Development of fusion inhibitory lipopeptides against airborne viral infections

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Airborne infection is transmitted through small aerosolised particles suspended in the air, and is responsible for the transmission of many infectious diseases of the considerable importance in both human and veterinary medicine. Although vaccination is supposed to be the best prevention for these infectious diseases, vaccines are not always available, particularly for new emerging pathogens and in some cases not sufficient in the prevention of new outbreaks. For an enveloped virus it is possible to identify candidate lead molecules directly from the genetic information, in the form of peptides corresponding to key domains of the viral fusion machinery. These peptides could specifically block viral fusion and consequently inhibit viral infection. Recent results have demonstrated that cholesterol tagged peptides provide highly potent fusion inhibitors with prolonged circulatory half-life *in vivo*. In addition, the dimerization was shown to increase the antiviral potency of some of these peptides. We have focused on 3 important human pathogens: Measles virus (MeV), Niph virus (NiV) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). MeV is highly contagious virus which remains the leading cause of death among vaccine-preventable diseases and continues to resurge worldwide. NiV is highly pathogenic zoonotic virus recently emerged in South-East Asia, causing up to 100% lethality in humans. SARS-CoV-2 is the etiological agent of the coronavirus disease 2019 (COVID-19) which is responsible for the current pandemic. These viruses infect target cells by coordinated action of viral envelope glycoproteins.

After binding of MeV hemagglutinin to the cell receptor, MV fusion protein initiates the virus-cell fusion, leading to the cell infection. We have developed the lipopeptide HRC4, capable of inhibiting MeV fusion and preventing measles infection in rodent models. We have further advanced this approach by administration of aerosolized peptides capable of inhibiting respiratory MeV infection in non-human primates, cynomolgus monkeys. We have developed an inhalation strategy using a customized nebulizer with the specific mesh size and interface ensuring a functionality of nebulized particles and their distribution in respiratory tract. Nebulization of HRC4 efficiently prevented MeV infection in monkeys, leading to the complete absence of MeV-RNA, MeV-infected cells and MeV-specific humoral response in treated animals housed separately from untreated. Furthermore, similar strategy has been used to analyze aerosolized peptide capable of inhibiting NiV fusion and entry into the cell. Using the similar customized nebulizer as for MeV, we have demonstrated in BSL4 laboratory in Lyon that aerosolization significantly delays the lethal outcome of NiV infection in African green monkeys, the nonhuman primate model which reproduces NiV infection as seen in humans. These results open new perspectives for antiviral prevention strategy against measles as well as the other airborne viruses, including SARS-CoV-2.

Entry of SARS-CoV-2 into the host cells is mediated by ACE-2 receptor, which is a component of the angiotensin-regulating system. This virus binds ACE2 via its envelope Spike (S) glycoprotein, leading to the virus-cell fusion and consequent SARS-CoV-2 entry and replication. SARS-CoV-2 S is a homotrimer in which each monomer contains 2 subunits, S1 and S2. S2 is responsible for the fusion and presents two heptad repeat domains (HR) in N and C amino termini. The interaction between these domains (HRC and HRN) is critical for the membrane fusion. We have analyzed the capacity of fusion inhibitory peptides, derived from the heptad repeat domains in C-amino terminus (HRC) of Spike, to inhibit SARS-CoV-2 infection in a murine model, both *ex vivo* and *in vivo*. We observed that the peptide causes significant inhibition of SARS-CoV-2 infection in organotypic cultures prepared from lungs of mice expressing human ACE2. *In vivo*, while SARS-CoV-2 is provoking 100% lethal infection within 10 days post-infection in K18-hACE2 mice, intranasal administration of peptides reduced the body-weight loss and protected between 80% to 100% of mice from the SARS-CoV-2-induced lethality. These results were associated with high decrease of viral load in lungs compared to the mock-treated group. These findings indicate that fusion inhibitory peptides highly reduce the clinical impact of SARS-CoV-2 infection in the animal model, thus providing a proof of concept for a new complementary approach of antiviral prophylaxis to be developed as part of the global effort against the current SARS-CoV-2 pandemic. This antiviral strategy may form the basis for an efficacious and timely emergency response, immediately following identification of potentially dangerous new virus which uses a similar fusion mechanism for viral entry.