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Combined therapy of influenza with antiviral drugs with a different mechanism of action in comparison with monotherapy

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Abstract

Increasing of drug-resistance in influenza virus is a new serious challenge for global healthcare. Existing antiviral therapy is incompletely effective and there is a need for improving the efficacy of current treatment strategies. One of the possible ways to overcome the resistance of influenza virus is to combine the use of antiviral drugs with various mechanisms of action. To assess the efficacy of influenza A (H1N1) pdm09 monotherapy with antiviral drugs (oseltamivir or umifenovir) versus combination of two antiviral drugs with different mechanism of action (oseltamivir+kagocel and umifenovir+kagocel), an open comparative study was conducted. All 200 patients with confirmed by RT-PCR analysis diagnosis influenza A (H1N1) pdm09 were randomly sorted to four group of 50 persons. Group 1 received monotherapy with an influenza virus fusion inhibitor umifenovir (Arbidol); Group 2 - monotherapy with a competitive inhibitor of influenza´s neuraminidase enzyme oseltamivir (Tamiflu); Group 3 - an influenza virus fusion inhibitor umifenovir (Arbidol) in combination with an interferons inductor Kagocel; Group 4 - a competitive inhibitor of influenza´s neuraminidase enzyme oseltamivir (Tamiflu) in combination with an interferons inductor Kagocel.

The clinical efficacy of antiviral therapy was assessed in dynamic with the following parameters: the time of temperature normalization, the time of intoxication and catarrhal symptoms reducing, the frequency of the main clinical manifestations and the incidence of complications. The end point in the duration of symptoms assessing was the absence of symptoms of the disease within 24 hours. All therapy was within the limits of registered medical use for the drugs. Patients groups were equivalent in terms of admission to hospital, age, sex, and the length of treatment from the onset of the disease.

The study demonstrated that the combination of drugs oseltamivir (Tamiflu) and umifenovir (Arbidol) with the antiviral agent Kagocel significantly increases the therapeutic efficacy compared with the corresponding monotherapy. Efficiency was proved with reducing the incidence of major clinical manifestations of indicators, reducing the duration of clinical symptoms and a decrease in the frequency of aggravations.

Keywords: influenza, A (H1N1) pdm09, resistance, combined therapy, monotherapy, kagocel, umifenovir, oseltamivir

INTRODUCTION

Influenza and other acute respiratory viral infections are the most mass socially significant diseases. In recent years, epidemics caused by respiratory viruses have been characterized by a severe course of the disease and a high mortality rate from these infections worldwide. According to the World Health Organization, in spite of the mass vaccination against influenza in developed countries, 3–5 million people every year become ill only with severe forms of influenza in the world. In the Russian Federation, the incidence of influenza and other ARVI is 20–40 million people per year, including 40–60 % of children. Influenza infection (caused by *Influenzavirus*, *B* and *C* genus of the *Orthomyxoviridae* family) plays an important role in exacerbating chronic diseases and developing complications, which are often the cause of patient's death: it has been shown that the presence of chronic cardiovascular or lung diseases increases tenfold the risk of death due to influenza [1–3].

Given the epidemiological relevance, WHO recommends vaccination against influenza. However, there is a number of factors that put in doubt the vaccination as the only method of influenza prevention. First, incomplete population coverage by vaccination. Second, vaccines cannot completely eliminate the incidence of influenza, but reduce the risk of severe forms of disease, complications and fatal outcome. Third, the epidemic process can be caused by various variants of influenza viruses, and the strain of vaccines, which is recommended by WHO twice a year, does not always correspond to the current circulating epidemic strains. Fourth, under the action of collective immunity, selection of the virus escape mutants ("genetic drift") occurs, so that by the end of the current epidemic season influenza viruses may differ markedly from their predecessors at the beginning of the same epidemic season. Fifth, as a result of the genetic reassortment and adaptation of zoonotic influenza viruses to mammalian cells, new subtypes of the virus with a pandemic potential may occur. The most epidemic and pandemic potential belongs to type A influenza virus, which circulates both in human and in animal population (wild birds and poultry, pigs, bats) in the form of various subtypes. In recent decades, it has become apparent that overcoming the species barrier for these viruses is not a rare phenomenon [4–5]. Development of vaccines against new strains of influenza virus would require a certain amount of time, financial costs; and chemotherapy will be the only means to restrict the spread of the disease.

