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Minireview

Novel Binary Drugs Against Respiratory Viruses: Combined Virus- And Host-Targeted Approach.

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Abstract

Influenza and coronaviruses are relevant respiratory infections that cause significant damage to people's health and healthcare systems. Modern antiviral drugs with direct viral action (VTA – virus-targeting action) have several disadvantages related to (i) a narrow spectrum of antiviral action, (ii) the rapid appearance of drug-resistant mutants, (iii) a lack of drug action to suppress the pathogenesis of viral diseases, and (iv) high therapeutic dosages and side effects. This minireview focuses on the design of drugs that target both viral (VTA) and cellular pathogenetic mechanisms (HTA – host-targeting action). This type of binary drug is designed to overcome the above limitations of the monotarget approach. An effective binary drug combining aprotinin, a natural polypeptide protease inhibitor, and ribavirin, a nucleoside analogue interfering with viral polymerase, is considered. Aerosol inhalations of this combined formulation were found to prevent virus dissemination and develop high therapeutic efficacy in mice infected with the highly pathogenic H7N2 avian influenza virus. This type of pharmaceutical formulation is applicable for aerosol delivery using a propellant metered-dose inhaler in patients at the earliest signs of respiratory infection. Thus, binary antiviral drugs open a promising avenue for creating reliable drugs to combat new, unknown viral threats of future highly pathogenic viral diseases "X".

Keywords: respiratory infections, viruses, antivirals, disease "X", aprotinin, ribavirin, inhalers, host-targeted, virus-targeted, drugs.

ACTUALITY OF RESPIRATORY VIRUS INFECTIONS

Respiratory viral infections continue to be a leading cause of acute illness, contributing to the global healthcare burden, particularly for infants, the elderly and immunocompromised individuals. Among these infections, the most prevalent are those caused by influenza viruses, coronaviruses, respiratory syncytial virus, human rhinoviruses, and human paramyxoviruses. Over the last decade, lower respiratory tract infections have caused 2–3 million deaths worldwide annually [1]. Additionally, there is a potential medical threat: the emergence of novel, unknown, and dangerous pathogens, such as highly pathogenic avian influenza viruses, severe acute respiratory syndrome coronaviruses, monkeypox virus, and others [2, 3]. The above-mentioned facts highlight the need for broad-spectrum antiviral strategies to design effective universal drugs for easy use in family and clinical practice as a first line of defense for the human population and attending physicians against such highly pathogenic viruses. For example, mortality risks of highly pathogenic influenza viruses of the H5 subtype in humans are estimated at >50% [4, 5], which is similar to the Spanish flu pandemic situation in 1918–1919, with 50–100 million deaths globally [6]. Thus, respiratory viral infections and future pandemics are major global public healthcare problems, and healthcare systems must remain prepared on a global scale for potential viral mutations that can alter viral adaptation and pathogenicity for humans, increase viral transmission patterns from birds and animals to the human population, and initiate the pandemic process.

TYPES OF ANTIVIRAL DRUGS

Currently, the arsenal of antiviral agents consists of two major groups: VTA (virus-targeting antivirals) and HTA (host-targeting antivirals). The first group consists of substances that directly target viral proteins or the viral genome, inhibiting viral replication and viral suppressive mechanisms acting on the cellular defense response. These targets include viral functional proteins and RNAs (polymerases, receptor proteins, viral ion channels, viral proteases, viral mRNAs, etc.) and structural

