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THE STUDY OF THE ANTI-NEURAMINIDASE AND INTERFERON INDUCING ACTIVITY OF ALTABOR SUBSTANCE

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Abstract: the antiviral activity of Altabor substance has been studied. It has been determined that Altabor in the dose of 1 mg/ml completely inhibits 1/6 of enzyme units or 51.5 γ/ml/min of neuraminidase, reduces the neuraminidase activity of A/Hong Kong/68/H3N2/, A/Victoria/75/H3N2, A/Khabarovsk/H1N1/ influenza viruses by 84.2 and 85.0 %. The drug also stimulates dose-dependently the production of interferon in human leukocytes. It has been found that the mechanism of action of altabor is due to inhibition of the neuraminidase activity of the virus with simultaneous induction of interferon by human cells.
Key words: altabor, antiviral activity, influenza, neuraminidase, interferon inducing activity.

Influenza is acute respiratory disease of viral etiology characterized by the phenomena of general intoxication and respiratory tract lesions [1, 2]. Hemagglutinin and neuraminidase, surface antigen of influenza virus are the factors of aggression. Hemagglutinin is a polypeptide, a surface protein of the viral envelope, due to it the virus attaches to the surface of the host cell. Neuraminidase is an enzyme, a glycoproteid complex that determines the enzymatic activity and is responsible for the ability of a viral virion to penetrate the host cell and leave it after propagation [3]. In other words, neuraminidase helps viral particles to penetrate the secretions of the mucous membranes to achieve epithelial cells of the respiratory tract by virions. In addition, the properties of hemagglutinin determine the intensity of intoxication in disease, and neuraminidase exhibits a marked immunosuppressive effect [4].

The entry of influenza viruses is the epithelium of the respiratory tract. The defense mechanism of the body (the 1st line) is nonspecific factors (mucus respiratory cilia, macrophages that capture the virus, secretory IgA). In order to infect the virus has to "overcome" the factors of nonspecific resistance of the respiratory tract. The causative agent getting into the nasopharynx is exposed to highly active secretion of cells that are capable of inhibiting the hemagglutinate and infective virus activity due to glycoproteins containing N-acetylneuraminic acid. In the submucosal layer as a result of release of biologically active substances (histamine, serotonin, kinins, prostaglandins) the reaction of vessels, blood cell elements, formation of small blood clots and hemorrhages, edema, local metabolism disorders, changes in the pH to the acidic side were observed; inflammatory process was formed [3,4].

Penetrating into the deeper layers of the epithelium, the virus meets the 2nd line of specific defense (interferon – IFN, circulating antibodies of IgM, IgG, IgE class, temperature reaction). In response to infection the early cytokine reactions as the fastest response to the virus are developed. Thus, we are dealing with the natural and most common variant of action on the influenza virus as an intracellular parasite when the virus itself turns on the IFN system acting as a natural inducer. As a result, at this stage of viral infection three interrelated steps are locally performed. They are: intracellular inhibition of IFN reproduction of viruses, removing the infected material and protection of uninfected cells with the newly formed IFN from a new infection. However, the effects of IFN are often insufficient to complete the infection process.

A lot of medicines used to treat viral infections work on one of the following principles [5, 6]:

- stimulation of the own body defences;
- destruction of the structure of the viral parts (drugs due to similarity to components of the virus are embedded in the genetic basis of the virus, and it leads to creation of defective parts that are not able to affect the healthy cells of the body);
- prevention of the viral entry into the cells of the body. If the virus has penetrated into the cell, then the only way of combating with it is destruction of a diseased cell together with the virus. On the one hand, it certainly does not allow viruses to multiply and affect other cells, but on the other hand, destruction of the own cells is also not the best way.

Therefore, for prevention and treatment of influenza diseases it is advisable to use two schemes, which would affect two elements of the infectious process, namely the use of drugs that would affect influenza virus destroying it, and medicines for strengthening the body's defences by stimulating the interferon inducing activity. Thus, the search for drugs that would have a combined scheme of action is appropriate and promising.

The aim of the work was to study the anti-influenza activity of Altabor substance obtained from the collective fruit of black alder (*Alnus glutinosa*) and grey alder (*Alnus incana*) in order to determine its effect on the virion of influenza virus concerning its destruction and on a human in relation to increase of the body defences.

Materials and methods

The research of the antiviral activity of Altabor substance was conducted by studying its effects on isolated neuraminidase and different strains of the influenza virus. The effect of the drug on interferon production was also studied.

The antineuraminidase activity of Altabor substance was studied on the example of inhibition of neuraminidases of different types of influenza viruses (A/Hong Kong/68/H3N2/, A/Victoria/75/H3N2, A/Khabarovsk/H1N1/) and neuraminidase isolated from Astrobacter ureafaciens 1 unit Calbiochem, Hoest in the

concentrations of 105 and 51.5 γ/ml/min with Altabor solution in the concentration of 1 mg/ml.

