon infection with the virus at doses of 2.0-3.0 lgTCID50 (4-19%). In the experiment with the XTT, the cytoprotective effect was manifested in the case of cells infection with the virus at doses of 1.0-2.0 lgTCID50 (increase in the proportion of surviving cells by 23-52% and 4-18%, respectively). Findings obtained from this study confirm that Influcid has strong ability to protect cells infected with various viruses and are compatible with findings of the studies performed by the Federal State Budgetary Institution "Smorodintsev A.A. Research Institute of Influenza" of the Ministry of Health of the Russian Federation led by M.Yu. Eropkin. In estimating of optical density in the MTT test it was found the strong cytoprotective effect of Influcid against the cells infected with pandemic strain A/California/07/09 (H1N1)pdm09. Substantial decrease in CPGE of the virus was noted not only at "preventive" administration (cells incubation with Influcid within an hour before their infection with the virus) but also at "therapeutic" option: Influcid adding an hour after cells infection with the virus (Fig. 3b, c). With the "preventive" scheme the cytoprotective effect of the drug was characterized by the strong dose-dependence - Influcid at the concentration of 1.0% definitely had stronger effect than at the concentration of 0.5% (Fig. 3b). In samples treated with Influcid 1.0% an optical density was found at the level of fully living and healthy cells (Fig. 3b) [12, 13].

Moreover, Influcid reduced substantially the CPGE of herpes viruses types I and II, adenovirus type II, parainfluenza virus, seasonal coronavirus and respiratory syncytial virus* in estimating of the cytopathic reaction by the MTT test and with the "preventive" scheme of the drug administration (Table 1, Fig. 4)** [13].

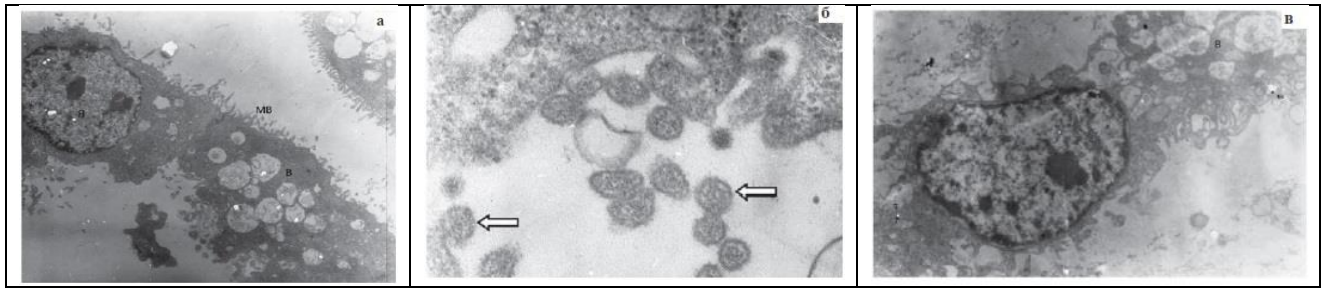
In the course of the scientific research there was studied the effect of Influcid at the concentrations of 0.125 and 0.5% on morphological changes in the MDCK culture cells infected with the pandemic human influenza strain A/IIV-Moscow/01/2009 (H1N1)pdm09 by the method of Transmission Electronic Microscopy (TEM). During the study of ultrathin section of cells there were founded viral particles both gemmated of plasma membrane and in intercellular space (Fig. 5a, b). MDCK cells, infected with influenza virus and treated with Influcid solution of 0.125% vol., usually preserved

their ultrastructure, at the same time viral particles in the cells were not found (Fig. 5c).

It is known that both in vivo and ex vivo Influcid has the ability to induce the interferons (IFN) production in white outbred mice at a level comparable to the known synthetic inducers of IFN. Production levels in vivo were 40-160 U/ml and up to 80-320 U/ml 24 and 48 hrs after administration, respectively, and ex vivo - in the titre 64-128 U/ml [17, 18].