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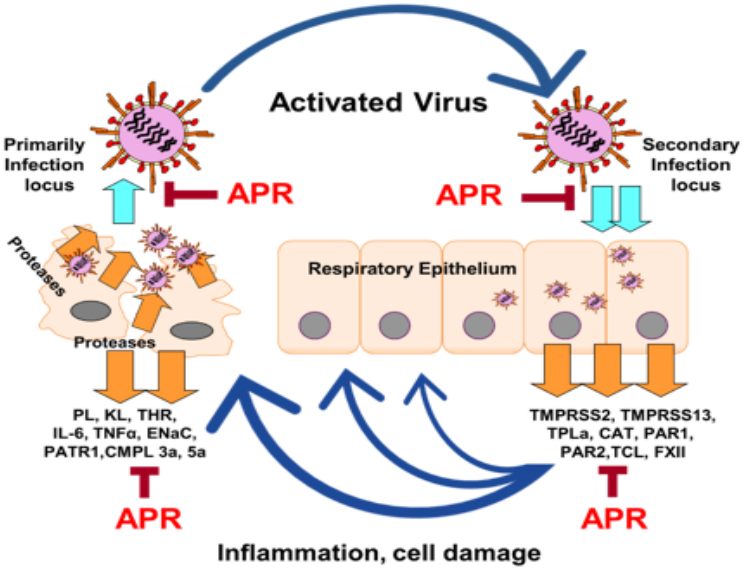
viral proteins involved in the morphogenesis and assembly of viral particles. The best-known representatives of this VTA class include rimantadine, baloxavir, oseltamivir, Relenza, favipiravir, ribavirin, molnupiravir, miRNA, recombinant nanobodies, and others used to treat RNA-containing viruses, including influenza and coronavirus infections in humans [7-12]. The VTA class of drugs has two major limitations: (i) a narrow spectrum of specific activity against closely related viruses due to significant virus-specific genomic variations, even among similar viruses, and (ii) the rapid development of viral resistance to such drugs. This resistance develops due to the high genetic error rate of polymerases of RNA-genomic respiratory viruses, amounting to 10-3–10-4 mutations per round of viral genome replication and producing extremely high amounts of viral quasi-species [13-14]. The second group consists of HTA compounds, which act on cellular targets. This group of drugs is characterized by a wide spectrum of activity against many viruses and a high barrier to resistance due to the inability of viruses to quickly adapt to new host cell metabolism [15-19]. However, this class of compounds acting on the host has an achievable obstacle due to potential cellular toxicity and, consequently, a narrow therapeutic dose window. Nevertheless, these drugs seem to be particularly valuable in the acute phase of respiratory diseases with short treatment courses. The development of this chemotherapeutic class is realized through several major avenues: (1) inhibitors of cellular receptors used by viruses for penetration into target cells; (2) inhibitors of cellular protein kinases that regulate cellular pathways used by viruses for their replication but do not interfere with defensive cellular processes against viruses; (3) drugs targeting host nucleotide biosynthesis, restricting viral replication and decreasing the emergence of virus drug-resistant mutants; (4) inhibitors of lipid biosynthesis and membrane biogenesis, interfering with viral entry, replication, and viral assembly on lipid membrane rafts; (5) inhibitors of host helicases involved in cellular regulation and IRES-dependent translation of viral mRNA in host cells; and (6) natural and synthetic inhibitors of host-specific proteases involved in the maturation of viral proteins and activation of viral particle infectivity [11, 20-24]. Currently, this HTA research direction is widely developed and is superior to the traditional “magic bullet” approach discovered by P. Ehrlich at the beginning of the 20th century [25].

BINARY ANTIVIRAL DRUGS AND INHALATION DELIVERY

We previously studied the antiviral activity of aprotinin, a low-molecular-weight polypeptide inhibitor of a broad spectrum of serine proteases [26, 27]. This protease inhibitor was found to demonstrate a marked inhibitory effect (i) on the virus itself, preventing its activation from a non-infectious to an infectious form [20, 28-34] and (ii) on host processes involved in the activation of thrombocyte receptor 1, epithelial Na+ channel (ENaC), kallikrein-activated kinins, and proinflammatory cytokines such as IL-6, TNF-α, thereby reducing clinical thrombosis, coagulation abnormalities, lung edema, and inflammation [35-47]. Both inhibitory activities (VTA and HTA) of aprotinin proved to be useful for its therapeutic effects in the body. Aprotinin was proposed as the first binary chemotherapeutic agent possessing a combined VTA-HTA mechanism [20]. The scheme of proteolytic activation of the influenza virus and the binary therapeutic mechanism of aprotinin are illustrated in Fig. 1. Clinical studies conducted in Russia and Spain between 1990 and 2024 demonstrated the antiviral and therapeutic efficacy of aprotinin inhalations in patients suffering from acute respiratory infections caused by influenza viruses, paramyxoviruses, adenoviruses, coronaviruses and others [29, 46, 48-54].

Figure 1. “Vicious cycle” developing influenza pathogenesis and aprotinin targets.