Determination of the neuraminidase activity was conducted by the method of Aminoff. To neuraminidase dilutions (0.1 ml), 0.1 ml of the drug was added in the dose of 0.2 mg/ml, incubated for 1 h at 37°C, fetuin was added and allowed to stand at 37 °C for 18 h. Then to each test tube 0.25 ml of sodium periodate was added and incubated for 30 min. After that 0.4 ml of sodium arsenite was added, shaken, and 2 ml of thiobarbituric acid was added, boiled for 7.5 min, cooled using ice. Then 5 ml of acidified butanol was introduced, shaken, centrifuged for 10 min at 1500 rev/min. The neuraminidase activity was expressed in units of UV-absorption at the wavelength of 549 nm or in percentage when comparing the test samples.

The interferon inducing activity of Altabor was studied in the culture of human leukocytes. The solution of the drug in different doses was added to 3 mln of human leukocytes and cultured at 37° C for 24 hours. After that the supernatant was collected, and the pH value was adjusted to 2.0, left at 4 °C for 48-72 h, then the pH of the liquid was restored to 7.3, and the level of interferon was determined.

Results and Discussion

The results of the studies on the antineuraminidase and interferon inducing activity of Altabor substance are presented in Tables 1 and 2.

Table 1 - The antineuraminidase activity of Altabor substance

Neuraminidase, γ/ml/min	Indicators of optical density		% inhibition
	neuraminidase	neuraminidase + Altabor	
105	0.990	0.210	78.8
51.5	0.595	0.090	100
A/ Hong Kong//68/ H3N2/	0.820	0.120	84.2
A/ Victoria/75/ H3N2,	0.690	0.110	84.2
A/ Khabarovsk/ H1N1/	0.700	0.105	85.0

Thus, based on the results it can be concluded that Altabor substance in the dose of 1 mg/ml completely inhibited 1/6 enzyme units or 51.5 γ/ml/min of neuraminidase (Astrobacter ureafaciens 1 unit Calbiochem, Hoest), and there is 84.2 and 85.0 % reduction in the activity of neuraminidase of A/Hong Kong/68/H3N2/, A/Victoria/75/H3N2, A/Khabarovsk/H1N1/ influenza viruses.

Table 2 – The interferon level produced by Altabor in the human leukocyte culture

Altabor in doses, mg/ml	The activity of IFN, EI_{50}/ml
2.0	80
1.0	160
0.5	160
0.25	320
0.125	640
0.032160 0.064	20
PolyI PolyC	2560

These data show that Altabor dose-dependently stimulates the production of interferon in human leukocytes indicating the activity of the drug and its prospects as an interferon inducer.

Conclusions

The results of the studies conducted on the antiviral activity of Altabor substance have shown that this substance effectively inhibits the reproduction of influenza viruses. The mechanism of the anti-influenza action of Altabor is due to inhibition of the neuramidase activity of influenza viruses with simultaneous induction of interferon by human cells.

Prospects for further research

The results of the anti-influenza activity obtained for Altabor substance allow to consider it as a promising substance for creating drugs to treat influenza and influenza-like infections.

References

1 Genetic analysis of influenza A virus H1N1 "pandemic" in the epidemic / E.S. Kostyukova, N.B. Zakharzhevskaya, P.A. Kostin [et al.] [Text] // Therapeutic archive. - 2012. - № 3. - P. 48-54.

2 Hrynevych O.Y. Review of the epidemiological situation in the world regarding the incidence of seasonal

and avian influenza in early 2012 [Text] / O.Y. Hrynevych, I.G. Markovic // Family Medicine. - 2012. - № 1. - P. 32-34.
3 Obertynska O.V. Modern therapy of influenza of inhibitors of neuraminidase [Text] / O.V. Obertynska // Family Medicine. - 2014. - № 1. - P. 111-114.
4 Dibrova V.A. Aspects of the pathogenesis of influenza A (H1N1) [Text] / V.A. Dibrova, E.V. Tsema // Modern infection. - 2010. - № 2. - P. 12-21.
5 Markovic I.G. Analysis of influenza diseases population of Ukraine in 2009-2013 years [Text] / I.G. Markovic, O.Y. Hrynevych // Ukraine. Health of the Nation. - 2013. - № 2. - P. 118-124.
6 Moskaliuk V. D. Dynamics of cellular immunity of acute respiratory viral infections patients against an aerosol interferon treatment background [Text] / V. D. Moskaliuk, V. D. Sorokhan // Infectious diseases. — 2010. — № 4. — P. 14-18.

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