Etiotropic drugs are the basis of antiviral influenza chemotherapy. However, their widespread use in clinical practice leads to the emergence and rapid increase of resistant viral strains. Therefore, the issue of finding new and optimizing use of existing antiviral drugs remains relevant [6–10]. According to experts, one of the possible ways to overcome the development of resistance is the use of combinations of two or more antiviral drugs with different mechanisms of action. The advantages of monotherapy in this case may include not only a synergy of action, but first of all the complexity of the formation of a genetic barrier for the emergence of resistance, which in this case requires multiple mutations in the genetic structure of the virus.

The purpose of this work is to compare the therapeutic efficacy of oseltamivir (Tamiflu®) and umifenovir (Arbidol®) in the monotherapy and in combination with an antiviral drug, widely used in Russia and in a number of other countries, in the composition of etiotropic ARVI and influenza therapy, namely an inducer of interferons Kagocel ® [11].

METHODS

Scientific and research work was carried out from December 2013 to March 2016 by the staff of the Department of Infectious Diseases, located on the basis of Regional Clinical Hospital No. 2 of Vladivostok. The study included 200 adult patients admitted to the hospital with a preliminary diagnosis of "moderate influenza". Of 200 patients aged from 21 to 60 years (26.5□4.6 years), 100 (50 %) were men (21–60 years old, 31.2 □ 4.2 years), 100 (50 %) – women (23–60 years old, 34.3□4.3 years). Influenza-infected pregnant women were not included in the study. All patients had no history of influenza vaccination over the current epidemic period. The presence of influenza A infection was confirmed by a nasopharyngeal swab test using real-time reverse transcription polymerase chain reaction (RT-PCR). Patients were hospitalized at various periods from the disease onset: from several hours to 3 days.

Therapy: All schemes of antiviral drugs use were prescribed strictly according to the instructions for the drugs use. Umifenovir (Arbidol, *Pharmstandard*, Russia) is an inhibitor of influenza virus fusion and endosome membranes. It was used in the form of tablets (100 mg) in the monotherapy according to the following scheme: 200 mg 4 times/day × 5 days. Neuraminidase inhibitor oseltamivir was used in a 75 mg capsule formulation (Tamiflu, *F. Hoffman-La Roche Ltd*, Switzerland) in the monotherapy according to the following scheme: 75 mg 2 times/day × 5 days. Interferons inducer kagocel (Kagocel, no INN, only the trade name, *Nearmedic Pharma*, Russia) was used in the form of tablets (12 mg) only in combination with umifenovir or oseltamivir (see above) according to the scheme: 24 mg 3 times/day × 2 days, 12 mg 3 times/day × 2 days.

Study design: prospective open comparative study. All patients were randomly divided into 4 groups of 50 people after signing the informed consent form for participation in the study. Group 1 (30 men, 20 women, age 28.1 ± 2.7 years; hospitalization from the onset of the disease 2.0 ± 0.5 days) received monotherapy with umifenovir; group 2 (23 men, 27 women, 29.2 ± 3.1 years, 2.7 ± 0.8 days) – monotherapy with oseltamivir; group 3 (26 men, 24 women, 23.6 ± 2.9 years, 1.9 ± 0.6 days) – combined therapy with umifenovir and kagocel; group 4 (21 men, 29 women, 26.8 ± 3.0 years, 1.7 ± 0.6 days) – therapy with oseltamivir and kagocel. The control group was not formed for ethical reasons.

Clinical efficacy criteria for antiviral drugs: An assessment/analysis of the term of temperature normalization, reduction of intoxication, catarrhal symptoms, frequency of the main clinical manifestations and frequency of complications after the end of treatment compared with the condition before the start of treatment. For the assessment of the duration of symptoms, the end point was considered to be their absence within 24 hours.

Statistical processing of the obtained results was carried out using the methods of the empirical (calculating mean values and standard deviations) and parametric (Student's *t*-test) approaches using STATISTICA 6.0 software package (*StatSoft*, USA).

