Development of anti-influenza agents: experience, findings, perspectives

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WHO recommends each country to create the necessary reserve of therapeutic chemical agents to be used for prophylaxis and treatment in situations of a sharp increase in influenza patients among the population. Practical measures should be aimed at reducing the disease incidence and mortality rates, as well as minimizing social disorganization. Developing active therapeutic chemical agents against influenza and their implementation schemes remains an urgent and promising task.

Though the last decades have seen the saturation of the pharmaceutical market with dozens of new antiviral agents, the question of prophylaxis and treatment of the most widely spread viral infections is far from being solved. It is common knowledge that the development of new pharmacological substances is rather a time consuming and financially burdensome way of creating medicines. Within the framework of pharmacoeconomics – a new branch of modern pharmacy that compares the efficacy, safety and costs of medicinal products under various medical treatment schemes – the most promising and cost-effective strategy is to uncover antiviral properties of the drugs that are already commercially manufactured (though intended for different medical uses), their activity and side effects being well-known due to their long-term use. Besides, this approach allows to extend the indications for using the existing medicinal products.

The Laboratory of immunobiological and therapeutic chemical agents of the Ukrainian I.I. Mechnikov Anti-Plague Research Institute of the Ministry of Health of Ukraine (UAPRI) has been conducting continuous research focused on influenza for many years. Numerous studies in this field are carried out in cooperation with colleagues from other institutes, which has enabled us to obtain practically applicable and fundamental results, including those regarded as top priorities.

It has been established that the interaction between influenza virus and cell membranes with high sensitivity results in increased proteolysis that leads to cleavage of influenza virus hemagglutinin. This facilitates its entry into the cell and stimulates viral deproteinisation and subsequent replication. Proteolytic activation of influenza viruses can localize not only at a subcellular level, in cells or individual organs (lungs), but also affect the entire body (blood). Proteolysis inhibitors (PIs), in particular, epsilon-aminocaproic acid (EACA), block proteolysis activation and the viruses' entry into the cells, which reduces the viral harvest. When dealing with whole-body localization in experimentally induced influenza, EACA exerts not only etiotropic (direct antiviral) action, but also positive pathogenic and immunomodulating action.

EACA has been proved to inhibit multiple subtypes of influenza A virus (serotypes H1N1, H2N2, H3N2 of human influenza viruses, as well as H5N3, H7N3 of avian influenza viruses), influenza B virus, parainfluenza and adenovirus infection. This is a convincing argument in favor of topical administration of aminocaproic acid (delivered through intranasal or inhalation routes) for treatment and prevention of influenza and other respiratory infections in human and veterinary medicine.

Further clinical observations confirmed the prophylactic effect of the ACA medicinal product (aminocaproic acid) when administered through intranasal or inhalation routes.

Research projects carried out in collaboration with the Research Institute of Influenza (St. Petersburg, Russian Federation) have demonstrated that the use of ACA promotes lung aeration, preserves pulmonary vascular structure and blood flow, blocks the development of lung perivascular edema and hemorrhagic syndrome, thus preventing failures of aerohematic barrier. The medicinal product under consideration precludes proteases from damaging such local pulmonary antiviral defense mechanisms as those provided by secretory antibodies and non-specific viral inhibitors (which is crucial for local administration of the drug), as well as by interferon. Administration of aminocaproic acid to models with experimentally induced influenza activates their defense reactions by stimulating lung secretion, inducing perivascular and peribronchial lymphoid infiltration, and by increasing the number of pulmonary granular cells.

An integrative result of etiotropic (antiviral), positive pathogenic and immunomodulating actions of aminocaproic acid administered to models with experimentally induced influenza is decreased lethality, as well as a substantial reduction of infectious virus titers in the lungs of mice both infected with a non-lethal strain of A/Hong Kong/1/68 (H3N2), and with a lethal strain of the highly virulent virus A/PR/8/34 (H1N1).

The results above were obtained following intranasal, peroral or parenteral administration of aminocaproic acid to mice with experimentally induced influenza according to therapeutic scheme (during the first 5 days after primary infection), emergency prophylaxis scheme (1-2 days before the exposure), and long-term prophylaxis scheme (for 5 days with re-infection 15-45 days later).

Therefore, the results above can be viewed as convincing evidence in favor of combined enteral and intranasal administration of aminocaproic acid.