* Adenovirus - HAdV3/etalon/4120 strain causing ARVI, febrile pharyngitis and pharyngoconjunctival fever; parainfluenza type III is associated with bronchiolitis and pneumonia, strain HPIV-3/2235; seasonal coronavirus - strain HCoV-lp/3482; respiratory syncytial virus - strain RSV/4317; herpes virus type I - strain HSV1/SPb/248/88; herpes virus type II, associated with immunodeficiency states and responsible for frequent recurrences, often resistant to administered etiotropic treatment - strain HSV2/etalon/2000.

** Data on influenza viruses obtained from the MDCK cell culture, on adeno- and herpes viruses from the L-41 cell culture (a human macrophage line of the monocytic and leukemia origin), the others - from the A-549 culture (an epithelioid line originated from the human lung carcinoma).



а - a, б - b, в – c.

Fig. 5. Photos made by TEM: a - ultrastructure of the intact cells monolayer of the MDCK tissue culture; b - ultrastructure of the cytoplasm region and plasma membrane with budding particles of the influenza virus and virions in the intercellular space (marked with arrows); c - ultrastructure of the cell of the MDCK tissue culture infected with the influenza virus with 0.125% vol. of Influcid. General view of the cell with vacuolated cytoplasm, multiple growths on the changed plasma membrane. Viral particles in cytoplasm or plasma membrane are not found. MB [MV] - microvilli, Я [N] - nucleus, B [V] - vacuole.

At the same time in literature there are no data on the ability of Influcid to have a direct damaging effect on respiratory viruses. This fact became the ground for studying the viricidal action of Influcid against the pandemic influenza virus A/IIVMoscow/01/2009 (H1N1)pdm09. Evaluation of the drug's ability to inhibit influenza virus in solution was carried out by comparing the results of the infectious activity of the virus exposed to Influcid at concentrations of 1.0%, 5.0%, 10% for 3 hours and 24 hours with a negative control (intact virus). In view of the fact that formulation of Influcid (drops) contains ethyl alcohol, simultaneously there was carried out a control of reaction with the ethyl alcohol at concentrations equivalent to the drug concentrations chosen for the test (positive control) to take into account possible inhibitory effect of the ethyl alcohol on the viral viability. Infectious titer of the virus was determined in the hemagglutination reaction.

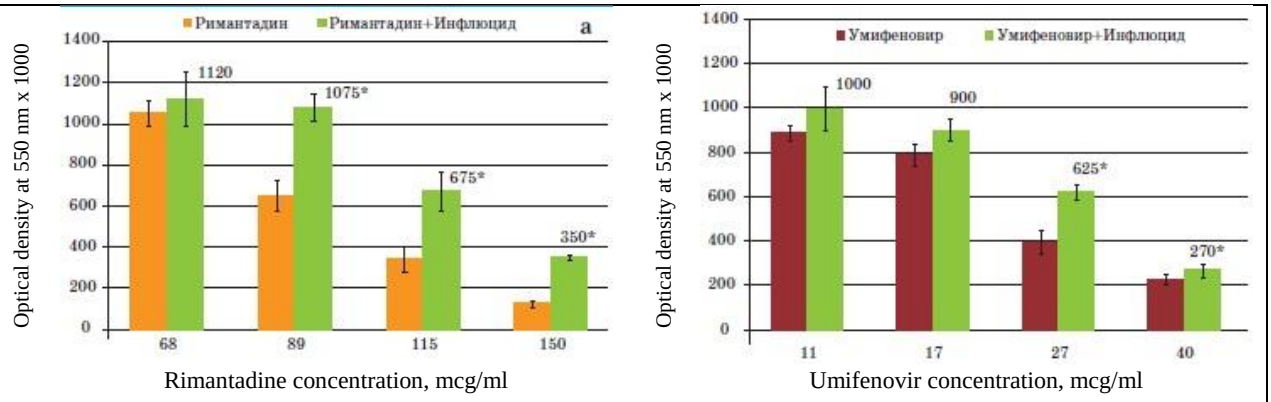
According to the data obtained in 1st and 2nd incubation regimens Influcid decreased the infectious titers in all studied concentrations beginning from 0.5 lgTCID50 at the concentration of 1.0%. Maximum decrease in the infectious titer compared to the negative control (1.6 lgTCID50) could be achieved at incubation for 24 hrs at the Influcid concentration of 10.0%. Compared to the effect of the equivalent concentrations of alcohol in the positive control the difference was 1.6-1.8 lg TCID50 on Influcid exposure at the concentration of not less than 5%.