RESULTS

The severity of the disease was average in all patient groups and was characterized by typical clinical manifestations of influenza [3–7]. The clinic was characterized by acute onset (100 %), rapid rise of temperature to subfebrile (34.5 %) or febrile (65.5 %) values. In the acute period of the disease before the start of treatment, patients had symptoms of general intoxication: weakness (100 %), headache (70 %), myalgia (61 %), loss of appetite (54 %), and less rare pain in the eyeballs (9 %). Since day 1–2 of the disease, most patients had catarrhal symptoms: runny nose (89 %), cough (72 %), and sore throat (44 %). Peripheral blood parameters at the height of the disease was characterized by normocytosis (71 %), less frequently leukopenia (15.5 %) and leukocytosis (13.5 %). Clinical symptoms before treatment in all four groups of patients were comparable.

No side effects were observed with chemotherapy use in all patient groups. The decrease of the frequency of clinical manifestations of influenza after the end of treatment is presented in Table 1, the duration of the main symptoms of the disease – in Table 2, the incidence of complications – in Table 3.

Table 1. Frequency of clinical manifestations of influenza before and after chemotherapy [*,] (%)**

Syndrome	Symptom	Before the start of treatment				After end of treatment			
		Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Fever	Subfebrile (37.0 –-37.9 ºC)	36	32	34	36	4	-	-	-
	Febrile (38.0 ºC and above)	64	68	66	64	-	-	-	-
General intoxication	weakness	100	100	100	100	8	2	-	2
	headache	60	72	80	68	-	-	-	-
	pain in the eyeballs	10	14	6	6	4	2	-	4
	myalgia	62	76	76	30	-	4	-	-
	loss of appetite	54	64	50	48	8	2	2	2
Catarrhal	cough	72	86	70	60	6	4	-	2
	runny nose	96	86	80	94	4	-	-	-
	sore throat	50	42	36	48	-	4	-	-

Notes: * Patients of group 1 received monotherapy with umifenovir; group 2 – monotherapy with oseltamivir; group 3 – combined therapy with umifenovir and kagocel; group 4 – combined therapy with oseltamivir and kagocel. Each group included 50 patients.

Table 2. Duration of clinical manifestations of influenza, depending on the antiviral therapy [*]

Parameter	Duration [**], days			
	group 1	group 2	group 3	group 4
Fever	6.0 □ 0.9	3.3 □ 0.6	2.0 □ 0.6	1.9 □ 0.5
General intoxication	5.2 □ 0.5	4.0 □ 0.5	3.0 □ 0.6	2.3 □ 0.6
Catarrhal syndrome	8.2 □ 0.8	5.8 □ 0.6	3.3 □ 0.7	3.3 □ 0.5

Note: * For description of patient groups see the note to Table 1.
** Format of data representation: {mean value} □ {mathematical expectation of variance}.

Table 3. The incidence of complications of influenza, depending on the antiviral therapy *

Complication	Occurrence, %			
	group 1	group 2	group 3	group 4
Pneumonia	10	6	0	2
Myocarditis	2	0	0	0
Sinusitis	8	6	2	2

Note: * For description of patient groups see the note to Table 1.

DISCUSSION

Each stage in the life cycle of the influenza A virus can become a target for the action of antiviral chemotherapy drug. However, under the influence of selective pressure from the chemotherapy, the viral population is gradually enriched with resistant viral variants, and this effect is manifested at all levels of the system organization – from an individual infected cell to large human populations [2, 8–10, 12–14]. The latter effect is most undesirable, since it sharply reduces the possibilities of chemotherapy as a tool for controlling the epidemic process. In order to reduce the intensity of the formation of resistant viral strains, it is recommended to use combinations of etiotropic chemotherapeutic agents with different mechanisms of action or a combination of etiotropic and immunomodulating drugs [15].

Umifenovir was originally considered as an immunostimulant, which increases the activity of phagocytes and normalizes the absolute and relative parameters of immunocompetent cells. However, over the last decade, new experimental data have been obtained and accumulated suggesting that umifenovir has a direct inhibitory effect on the reproduction of influenza A virus, disrupting the fusion of the virion and endosome membranes, and thereby inhibiting the penetration of the nucleoprotein into the cytoplasm of the target cell [16–19].

Oseltamivir is an inhibitor of viral neuraminidase whose tetramers form peplomers on the surface of the virion and whose main function is the enzymatic cleavage of the terminal residue of neuraminic acid from glycans capable of acting as receptors for viral hemagglutinin. The latter is necessary for detachment from "false cellular receptors" and budding daughter virions from the infected cell [20–21].

