Materials of Conferences

DYNAMICS OF HUMAN INFLUENZA VIRUS RESISTANCE TO CHEMOTHERAPEUTIC DRUGS FROM 2000 TO 2009

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The proportion of resistant strains of human influenza viruses to rimantadine was increasing up to the 2005–2006 epidemic season, reached its maximum, and then gradually started to decline. The beginning of the 2007–2008 epidemic season has experienced an unprecedented increase in the oseltamivir resistance in A (H1N1) viruses circulating in some European countries. At that the specific number of this maximum ranged from 100 to 70%, and its decrease in different regions took place with a different intensity. In 2009 the circulation of so-called "swine" A(H1N1) influenza broke out, the rimantadine resistance among isolates is kept at the level of 100%. Influenza viruses of subtypes A(H1N2) and A(H3N2) are more susceptible to oseltamivir than to zanamivir. The situation with influenza viruses A(H1N1) and type B is reversed.

Currently, the main ways to combat the influenza infection are vaccinal prevention and chemotherapy. Both methods have their advantages and disadvantages, so the best result can be obtained in their combined application. This paper describes the latest advances in chemotherapy, demonstrates the dynamics of resistance and mechanisms of action of antiviral drugs against influenza viruses.

Vaccination covers the preventative methods to combat the influenza. Recently, the quality of vaccine agents was significantly improved, their range extended. With timely vaccination it is possible to prevent influenza infection in 80–90% of children and adults, at that the disease in the vaccinated persons usually proceeds in a milder form [1].

Influenza chemotherapy has long been considered an unpromising sphere as compared to vaccinal prevention, but immunity against a particular strain does not protect the body from repeated illness caused by an another strain. Therefore, the production and use of chemotherapeutic drugs is not only permissible but also necessary [2].

Currently, there are two groups of chemotherapeutic drugs:

– M protein-inhibiting

– neuraminidase-inhibiting

Historically, the very first anti-influenza chemotherapeutic drug was amantadine, discovered in 1933 by Czech researchers S. Landa and B. Mihachek when studying the oil properties.

The great disadvantage of amantadine is its high toxicity, and a large number of side effects, identified in a number of patients. This led to the creation of rimantadine, which is an alpha-methyl-1-adamantane.

Dynamics of rimantadine resistance among circulating strains

Rimantadine as well as amantadine is an inhibitor of influenza virus uncoating. It irreversibly inhibits the M2 protein, and thus stops the flow of protons through the virion membrane [3]. Rimantadine blocks the functions of ion channels and thereby disturbs the process of the virus "stripping".

However, the rimantadine action is directed only against influenza A virus, because influenza B virus does not have the M2 protein at which the drug-induced action is aimed. Analogue of the M2 protein in influenza B virus is the NB protein, encoded by the open reading frame in the neuraminidase gene and by its structure fundamentally differing from the M2 protein. There is no adamantane-binding site in the NB protein. However, despite the absence of the M2 protein, rimantadine can improve the condition of patients with influenza B, as it mitigates the toxic effects of influenza.

At present, the search for new chemical compounds among the adamantane derivatives is going on. For example, the antiviral activity against influenza virus for pyrrolidine and aminoethyl derivatives of aminoadamantane was shown, as well as for derivatives with metal ions [4].

Influenza A viruses, unsusceptible to the action of drugs of adamantine series, usually carry the following mutations in the amino acid sequence of the M2 protein: L26F, V27I, V28I, A30T, S31N, G34E. The greatest number of rimantadine-resistant strains carries the S31N mutation [5].

Mutations in the hemagglutinin gene are poorly investigated, however, for rimantadine-resistant strains it was shown that it has the following mutations: in HA2 – N49S, M58L, S70C, R75K, M58I, F109S, and in HA1 – L315P, S323P [6].

There are also mutations in the hemagglutinin gene associated with the S31N mutation in the M2 protein gene. According to Pontoriero et al. [7], those are the mutations S193F and D225N, which are present in rimantadine-resistant strains on the par with the S31N mutation in the M2 protein gene.