Taking into consideration the showed cytoprotective effect of Influcid, we studied the drug effect on the vital characteristics of cells when the drug administered concurrently with a range of other medications most frequently used as a therapy for ARVI and influenza.

In the Federal State Budgetary Institution "Smorodintsev A.A. Research Institute of Influenza" of the Ministry of Health of the Russian Federation, using photometric method involving MTT and Vario-scan analyzer at the characteristic wavelength of 550 nm, there was studied the cytoprotective effect of Influcid (0.125%) against the toxic cellular response in the culture to the high doses of the antiviral preparations of Rimantadine (L-41 cells) and Umifenovir (A-549 cells). According to the data obtained, at the boundary concentrations of Rimantadine and Umifenovir, Influcid in the treatment regimen protected the cells against dysfunction and death - an optical density in the samples affected by Influcid was statistically higher than in the samples affected only by cytotoxic concentrations of the antiviral preparations (Fig. 6).

In the experiment using the toxicity test with the MTT (Sigma) dye there was studied the toxicity of the co-use of Influcid with antipyretic drugs recommended for pediatric use like paracetamol (acetaminophen) and ibuprofen. Based on the results of literature search we used concentrations within the range of 0.03-1.0 and 0.045-1.0 mcg/ml for acetaminophen and ibuprofen, respectively [19].

In all series of the experiments at the simultaneous effect on the antipyretic drugs' cells and Influcid there were found no cytotoxic effects (neither visually under inverted microscope, nor at the colorimetric test design). It may be account for by the fact that



Римантадин - Rimantadine; Римантадин+Инфлюцид - Rimantadine+Influcid; Умифеновир - Umifenovir; Умифеновир+Инфлюцид - Umifenovir+Influcid;

Fig. 6. Change of optical density in MTT test at the simultaneous addition of Influcid (0.125%) as well as toxic concentrations of Rimantadine (a) and Umifenovir (b) to the cell culture.

Values are presented as M±SD (where M is point value, whiskers' range - SD meaning); *intergroup differences are statistically significant (U-test, $p<0.05$).

Influcid having high security and absence of cytotoxicity, even at the highest concentration (1.0%), does not potentiate the toxic effect of the antipyretic drugs used at the maximum cytotoxic concentrations (1.0 mcg/ml).

Discussion

This article was written during the height of first wave of contagion caused by the novel for human population virus - SARS-CoV-2. At the beginning of June 2020 when searching PubMed* using keyword "COVID-19" among titles or abstracts of articles, 19.001 publications have been reported for the last year. In more narrow search using combinations "COVID-19 + therapy" and "COVID-19 + treatment" 1050 and 2598 results were found, totally almost 20% of all publications were devoted to the different aspects of treatment of this infection. By comparison, in the search for publications for 2009-2010 using key combination "pandemic H1N1" 2275 results were found, of which with the refiners "therapy" and "treatment" totally 113 results were found (4% of the overall number of the findings).

As is seen from the given data, even after the world already have faced the pandemic threat in 2009, emergence of new and pretty much dangerous infection has raised before us the necessity to find not only individual effective etiotropic medicines but also strategically important task to develop a full complex of the treatment. Within this task special attention is paid to the combination of efficiency and safety not only in view of direct toxic effects but also potential for use in different patient population, possibility to combine with other types of treatment, ease of use, issues of the pathogen resistance etc. Under these circumstances relevant are not only the newly developed therapeutic decisions but already well-established medicinal products. Related to such medicinal products a significantly bigger scope of knowledge is accumulated. And what is especially important apart from the data obtained in the course of well-controlled clinical trials, there is a great experience in routine use, which to an important degree update the common knowledge base due to use in different patient population and information about safety of the treatment.

According to the results presented in this review, Influcid showed the ability to have specific antiviral action against influenza viruses. It is important that over the past decade the drug has stable antiviral activity and broad spectrum of action against influenza, including against the most recent strains to date - A/Moscow/225/2019 (H1N1)pdm09 and B/Moscow/17/2019.

