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Influenza A Virus Inhibition through Multivalent systems having Dual Mechanism of Action

1. Background and significance:

Influenza Virus and Healthcare Challenges: Influenza (flu) virus is an enveloped RNA virus whose membrane anchors two viral proteins, hemagglutinin (HA) and neuraminidase (NA) that regulate interactions of virion with host cells. The viral infection develops in several steps that starts with (1) the attachment of the virus to the sialic acid (SA) receptor on the cell surface through HA. (2) The virus is then taken up by the cell through endocytosis. This initiates a complex process involving (3) the delivery of genetic material, (4) multiplication to make new copies, (5) expression of viral proteins, and the budding of the new virions. (6) The final step involves the release of newly formed virions by cleavage of SA by viral NA sialidase protein (**Figure 1**).

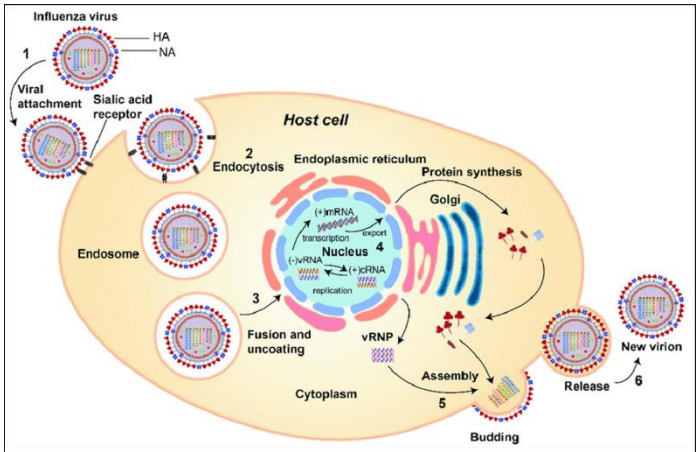

Figure 1. Life cycle of influenza virus involving its attachment and release from the host cell.

In recent years, the emergence and reemergence of infectious diseases caused by influenza virus have posed significant challenges to global health.[1] Influenza virus (flu) can cause a wide range of illnesses, from mild infections to severe and life-threatening diseases. It causes 3-5 million cases of severe illness and an estimated 500,000 deaths every year around the globe. Recent studies have illustrated that influenza A virus pre-infection can significantly promote the infectivity of SARS-CoV-2 infection.[2,3] Together with disease burden of influenza, the economic impact to the society and healthcare system due to direct medical expenditure and indirect losses caused by absenteeism and dropped productivity is substantial.[4]

The Existing Treatments: One practical approach to prevent infections is through active immunization using vaccines that target and eliminate causative agents. However, the low spectrum efficacy, associated high cost, highly variable viral genomes, and necessity of vaccine for entire population, make it necessary to develop effective antiviral drugs.[5] Moreover, the administration of vaccine to the population which is already at risk is also challenging.

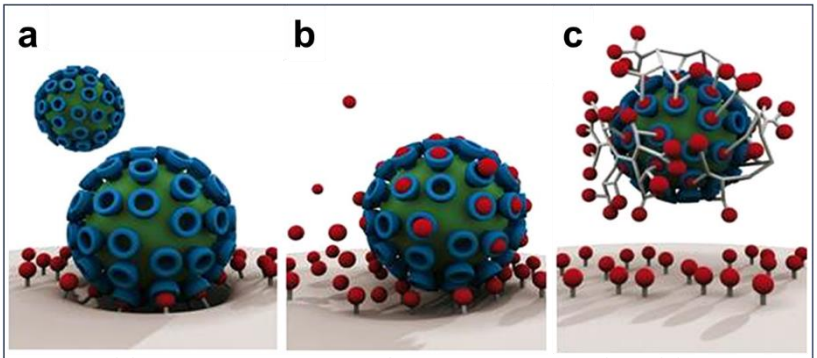

Figure 2. (a) Multivalent interactions of a virus to the cell surface. (b) monovalent inhibitors (red) are not able to completely inhibit the virus attachment to the cell. (c) multivalent inhibitors can significantly inhibit the virus, preventing its spread.