It is important to note that animals treated with aminocaproic acid displayed a considerably higher resistance to re-infection after a 30-day interval, compared to those in the placebo-treated control group. What is more, in contrast to mice in the control group, the lungs of the former did not contain the infectious virus following 24 hours after re-infection; they also demonstrated a substantially higher increase of hemagglutinin blood titers compared to the control group.

Furthermore, the results testify to viability of the approach based on using aminocaproic acid for immunization with live attenuated vaccines.

The results of our experimental studies and the analysis of publications enable us to develop the concept of a 'vicious circle' existing in situations with viral infections, particularly influenza: the interaction between the host and the virus activates proteolysis at different structural and functional levels; this in its turn increases infectious agents in a virus population and stimulates the development of pathogenetic phenomena. That results in the advance, complication and generalization of the infection. The use of proteolysis inhibitors, in particular, aminocaproic acid, can prevent the development of this 'vicious circle' (prophylaxis) or break it (therapy).

Further clinical observations conducted jointly with the researchers of Odesa State Medical University demonstrated the efficacy of aminocaproic acid preparations used for treating children of various ages diagnosed with influenza and other acute respiratory viral infections (ARVI).

For complicated influenza and ARVI, aminocaproic acid was prescribed with the daily dose constituting 0.1-0.2 g/kg body mass, administered enterally (through the mouth). The daily dose was divided into 4 intakes, with the preparation previously dissolved in boiled and cooled water, or using a pre-made 5% solution. In case of a severe disease course, the ACA daily dose was increased to 0.3-0.5 g/kg body mass, with half of the daily dose administered through intravenous infusions. The study proved the efficacy of placing into the nostrils wound cotton swabs soaked with a 5% solution of aminocaproic acid for 5 to 10 minutes, or dripping a few drops of this solution into each nostril every 3 to 4 hours. The course of treatment lasted 3 to 7 days.

ACA preparations turned out to be particularly effective when administered at early stages of the disease, due to their ability to prevent the development of life-threatening toxic shock syndromes in children, as well as to reduce the occurrence of secondary bacterial infections by 50% and more. Locally administered ACA promotes speedy restoration of the ratio of cylindrical and flat epithelium, reduces the viral antigen host period and the duration of cytotoxic effect of the virus. In infants with complicated influenza and ARVI, as well as in those prone to catching the viruses, the best results were gained by means of enteral administration of aminocaproic acid (through the mouth) combined with its intranasal administration (through the nose), with the course of treatment lasting 5 to 7 days, and simultaneous administration of immunoglobulin with directed effect: 0.2 to 0.3 ml/kg body weight 2-3 times daily. The combination above contributes to reducing the duration of infection-induced toxicosis by 3 to 4 days, and to shortening the duration of fever by up to 2 days. Simultaneous administration of ACA and the immunoglobulin above is most effective if started in the first 3 days of the disease.

The prophylactic efficacy of aminocaproic acid in 3 to 6-year-old children with recurrent infections was revealed in the course of observations carried out in Tallinn preschools jointly with the scientists of Estonian Institute of Preventive Medicine. The first prevention course was held for 2 weeks in September. The children were immunized with 0.5 ml of a 5% ACA solution two times a day (in the morning and evening), through a device to secure intranasal delivery. The second analogous prevention course was carried out during the period when the influenza epidemic was at its height. According to the analysis of epidemiological efficacy of aminocaproic acid, children who were administered ACA tended to display lower incidence rates, compared to those displayed by children not immunized with it. The tendency persisted for the following 4 months. The number of children who caught influenza and ARVI up to 6 times a year was reduced by 60%. The prevention course shortened the duration of one disease by 2.7 days on average.

In cases of influenza circulating in household settings, the medicinal product under consideration was administered through enteral and intranasal routes, according to the therapeutic scheme. This was due to the fact that emergency prophylaxis must be combined with early treatment. In order to be provided with prophylactic protection during influenza epidemics, children and adults should be administered a 5% ACA solution by dripping it into each nostril 3 to 5 times a day, or by consecutive placing into each nostril a wound cotton swab soaked with a 5% solution of aminocaproic acid for 5 to 10 minutes, every 3 to 4 hours. Contraindications for aminocaproic acid are a tendency to thrombosis and kidney diseases. However, when used for purposes of treating influenza and ARVI, ACA doses were several times lower than a daily hemostatic dose which yielded no adverse effects.

The results provided by the study led the Pharmacological Committee of the Ministry of Health of Ukraine to approve Regulation No.2 of 25 February 1993 used by the Ministry of Health of Ukraine to pass Order No.225 of 03 November 1993 on allowing enteral and topical administration of aminocaproic acid against influenza and acute respiratory viral infections in children predominantly with moderate and severe course of the disease.