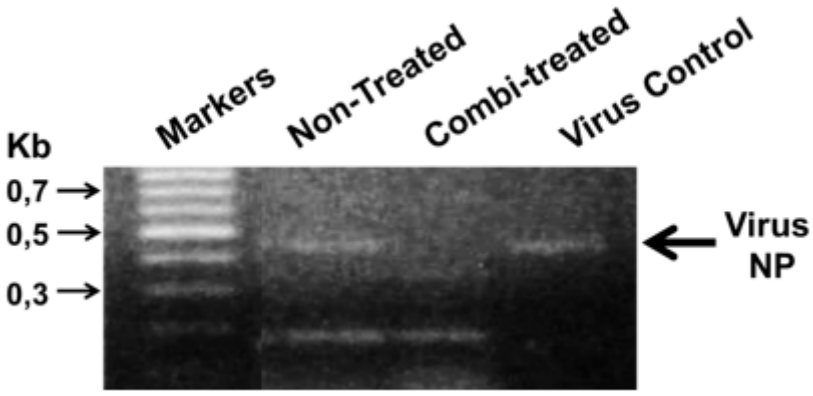


Virus stimulates hot proteases, which is one of the necessary factor of virus multicycle replication and influenza pathogenesis. Up-regulated host activate cellular fusogenic activity and infectivity of synthesized virus through the specific cleavage of the viral receptor protein hemagglutinin HA0 (m.w. 80 kD) into two subunits HA1 (55 kD) and HA2 (25 kD) [20]. Virus-induced cell damage provokes inflammation that induces a leakage of plasmin, kallikrein, thrombin and transcytosis of leucocytes into tissues causing inflammatory processes. Aprotinin suppressing host proteases normalizes proteolytic balance in the infected organism, decreases a spread of virus and development of inflammation to provide multivalent therapeutic action. Designations: KL (kallikrein), PL (plasmin), THR (thrombin), PATR1 (protease-activated thrombocyte receptor 1), ENaC (epithelial Na+ channel), TMPRSS2, TMPRSS13 (transmembrane serine protease 2, and 13), uPA (urokinase plasminogen activator), CMPL 3a, 5a (protease-activated compliment components 3a and 5a), TNF-α (tumor necrosis factor alpha), CAT (cathepsins), TCL (secretory bronchiolar serine protease - Tryptase Clara), PAR1,2 (proteases activating receptors 1 and 2).

There are many ways to further modernize binary drugs through numerous combinations of different agents acting on multiple cellular and viral targets [21, 22, 55, 56]. Such drug combinations will further enhance their synergistic therapeutic efficacy and antiviral spectrum against a wide range of viruses, while reducing therapeutic dosages and side effects. These properties of combined drug formulations will be particularly useful in the fight against new, unknown viral challenges, including avian influenza, highly pathogenic coronaviruses, and novel, still unknown viruses [57-59]. One real threat of this nature currently exists in the form of highly pathogenic variants of the avian influenza virus, predominantly viral hemagglutinin HA antigenic subtypes H5 and H7 containing a highly cleavable multibasic proteolytic site [60-62]. Influenza viruses are maintained in natural avian reservoirs, including aquatic bird species. From this reservoir, the viruses are transmitted to other migratory birds, domestic poultry, swine, cows, and a variety of other mammals. There is a risk that the avian influenza virus will adapt to humans and cause a pandemic with unpredictable consequences [5]. To counter this threat, it is necessary to have highly effective drugs with reasonable and predictable therapeutic and antiviral efficacy in the official arsenal, available in the general healthcare system.

One such possibility is demonstrated in Figs. 2 and 3. The figures show an assay of aprotinin, a protease inhibitor, combined with ribavirin, a viral polymerase inhibitor. Both of these drugs have been approved for clinical use in humans [20, 63, 64]. This drug combination affects multiple targets in viral replication and disease pathogenesis [8, 30, 65-67]. Four daily 7.5-min aerosol inhalations of low dosages of this combination provided achievable therapeutic efficacy in a mouse model of infection caused by the highly pathogenic avian influenza virus subtype H7N2, analogous to the modern avian influenza virus subtype H5N1. Furthermore, this treatment reduced the most serious generalized syndrome of avian influenza-virus dissemination in the infected host and its penetration into the mouse brain. It is important to mention that the inhalation delivery doses used provided high antiviral concentrations in the respiratory epithelial lining fluid, and these doses were more than 100 times lower than toxic concentrations for animals and humans (see the legend to **Fig. 2)**.