Initially, the number of resistant strains was low (less than 1%). At the same time, in Greece the 2004–2005 epidemic season did not reveal any resistant strain. Later, in Europe, there was a slight increase in the proportion of resistant strains. In 2005–2006 their number increased to 12%, and in 2006–2007 quite to 25%. A similar situation was observed in the Asian region. For example, among the influenza virus strains circulating in Hong Kong from 2003 to 2005, a share of rimantadine-resistant isolates increased from 20% to 83%. Among the Asian strains of subtype A(H3N2), isolated in the

2005–2006 epidemic season, the resistance reached 100% [8]. By the 2005–2006 epidemic season the proportion of resistant strains of subtype A(H3N2) in Japan fluctuated, according to various authors, from 90% to 72.2% (25,9), while in the subtype A(H1N1) there was not found any resistant strain. In the 2006–2007 epidemic season in the Japanese population of subtype A(H3N2) the number of resistant strains came to 79,4%, and among A(H1N1) – only 48,2% (26–10). In the North America, despite the geographical distance from Eurasia, the overall tendencies were similar. For example, in the USA over the 2005–2006 epidemic season, 92,3% of isolates of subtype A(H3N2) were resistant to rimantadine according to the data of a genetic test, that is, by the existence of the S31N mutation. Of eight strains of subtype A(H1N1), isolated in the same epidemic period, this mutation was carried by two strains [9].

Neuraminidase breaks up the sialic acid component of hemagglutinin receptors of respiratory tract epithelial cells, helping to release newly formed virus particles from the cells and infect new cells with them.

Another function of neuraminidase is its ability to break down neuraminic acid in the nasal mucus, in that way making easier the virus penetration through the respiratory tract [10].

The drug Zanamivir was the first neuraminidase inhibitor. Due to the low bioavailability of zanamivir (Relenza) (less than 5%), it is effective and used in the form of aerosol inhalation or intranasal spray, which ensures its delivery to the place of direct viral replication in the cells of the respiratory tract. In addition, there is a danger of spasm development in patients with bronchial asthma [11].

Therefore, the pharmaceutical company F. Hoffmann – La Roche (Switzerland, Basel) has initiated a study to find another neuraminidase inhibitor, which would be effective for the systematic use. As a result of the synthesis and study of a large number of neuraminidase inhibitors in the pharmaceutical market oseltamivir (Tamiflu) has appeared. Unfortunately, it is impossible to use oseltamivir in serious cases when patients are unable to use the tablets. In 2009, the firm BioCryst Pharmaceuticals has registered the third neuraminidase inhibitor – an experimental drug peramivir. Peramivir was approved for use in critical situations to treat heavily ill patients infected with pandemic influenza A(H1N1) ("swine influenza") [12].

Currently trials of a new neuraminidase inhibitor laninamivir are being carried out. This drug has revealed antiviral activity against influenza A and B viruses, including subtypes N1-N9, and against viruses resistant to oseltamivir. Also, it revealed efficiency against swine-origin influenza A(H1N1) and highly pathogenic avian influenza A(H5N1) [13]. Studies on the development of anti-influenza drugs in the group of neuraminidase inhibitors are in progress. In addition to the above, which are derived from cyclohexene, the cyclopentane and pyrrolidine derivatives have been developed, which also possess inhibitory activity against influenza virus neuraminidase. 7-alkyl ether and bicyclic ether derivatives of zanamivir have also been synthesized, which revealed a higher, compared to the drug itself, level of activity *in vitro* and *in vivo* under oral administration in the model of influenza pneumonia in white mice [14].

Mutations resulting in the resistance to oseltamivir have been discovered mainly in the influenza A viruses, and they differ depending on the subtype: the most common mutations in the viruses carrying N2, were R292K and E119V, while the most frequent mutation for N1 was H275Y (often referred to as H274Y, which is consistent with the numbering in N2) [15].

Laboratory studies to detect the virus resistance have demonstrated that the stable mutations during treatment with oseltamivir occur rarely. However, they are specific to the subtype: in neuraminidase subtype N1 the H274Y mutation was revealed, and in N2 – R292K [16]. In addition, the E119V mutation was identified with a very low frequency (only in neuraminidase type N2) [17].

Dynamics of oseltamivir resistance

In the first three years of oseltamivir appearance in the market (1996–1999), there have been no registered cases of influenza viruses with reduced susceptibility to oseltamivir. Pooled data obtained from 2000 patients taking oseltamivir, demonstrate a low percentage of the drug-resistant strain appearance (0,33% for adults and 4,0% for children). Monitoring of influenza during the 2000–2001 and 2001–2002 epidemic seasons in 22 European countries revealed that less than 1% of the strains in every season possessed a reduced susceptibility to oseltamivir. The worldwide number of influenza viruses with reduced susceptibility in the period from 2004 to 2007 was also low (12/3261, 0,4%) [18].