Kagocel (no INN) is an antiviral drug whose main mechanism of action is the ability to induce the production of its own interferons in the body. The active substance kagocel is a copolymer of gossypol with carboxymethylcellulose (gossypol is a natural polyphenol obtained from cotton); it has the ability to stimulate the production of interferons-alpha and -beta, which have high antiviral activity [13, 22–23].

The findings suggest that umifenovir has less therapeutic efficacy in relation to influenza A virus compared to oseltamivir: the duration of fever (Table 2) and the incidence of pneumonia (Table 3) for group 1 statistically significantly exceeds those for group 2 ($p < 0.05$ and $p < 0.05$, respectively). In the period of convalescence, in group 1, in comparison with group 2, such symptoms as low-grade fever, decreased appetite, rhinitis ($p < 0.05$, $p < 0.05$, $p < 0.05$, respectively) were more common.

The introduction of interferons inducer Kagocel into the therapeutic scheme compared with the corresponding monotherapy with oseltamivir and ufifenovir, reduces the duration of fever (Table 2) ($p < 0.0005$ for groups 1 and 3, $p < 0.005$, for groups 2 and 4), general intoxication ($p < 0.05$ for groups 1 and 3, $p < 0.05$, for groups 2 and 4) and catarrhal syndrome ($p < 0.05$, for groups 1 and 3, $p < 0.05$, for groups 2 and 4), reduces the incidences of pneumonia ($p < 0.05$) and sinusitis ($p < 0.05$). Also, the use of combination therapy reduced the frequency of preservation of such symptoms as weakness (for groups 1 and 3 $p < 0.05$, for groups 2 and 4 $p < 0.05$), decreased appetite ($p < 0.05$, for groups 1 and 3), cough ($p < 0.05$, for groups 1 and 3, $p < 0.05$, for groups 2 and 4) during the convalescence.

The most pronounced synergistic effect of kagocel was observed in combination with umifenovir (group 3 in Tables 1–3). At the same time, practically all the parameters of group 3 were comparable (statistically significantly indistinguishable) from the analogous parameters of group 4 (see Tables 1–3) which received combined therapy with oseltamivir and kagocel.

CONCLUSION

Treatment of influenza uses a complex of drugs aimed at combating the causative agent of the disease and intoxication, the elimination of inflammation foci, prevention of complications and enhancement of the body's immunological reactivity. For the treatment and prevention of influenza, WHO primarily recommends drugs with etiotropic action and direct inhibitory effect on viral reproduction [24]. Such drugs include, for example, M2 channel blockers (rimantadine, amantadine), influenza neuraminidase inhibitors (oseltamivir, zanamivir, peramivir, laninamivir), fusion inhibitors (umifenovir).

However, the issue of resistance of influenza viruses to these antiviral drugs is becoming more common. For example, since the beginning of 2008, there has been an increase of the number of resistant strains of influenza A viruses of the subtype H1N1 to oseltamivir [25]. If in 2001 the frequency of resistant strains of influenza A virus to oseltamivir did not exceed 0.32 % in adults and 4.1 % in children, in the 2007–2008 season, experts from the WHO unit reported on increased resistance to oseltamivir and zanamivir up to 64 % in various countries around the world. Umifenovir acts at the early stages of viral reproduction and inhibits the fusion of the viral lipid membrane with intracellular membranes, preventing the virus from entering the cell, but does not affect viral transcription and translation. Mutants resistant to umifenovir are currently obtained only during *in vitro* experiments, resistance is caused by mutations in the target HA2 protein [18].

One of possible solutions for overcoming the resistance of the influenza virus to known antiviral drugs is the use of combined regimens that include drugs with different mechanisms of action. The work carried out by the authors confirms this assumption. Combined use of drugs with direct antiviral action and interferons inducer, which stimulates the innate immunity system of the patient, showed its efficacy compared to monotherapy. The combination of etiotropic drugs oseltamivir (Tamiflu ®) and umifenovir (Arbidol ®) with Kagocel ® allows to significantly increase the therapeutic efficacy in comparison with the corresponding monotherapy, which results in a decrease of the frequency of the main clinical parameters, shortening the duration of clinical symptoms and reducing the incidence of complications. Good tolerability of ongoing therapy by patients should be noted. No adverse events and no negative changes of laboratory parameters were registered. The authors believe that the use of combinations of already known antiviral drugs can achieve not only a synergy of action and the corresponding increase of therapeutic efficacy, but it also complicates the formation of a genetic barrier to the emergence of resistance to such treatment regimen.

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