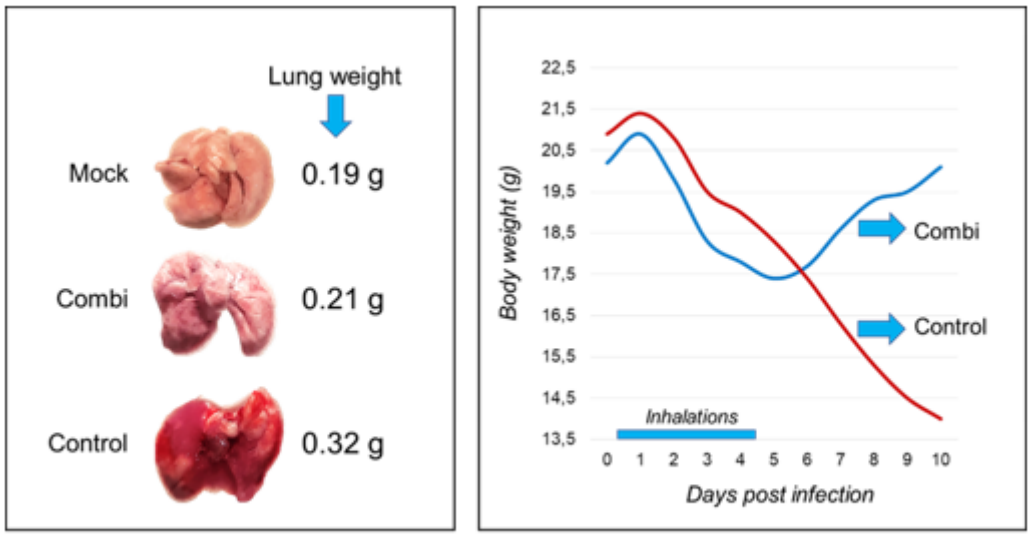
Figure 2. Effective inhalation therapy using a mixed aerosol of aprotinin and ribavirin in mice infected with the highly pathogenic avian influenza virus.



Mice (25-28 g) infected with the highly pathogenic avian influenza A/chicken/New Jersey/294598/04 (H7N2) virus were given four 8-min inhalations daily of a finely dispersed solution containing a combination of protease inhibitor (aprotinin - 0.3 mg/ml) and ribavirin (8 mg/ml), a combination therapy, or placebo (no drug aerosol treatment), for 4 days. This aerosol inhalation regimen provides 31.5 μg/g/day and 1.5 μg/g/day dosages for ribavirin and aprotinin, respectively. These doses correspond to 2.6 mg/kg/day and 0.15 mg/kg/day for ribavirin and aprotinin aerosol inhalation dosages in humans, which provides concentration in alveolar lining fluid is 30-60 times the half maximal response (EC50) of about 25 μg/ml (ribavirin) and 70 μg/ml (aprotinin)

observed against virus in cultured Caco2 cells test [65; 66]. Four days after the start of treatment, the mouse brain was removed, and RNA was isolated from the brain. Viral RNA was determined using a highly sensitive reverse transcriptase reaction (RT-PCR) and PCR with virus-specific primers for the viral NP gene, followed by analysis of the PCR product using agarose gel electrophoresis [68]. The figure shows the presence of the analyzed major viral gene NP (nucleoprotein) product (MW 0.470 kb) in the brain of control mice and its absence in the brain of mice receiving aprotinin-ribavirin combination treatment. Additionally, as shown in **Fig. 3,** combined aerosol treatment of mice infected with a lethal dose of the mouse-adapted highly pathogenic H7N2 avian influenza virus had a significant positive effect on disease dynamics: it significantly reduced lung inflammation, improved animal weight gain dynamics, and prevented mouse death. These data propose a prototype for an effective treatment approach against lethal avian influenza, which is known to cause a 60–90% mortality rate in humans [5, 69]. Developed combination therapies with rational multivalent action on a range of viral and cellular targets represent an attractive avenue for creating an effective class of drugs against the highly dangerous threat to humans that could arise should a similar epidemic variant of the avian influenza virus emerge in nature, capable of infecting humans via aerosol transmission. Currently, such variants of the highly pathogenic H5N1 influenza virus have emerged in nature only in certain mammalian species, such as cats, seals, sea lions, foxes, minks, cows, fur animals, etc., and domestic birds. [4, 5]. There is a real, high risk of such a virus adapting to and spreading among humans. If this scenario occurs, the designed combination therapy could be used as the first line of defense for the urgent protection of humans.

Figure 3. Therapeutic efficacy of inhalations with an aerosolized mix of aprotinin and ribavirin in mice infected with the highly pathogenic H7N2 avian influenza virus.



Mice (3 mice in each group) infected with highly pathogenic avian influenza virus A/chicken/New Jersey/294598/04 (H7N2) were given combined aprotinin-ribavirin inhalations for 4 days beginning on 12 hrs after infection, as described in the fig.2. On the day 6 after infection, the lungs were removed, weighed, and photographed (left panel A). The right panel B shows the mouse body weight daily dynamics of treated (Combi) and NoDrug-treated control (Control) mice.