However, the beginning of the 2007–2008 epidemic season has experienced an unprecedented increase in oseltamivir resistance of A(H1N1) viruses circulating in some European countries. Preliminary analysis data for isolates of the 2007–2008 epidemiological season showed an increase in the number of A(H1N1) strains carrying the H275Y mutation in comparison with the previous period (57/896 isolates, 6,4%), especially in the USA. Confirming this unexpected tendency, the European Centre for Influenza Surveillance (European Influenza Surveillance Scheme) reported that among strains of the influenza virus A (H1N1) the number of resistant to the drug increased to 23% (586/2533 tested samples), at that the ratio of resistent and susceptible strains was different for different countries, for example, 68% in Norway, 10% in England, and 1% in Italy. It is important that the majority of circulating influenza strains in Europe were susceptible to oseltamivir, as well as in the USA.

Further, in the winter 2008, a high level of resistant A(H1N1) strains was recorded in South Africa (100% of 225 isolates) and Australia (93% of 76 isolates), the lesser amount was observed in South

America (36% of 275 isolates) (WHO data, 2008). In the epidemic season 2008–2009 in most of Europe oseltamivir-susceptible A(H3N2) viruses were predominant [19], despite the presence of resistant A(H1N1) viruses. It should be noted that during this period oseltamivir-resistant influenza A(H3N2) and type B viruses were not registered, as well as that these viruses were susceptible to zanamivir. In the USA, however, A(H1N1) strains were the most numerous, and majority of them were resistant (~60%). In the course of the 2008-2009 epidemic the reports on the isolation of oseltamivir-resistant A(H1N1) strains came from 30 countries. 1291 of 1362 isolates (95%) proved to be resistant. At that, in Canada, Japan, Hong Kong, USA, Korea, and many European countries, the proportion of resistant strains was nearing or came to 100%. However, it should be noted that the appeared since 2009 in most countries, pandemic "swine" influenza A(H1N1) predominated over the seasonal and was more susceptible to oseltamivir (WHO data, 2009).

The next drug, Arbidol, is one of the most widely used anti-influenza drugs in Russia. As ribavirin, arbidol attacks the propagative influenza virus.

Mutations resulting in the development of resistance to arbidol have been mapped in hemagglutinin gene on the border between HA1 and HA2 subunits.

Ingavirin (2-(imidazol-4-yl)-ethanamide pentandioic-1,5 acid) is a new antiviral drug, a low-molecular peptidoamine, being an analog of natural peptidoamine.

A new drug is favipiravir (T-705) (6-fluoro-3-hydroxy-2-pyrazinecarboxamide), which revealed high activity *in vitro* against a number of RNA-viruses: seasonal influenza, highly pathogenic avian influenza. Presumably, favipiravir targets the RNA-dependent RNA polymerase [20]. Currently, there are no studies in which mutations, causing resistance to favipiravir, have been reported, but one may assume that they could be revealed in the viral polymerase gene, as it is the target of this drug.

Conclusions

In general, we can say that the proportion of resistant strains was increasing up to the 2005–2006 epidemic season, when it reached its maximum, and then gradually started to decline. At that the specific number of this maximum ranged from 100 to 70%, and its decrease in different regions took place with a different intensity. In 2009 the circulation of so-called "swine" influenza A(H1N1) broke out, the rimantadine resistance among isolates is kept at the level of 100%. Influenza viruses of A(H1N2) and A(H3N2) subtypes are more susceptible to oseltamivir than to zanamivir. The situation with influenza viruses A(H1N1) and type B is reversed. Findings of one investigation suggest that zanamivir is more effective than oseltamivir, against neuraminidases of subtypes N2, N3, N6, N7 and N9, while N1, N4, N5 and N8 are more susceptible to oseltamivir. Combined use of chemotherapy and vaccinal prevention is essential method for a successful combating viral infections.

References

1. Loginova S. Y., Borisovich S.V.; Semenova I.V., Maksimov V.A., Bondarev V.P., Nebolsin V.E. The study of Ingavirin antiviral activity against the influenza A(H3N2) infectious agent in vitro // Antibiotics and Chemotherapy, 2009, 54; 9–10.