The Order by the Ministry of Health of Ukraine established amendments and additions to the instructions for and patient's guide to medical use of aminocaproic acid, which recommended using the medicinal product for treating influenza and ARVI.

It is worth mentioning that aminocaproic acid as a medicinal product in the form of solution is produced by six companies, in the form of powder – by one company, in the form of tablets – by one company. The State Pharmacological Center of the Ministry of Health of Ukraine is currently working on adopting uniform directions for medical usage of aminocaproic acid which include recommendations on using the medicinal product against influenza. The directions above must be followed by all manufacturers. It should be pointed out that unlike all the existing therapeutic chemical agents which are allowed to be used by children no younger than 1 year of age, aminocaproic acid can be used without any age restrictions. Therefore, this therapeutic chemical agent is an effective instrument to protect infants in the first year of life – the most susceptible age group – against influenza and ARVI.

Another development carried out by the Institute and targeting children of this age group is the technology of producing bispecific immunoglobulin directed against influenza and staphylococcus. This anti-influenza bispecific immunoglobulin contains high levels of antibodies to current influenza virus strains and is also rich in anti-staphylococcal alpha-toxin antibodies. The medicinal product has undergone thorough experimental and clinical studies. Its clinical studies were carried out in the clinic of the Department of Children's Diseases at Odesa Medical University (OMU) and at the Research Institute of Influenza (RII, St. Petersburg). Those of

the children diagnosed with influenza and ARVI who were administered the immunoglobulin above, demonstrated a significantly lower number of complications and pneumonias, as well as a shorter duration of fever and inpatient care.

The OMU clinic has also conducted clinical observations that prove complex therapy with ACA and bispecific immunoglobulin with directed effect to be highly effective against influenza and ARVI in children. As revealed in the course of the studies, concomitant use of these medicinal products resulted in potentiating their anti-influenza effect. The results obtained in the course of clinical observations proved the expediency of recommending concomitant use of these medications for purposes of health care. That led to using significantly lower effective doses of immunoglobulin, lower drug loads and less expensive treatment.

It should be pointed out that the UAPRI has developed an approach which allows to make anti-influenza therapy more effective by means of using an integrated scheme based on purely nationally produced medicinal products, as well as imported products, which is especially relevant in view of rapidly spreading influenza pandemic in the modern world. Concomitant use of ACA and the nationally produced antivirus agent Amizon resulted in potentiating anti-influenza effect. The effect was observed *in vitro*, both for human and avian influenza virus; it was also found to be true for mice with experimentally induced influenza (severe lethal infection) when they were infected with the highly virulent virus A/PR/8/34 with the antigenic formula H1N1.

Concomitant use of ACA and the neuraminidase inhibitor Tamiflu also resulted in potentiating anti-influenza effect. In a mouse model of severe lethal infection with the highly virulent virus A/PR/8/34 (H1N1), the concomitant administration of Tamiflu and ACA in doses lower than minimum effective doses, demonstrated a statistically significant protective effect. This testifies to the fact that the combination therapy above is capable of: lowering drug loads, minimizing the incidence of toxic effects of using Tamiflu, and reducing the cost of treatment.

Furthermore, this combination therapy can allow more effective use of the 61 thousand doses of Tamiflu provided by the World Health Organization to combat influenza pandemic in Ukraine. Following the approach above, this rather expensive medicinal product can be used for preventing and treating influenza in a considerably bigger number of patients. Similar *in vitro* and *in vivo* studies have also been carried out with other authorized medicinal products – **Decametoxin (DT),** Ethonium (ET) and Unitiolum (UT). One of the mechanisms of antiviral effect produced by these medications can be considered inhibition of proteolytic processes caused by interaction between the virus and cell membranes with high sensitivity.

These medicinal products have been found to display a distinct anti-influenza activity against human influenza A and B viruses, as well as against the H5N3 and H7N3 avian influenza strains *in vitro.* That gives grounds to consider them prospective antiviral agents.