It is known, that Influcid among others pharmacological properties is the IFN inducer with activity comparable to the most common synthetic medicinal products: Tilorone (Amixin), Meglumine acridone acetate (Cycloferon), Sodium ribonucleate (Ridostin) [18]. At the same time, MDCK cells being IFN-competent (IFN-sensitive) themselves are not producers of the mentioned cytokines. In connection therewith quite rightly is the assertion that the pharmacological activity of Influcid more fully and clearly may be recognized during in vivo studies. Results of such works give the evidence that the coefficient of protection of laboratory rodents in the context of experimental flu-like infection was on the average of 1.7 and the protective index - 41% [11], and based on the other researchers' findings these values are even higher - 3 and 80%, respectively [18].

Based on the data presented in this article it is clear that intensity of the specific antiviral action cannot be explained only by the moderate virucidal

potential of Influcid. At the same time, it should be noted that the wide range of pathogens (RNA-viruses: influenza, parainfluenza, coronavirus, Dengue fever virus, DNA-viruses: adenovirus, herpes viruses type I and II), against which activity was shown,

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allows to single out the cytoprotective activity from the spectrum of the pharmacological properties of Influcid. That particular property has been proven in numerous studies with viruses and additionally confirmed in experiments using cytotoxic concentrations of series of antiviral and antipyretic drugs. It is clear that this effect is implemented due to non-specific mechanisms that may form the basis for the absence of signs of the formation of strains resistant to Influcid [11, 13].

One more important aspect of the pharmacological properties of Influcid is the ability to have complex immunomodulatory and anti-inflammatory action, proven by a number of clinical studies. So, the use of Influcid in the complex therapy of measles and chickenpox in children within the comparative clinical studies made it possible to reduce the duration of fever by 2.8 days, catarrhal syndrome by 5.4 days, and improve the hyperactivation of interferonogenesis faster, especially in relation to IFN γ, as well as apparent imbalance of the main pro-inflammatory and anti-inflammatory cytokines (IL-4, IL-1β, IL-6, IL-8, IL-2, IL-10, TNF-α) [20, 21]. In groups of children with ARVI who received Influcid as a part of the complex therapy, the duration of the disease was significantly reduced by 2-3 days, there was statistically reduced the frequency and the duration of use of antipyretics (paracetamol), mucolytics (ambroxol) and decongestants (oxymetazoline), with a less frequent need to use antimicrobial drugs, which allows to reduce the risks of polypragmasy and improve the safety of ARVI and influenza therapy [5-7]. In addition, against Influcid administration the improvement of hormonal and mediator balance took place faster, in particular recovery of functional activity of the adrenal cortex [9]. In all clinical studies conducted, all participants (physicians, patients and their representatives) noted the high safety and good tolerance of Influcid in both adults and children.

It should be noted that when monitoring the antiviral activity of Influcid in 2019–2020, all experiments were carried out on cell cultures, which, as mentioned above, may not fully reveal the antiviral potential of the drug, that is a certain restriction. Authors believe that the further in vivo study of the antiviral and cytoprotective activity of the drug is advanced.

Conclusions

1. Influcid has the specific antiviral activity against the wide spectrum of influenza viruses and also is active against a number of RNA- and DNA-containing viruses of ARVI and other infections which was proved in the course of studies carried out in three leading research centers in Russia.
2. The specific antiviral activity of Influcid against influenza viruses remains for the last 14 years, in particular Influcid is active against current strains of influenza virus during the 2019-2020 season.
3. Influcid possess the ability to protect cells against pathogenic action of influenza viruses and other ARVI pathogens (cytoprotective action).
4. Influcid does not increase the toxicity of the antipyretic drugs based on Ibuprofen and Acetaminophen widely used in particular in pediatrics, and protect the cells against the pathogenic influence of the toxic doses of a number of antiviral drugs (Rimantadine, Umifenovir).
5. While possessing combination of antiviral activity, cytoprotective action, the ability to exert a pathogenetic (immunomodulatory and anti-inflammatory) effect, as well as a favourable safety profile, Influcid can be a relevant and promising drug in the arsenal of practicing physicians.

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