2. Aoki FY, Boivin G, Roberts N. Influenza virus susceptibility and resistance to oseltamivir. Antivir Ther 2007;12:603–16.

3. Bridges C.B., Harper S.A., Fukuda K., Uyeki T.M., Cox N.J., Singleton J.A. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP) [published correction in MMWR Morb Mortal Wkly Rep 2003; 52(22):526]. MMWR Recomm Rep. 2003;52RR-81–34.

4. Bright R. A., Shay D. K., Bo Shu, Cox N.J., Klimov A.I. Adamantane Resistance Among Influenza A Viruses Isolated Early During the 2005-2006 Influenza Season in the United States // JAMA. – 2006 – V. 295. – P. 891–894.

5. Besselaar T.G., Naidoo D., Buys A., Gregory V., McAnerney J., Manamela J.M. et al. Widespread oseltamivir resistance in influenza A viruses (H1N1), South Africa. Emerg Infect Dis 2008;14:1809-10.

6. Borman S. Flu virus proton channel analyzed: structures of key surface protein suggest different drug mechanisms. Chem Eng News. 2008; 86:53–54.

7. Cyranovski (2005) Threat of pandemic brings flu drug back to life. Nature Medicine 11 (9): 909.

8. De Clercq E. Antiviral agents active against influenza A viruses.. Nat Rev Drug Discov. 2006 Dec;5(12):1015-25. Review.

9. Deyde V.M., Xu X., Bright R.A., Shaw M., Smith C.B., Zhang Y., Shu Y., Gubareva L.V., Cox N.J., Klimov A.I. Surveillance of resistance to adamantanes among influenza A(H3N2) and A(H1N1) viruses isolated worldwide. – 2007. – V.15. – P. 249–257.

10. García-Granda S., Gotor V., Gutiérrez-Fernández A. et al. Preparation of (R)-(1-adamantyl)glycine and (R)-2-(1-adamantyl)-2-aminoethanol: a combination of cobalt-mediated b-ketoester alkylation and enzyme-based aminoalcohol resolution. Tetrahedron: Asymmetry. 2000;11:4549–4557.

11. Hassan K., Drews S., Blair J. et al. Antiviral therapy improves outcome of influenza infections in patients requiring admission to intensive care. Abstracts of the Forty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy; 2009; San Francisco. Washington, DC, USA: American Society for Microbiology; Abstract V-537.

12. Jack A. Flu′s unexpected bonus. BMJ 2009;339:720-21.

13. Kubo S., Tomozawa T., Kakuta M., Tokumitsu A., Yamashita M. Laninamivir prodrug CS-8958, a long-acting neuraminidase inhibitor, shows superior anti-influenza virus activity after a single administration. Antimicrob Agents Chemother. 2010 Mar;54(3):1256-64. Epub 2010 Jan 4.

14. Leneva I.A., Russell R.J., Boriskin Y.S., Hay A.J. Characteristics of arbidol-resistant mutants of influenza virus: implications for the mechanism of anti-influenza action of arbidol. Antiviral Res 2009;81:132–140.

15. Moscona A. Oseltamivir resistance-disabling our influenza defenses. N Engl J Med 2005;353:2633-6.

16. Mereckiene J., Cotter S., Weber J.T. et al. Low coverage of seasonal influenza vaccination in the elderly in many European countries. Euro Surveill. 2008;13 pii=19001.

17. Pontoriero A., Baumeister E., Campos A.M., Moreno A., CadarioM.E., Savy V. Surveillance of adamantane resistance among influenza A H3 viruses isolated in Argentina between 2001 and 2007. //Rev Argent Microbiol. – 2008. – V.40(3). – P.180–184.

18. Poland G.A., Jacobson R.M., Ovsyannikova I.G. Influenza virus resistance to antiviral agents: A plea for rational use. Clin Infect Dis 2009;48:1254-6.

19. Scholtissek C., Quack G., Klenk H.D., Webster R.G. How to overcome resistance of influenza A viruses against adamantane derivatives // Antiviral Research. – 1998. – V.37. – P.83–95.

20. Smee D.F. et al. Intracellular metabolism of favipiravir (T-705) in uninfected and influenza A (H5N1) virus-infected cells. J Antimicrob Chemother. 2009;64:741–746.

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