As established by the UAPRI, aminocaproic acid prevents autolysis of viral antigens in the process of storing inactivated vaccines, thus prolonging their shelf life and increasing the protective effect of the vaccines (Patent of Ukraine No. 9574 of 17 October 2005. Bulletin No. 10.3 "The method of antiviral vaccine production"). Combination therapy with ACA and natural interferon inductors stimulates interferon production and increases the degree of protection in animal models with experimentally induced viral infection (Patent of Ukraine "Interferon induction method". Patent No. 14402 of 15 May 2006. Bulletin No. 5. International Patent Classification (2006) A61K 45/00). Joint projects carried out by the Laboratory in cooperation with the researchers of Odesa National University have resulted in the development of a plaster for transdermal delivery of Rimantadine, which lowers the medication dose, prevents its harmful effects on the digestive system and liver, and sustains its desired blood concentration over an extended period of time (Patent of Ukraine "The antiviral agent for prophylaxis and treatment of influenza". Patent No. 77974 C2 of 15 February 2007).

The results obtained prove high anti-influenza efficacy of the transdermal Rimantadine delivery system developed by the Laboratory. A comparative study shows that transdermal delivery of Rimantadine turns out to be more effective than its peroral administration. The major advantages of a transdermal drug delivery are the long-term effect of the medicinal product, its lower effective dose, lower fluctuations in the drug concentration and the drug concentration maintained within a therapeutic range, painless and non-invasive delivery of the drug, reduced side effects. Besides, the drug delivery can be terminated at any time, in any case of developing adverse effects, by means of removing the plaster from the skin. Transdermal drug delivery excludes the processes related to drug metabolism in the liver, with liver being the primary site for drug metabolism. This method of drug delivery can also be helpful in delivering highly active medications which irritate the digestive system or have a short half-life.

The system can be especially helpful for treating children.

Our Institute in cooperation with the researchers of the Institute of Physics and Chemistry of the National Academy of Sciences of Ukraine (Physico-Chemical Institute, Odesa) has developed the principle applied to the search for antiviral agents which is based on determining the correlation between the chemical structure of substances and their antiviral characteristics (quantitative structure – activity relationship model (QSAR model)). The hierarchical QSAR technology (HiT QSAR) is aimed at optimizing the process of developing new effective medicinal products. Due to the HiT, QSAR tasks can be performed not from scratch, but based on the information obtained at earlier stages of the research, by means of the system of continually improved solutions.

What makes the technology above unique and groundbreaking is its multifaceted hierarchical strategy applied to: models describing the molecular structure of compounds (1D → 2D → 3D → 4D); atom description models in molecular simplexes (descriptive → physicochemical → field model); structural parameters (local → integral); activity assessment scales (binary → nominal → ordinal → continuous); mathematical methods used to determine the structure-activity relationship (pattern recognition → cross-range scaling → multiple regression → PLS); final aims of the solution of the QSAR task (prediction → interpretation → structure optimization → molecular design).

The HiT application results in a set of various QSAR models which by complementing one another cope with virtual screening, estimating the influence of structural factors on activity, modifying the existing molecular structures and

designing new ones that guarantee high efficacy of potential antiviral agents.

The HiT above has been implemented in a range of computer software. The findings in this field have led to developing recommendations for directed synthesis of substances with a definite composition of predicted antiviral activity. Moreover, this method can be used to predict the antiviral activity of the existing authorized medicinal products used for other medical purposes.

In addition to experimental and clinical studies, the Institute is concerned with measures to control and prevent a potential influenza pandemic caused by current strains of viruses. For the last few years the Institute has been closely cooperating with national and international institutions such as the Vector Research Center (Russia), US Naval Medical Research Unit No. 3 (NAMRU-3), WHO Regional Avian Influenza Center (Memphis, USA). Within the framework of this coordinated research, the main vectors are to study the role of bird migration in the spread of avian influenza in Ukraine, as well as the major routes of its transmission to humans.

Pursuant to Orders No.560 of 25 October 2005 and No.661 of 20 November 2008 by the Ministry of Health of Ukraine, the Ukrainian I.I. Mechnikov Anti-Plague Research Institute is the leading center for diagnosing avian influenza and current influenza. The Center is provided with modern equipment and diagnostic test systems for detection and research of both pandemic strains of influenza virus and avian influenza viruses. Besides, this equipment (Real-time PCR) will also provide an opportunity to conduct a molecular genetic analysis of the identified pathogens. The Institute has two emergency brigades established to offer practical aid, consulting services and methodological support to health care facilities of Ukraine. The brigades are staffed by experts (virusologists and epidemiologists) of the highest qualification. Therefore, the Institute is ready to carry out anti-epidemic measures against influenza in Ukraine, as well as conduct medical research and development.

What must make a solid foundation for effective measures against a potential influenza pandemic caused by current strains of viruses is a combination of practical work and scientific developments.

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