FUTURE MODERNIZATION OF BINARY ANTIVIRAL DRUGS

The next step in the modernization of aprotinin-based binary drugs involved the development of a pressurized manual metered-dose inhaler (pMDI) containing the ozone-saving fluorocarbon propellant 134A. This propellant has several undesirable technological challenges, including the poor solubility of propellant A134 in aqueous solutions used to prepare the aprotinin polypeptide substance, as well as the possible inactivation of the aprotinin protein structure in the propellant mixture of the designed pressurized inhaler. All these limitations were successfully resolved by a three-component composition comprising propellant, ethanol, and glycerin [70], which was adapted for this aprotinin-containing inhaler. The resulting first compact manual metered-dose inhaler, containing polypeptide aprotinin as the active pharmacological substance, passed technical and clinical trials in Russia and was approved under the trademark AERUSR for clinical use in the treatment of acute respiratory viral infections [20].

In the modern era, the concept of novel unknown infectious diseases posing a threat to society, so-called "X" diseases, has emerged [2, 3, 5]. Examples of such pathogens include highly pathogenic avian influenza viruses of the H5 and H7 subtypes, which cause mortality rates of 50–90% in animals and humans, highly lethal coronavirus strains, and the highly pathogenic monkeypox virus. Combating such types of new, unknown viral diseases requires broad-spectrum, multi-targeted antiviral drugs as a necessary first line of defense to prevent, or at least reduce, the widespread transmission of these pathogens and high human mortality. In this area, we are developing a binary aerosol drug with two broad-spectrum active substances, for example, aprotinin and ribavirin, each of which is approved for clinical use in humans in the US, Canada, and Russia [20, 63, 64]. The initial pilot study conducted on mice infected with the highly pathogenic avian influenza virus supports this approach, demonstrating the high efficacy of this chemotherapeutic combination, as shown above in **Figs. 2 and 3**.

These data allow MDIs to be considered for rapid individualized medication for each patient at the earliest stages of illness and for early prophylactic use in family and hospital practices. MDI devices are currently undergoing development and improvement in several areas. First, new types of propellants are being developed that do not emit CO_2 and are completely harmless to the ozone layer, so-called green inhalers [71-75]. The first prototypes of such MDIs have already been approved for clinical trials [76]. Second, the use of nanobiotechnology in aerosol formulations allows for the employment of various types of nanoparticles with absorbed active substances, which stabilize aerosolized mixtures, enable targeted delivery of active substances to specific respiratory sections, including the distal domains of the respiratory tract, and provide prolonged therapeutic effects [77-79]. Third, advances in inhalation devices, including pressurized metered-dose inhalers (pMDIs), dry powder inhalers (DPIs), and different types of nebulizers, have established the pulmonary route for administering biologics, such as biologically active enzymes and peptide molecules, monoclonal antibodies, nanobodies, and their combinations. These devices, delivering inhaled biologics, optimize multivalent therapeutic mechanisms of drugs with their targets and reduce effective dosages and side effects [20, 78-80].

CONCLUSION

(i) The respiratory infection process caused by viruses, such as influenza viruses, coronaviruses, paramyxoviruses, etc., develops through the mechanism of a "vicious cycle". The virus activates host proteases, and upregulated proteases activate virus-dependent cell fusion and infectivity of the synthesized virus to promote the spread of infection. On the other hand, cell damage and an overbalance of host proteases, such as plasmin, kallikrein, thrombin, transmembrane serine proteases, PAR1, TMPRSS2, TMPRSS13, uPA, matriptase, tissue plasminogen activator, etc., provoke pathogenic inflammatory processes, lung edema, hyperactive coagulation, thrombosis, and other pathogenic mechanisms.

(ii) The exogenous protease inhibitor aprotinin, which suppresses a wide spectrum of proteases, normalizes the host proteolytic balance, decreases the spread of viral infection and the development of inflammation, and provides a marked therapeutic effect.

(iii) A combination of virus-targeted and host-targeted drugs, known as binary drugs, for example, ribavirin, an inhibitor of viral polymerases, and aprotinin, a protease inhibitor, provides protection against highly pathogenic avian influenza virus at low drug dosages and prevents virus dissemination throughout the infected organism.

(iv) These binary drug combinations, possessing a wide spectrum of antiviral activity, are proposed for use in hand-held metered-dose inhalers (MDIs) as effective drugs for the first line of defense against future new, unknown highly pathogenic viruses, referred to as diseases "X".

(v) The global healthcare system must remain prepared for the high potential risks of natural virus "X" mutations that can dramatically increase viral pathogenicity in humans and viral transmission from birds and animals to the human population, thereby initiating a new pandemic process.

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Competing interests

The author declares no known competing financial interests or personal relationships that could influence the work reported in this article.

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