Several NA-blocking anti-influenza drugs such as zanamivir (ZA), peramivir and oseltamivir are available,[6] but they usually fight symptoms and are active against only particular viral strains. Moreover, the prolonged use of these medications can also lead to the development of virus resistance as suggested by oseltamivir and zanamivir-resistant strains.[7]

Another approach to prevent virus infection involves the inhibition of early step of infection by targeting the viral HA proteins to prevent the entry of virus into the target cell membrane.[8] For efficient virus inhibition, the virus-inhibitor interactions should be stronger than the virus-cell interactions so that virus preferably interact with the inhibitor instead of the host cell receptor (**Figure 2**). Multivalent silaylated compounds (i.e., HA inhibitors) of different types such as polymers,[9] nanogels[10] and nanoparticles,[11] which not only binds to the several receptors on virus

simultaneously but also shield the virus surface to circumvent attachment to the host cell, have shown to prevent the virus infection. But, due to high structural variability of HA, broad activity against a different viral strain is still elusive for most of sialylated multivalent inhibitors.

The Need: A novel approach to treat influenza (flu) virus infection is needed, which facilitates (i) infection prevention at any stage of viral cycle, (ii) with a very low chances to cause resistance, and (iii) can treat drug-resistant viral strains as well. To meet such requirements, we proposed developing multivalent virus inhibitor having dual mode of action to target double binding sites on the virus surface.
We aimed for generating a highly potent influenza virus inhibitor by combining two different bioinspired designs: (i) functionalizing the polymeric backbone with SA, acting as a competitor to the natural SA present in the cell membrane, aims to block the viral HA proteins, (ii) complementing the polymer with zanamivir (ZA), an approved NA inhibitor to enhance the interaction by binding and blocking the NA, thus exhibiting dual mechanism of inhibition. Besides these mechanisms, the supplementary SA and ZA groups on the polymeric backbone not interacting with receptors may protrude on the virus surface and can cause a steric shielding effect. Compared with available drugs, the proposed systems exhibited double mechanism of action, targeting both the HA and NA viral surface proteins in multivalent manner. Because of the multivalency, the virus-inhibitor interactions remain potent even in the case where the attachment of the monomeric binder/drug becomes inefficient due to mutation.

2. Objectives:

This research proposal addressed the critical need for novel approaches to combat influenza virus infection. The application of multivalent interactions in virus inhibition represents an innovative strategy that could lead to the development of more effective and durable treatment.[12] The outcomes of this research contributed significantly to the field of medicinal chemistry, particularly in the design and development of next-generation antiviral agents. The primary objectives of this research proposal were as follows:

a. To design, prepare and characterize multivalent compounds targeting influenza A virus.
b. To evaluate the inhibitory efficacy and mechanism of action of the developed multivalent inhibitors against influenza A virus and thus preventing viral infection.
c. To contribute to the advancement of knowledge in the field of medicinal chemistry and infectious disease therapeutics.

3. Key Questions Answered

a. How effective are multivalent systems with dual mechanisms of action in inhibiting influenza A virus infection?
b. What is the optimal structural design for multivalent systems to enhance their antiviral efficacy?
c. How do multivalent systems interact with influenza A virus at the molecular level?
d. Can the dual mechanism of action prevent viral replication and entry into host cells simultaneously?
e. Can the Influenza A virus develop resistance to multivalent systems, and if so, how can this be mitigated?

4. Experimental design and methods:

Design and synthesis: For the efficient inhibition of influenza, targeting both the surface proteins i.e. HA and NA by multivalent system proved to be a useful strategy. The dual receptor multivalent inhibitors were synthesized through the functionalization of linear polyglycerol (IPG) with NA-binding drug ZA and natural HA-binding molecule SA. This is the first time dual-targeting low molecular-weight polymers were prepared. The study demonstrated the superior efficacy of the multivalent systems, which outperforms commercial drug by up to 10,000 times, marking a significant advancement in broad-spectrum antiviral therapy. In the follow up study, we extended the concept of dual targeting to nanoparticles, where nanoparticles, of matched topology with virus, were functionalized with IPG containing ZA and SA. The study introduced the innovative concept of "topology-matching design" for virus inhibitors. By designing nanoparticles that match the nanotopology of IAV virions, this study demonstrated a dual-action inhibition of the virus, achieving more than 99.999% inhibition even 24 hours post-infection, highlighting its potential as a potent antiviral treatment.

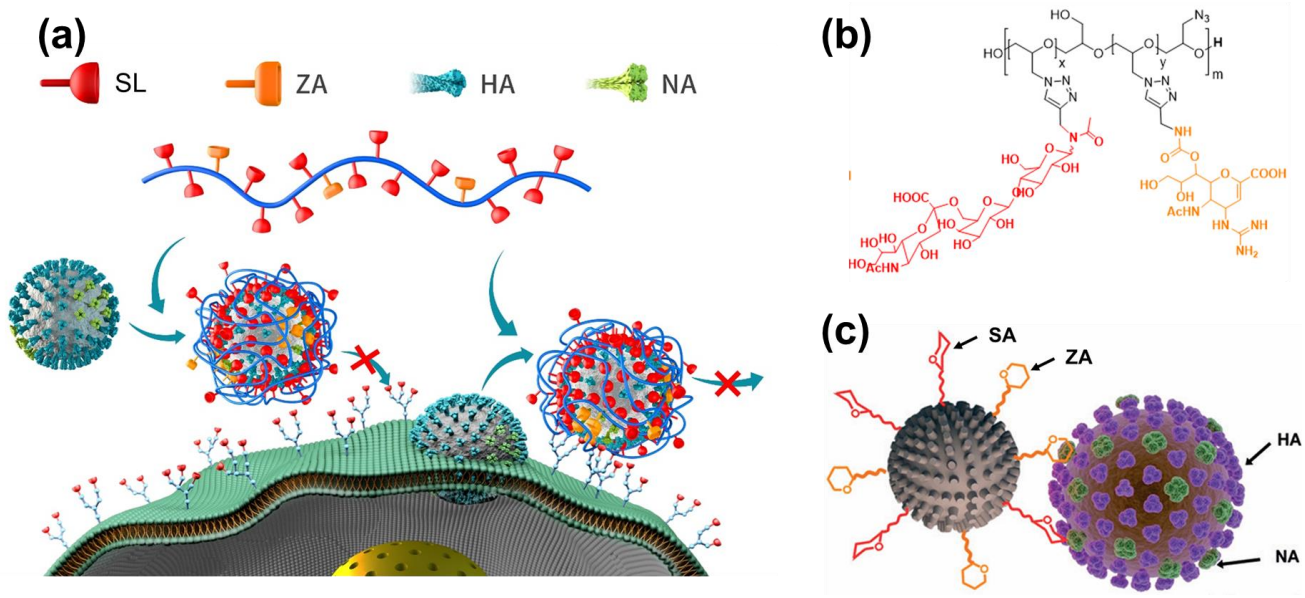

Figure 1. (a) Principle of the dual binding mode of multivalent polyglycerols. A linear polymer (blue) with covalently bound sialic acid (SA, red) and zanamivir (ZA, orange) ligands, does not only inhibit the binding of influenza A virus (grey ball with viral HA and NA glycoprotein) to the cellular surface, but also blocks the release of progeny virions by inhibiting SA cleavage from the cell surface. (b) Chemical structure of SA and ZA functionalized lPG (b) Proposed binding patterns between SA and ZA functionalized virus topology matched nanoparticle and influenza virus particle.

Biological evaluation and mechanistic studies: Inhibition studies were conducted using relevant influenza virus strains such as A/X31 (H3N2), A/Panama/2007/1999 (H3N2), and A/Bayern/63/ 2009 (H1N1pdm). The inhibitory efficacy of the multivalent inhibitors was initially evaluated through *in vitro* assays, such as enzyme activity assays, cell culture studies, and microorganism growth inhibition assays.
We first tested all compounds for the inhibition of NA activity of virions in a standard fluorescence-based assay using 2′-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA).[13] Next, the potential of these multivalent compounds to prevent binding of the seasonal virus to cells was studied by hemagglutination inhibition assay. The plaque reduction assay which is particularly useful for studying the efficacy of antiviral drugs, was employed to determine the inhibition potential of the synthesized systems. This assay provided quantitative data on the ability of polymers to inhibit viral replication. Cell viability of the synthesized multivalent systems was measured by *in vitro* MTS assay.

5. Outcomes:

Novel multivalent inhibitors: The research yielded novel multivalent inhibitors targeting influenza virus, potentially leading to innovative therapeutic agents. Research outcomes can help in designing potential inhibitors for other pathogenic microorganisms such as viruses (herpes simplex virus and SARS-CoV-2) and bacteria (E. Coli and P. aeruginosa), which interact with the host cells via their surface proteins.
Insight into mechanisms: The study provided insights into the mechanisms by which multivalent interactions exert inhibitory effects on influenza virus and can help us further understand the field to develop potential inhibitors which can block infection at any stage of the cycle.
Reduced drug resistance: This research demonstrated the potential of multivalent inhibitors to reduce the development of drug resistance, contributing to more sustainable treatment strategies.

6. References

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बद्री प्रसाद

Dr. Badri Parshad
Harvard Medical School
Harvard University
Boston, MA 02129, USA