Concerns of using sialidase fusion protein as an experimental drug to combat seasonal and pandemic influenza

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Sialidase fusion protein is reported to have great potential to combat seasonal and pandemic influenza, because it may prevent influenza virus infection by removing all sialic acid receptors from host cells. Meanwhile, recent studies have demonstrated that absence of α2-6 sialic acid does not protect a cell from influenza infection, and influenza virus can infect desialylated cells, suggesting that accessible surface sialic acid is dispensable for influenza virus infection. In addition, studies using animal models have shown that neuraminidase promotes adherence and invasion of Streptococcus pneumoniae, because cleavage of sialic acid from host cells exposes cryptic receptors for S. pneumoniae. The purpose of this article is to comment on the benefits and potential risks of using sialidase fusion protein as an experimental drug to combat seasonal and pandemic influenza.

Keywords: influenza virus, secondary bacterial pneumonia, animal model

Introduction

Influenza is an acute contagious respiratory disease that can result in illness ranging from mild to severe and life-threatening complications. A pandemic influenza is a global outbreak of influenza and occurs when a new influenza virus emerges and spreads in humans. The origin, spread and control of pandemic influenza have been well summarized by recent review articles. With the increase in global transportation, urbanization and overcrowded conditions, epidemics due to the new influenza virus are likely to spread quickly around the world. The World Health Organization (WHO) released the WHO Global Influenza Preparedness Plan in 2005 to assist WHO Member States and those responsible for public health, medical and emergency preparedness to respond to threats and occurrences of a future pandemic influenza. More than two dozen countries also prepared their own National Influenza Pandemic Plans. Main preparedness activities have focused on preparing and rehearsing response plans, developing a pandemic vaccine and securing suppliers of antiviral drugs.

Avian influenza viruses replicate in the intestinal tract and the respiratory tract of the host and preferentially recognize receptors with saccharides terminating in sialic acid-α2,3-galactose, whereas human influenza viruses target primarily cells in the respiratory epithelium of the human airway and preferentially recognize receptors with saccharides terminating in sialic acid-α2,6-galactose. Based on the WHO report of 12 November 2007, there have been 335 laboratory-confirmed cases and 206 deaths caused mainly by poultry-to-human transmission of avian influenza A (H5N1) in 12 countries since December 2003. The specificity for different receptors is one of the explanations for the species barrier between avian and human influenza viruses. However, a mutant or reassortant avian influenza virus could trigger the next influenza pandemic when it gains the capability to infect humans efficiently and spread easily from human to human. The epidemiology, virology, pathogenesis, hospital infection control, laboratory diagnosis and treatment of human influenza H5N1 virus infections have been reviewed by recent published articles.

Faced with the threat of the potential for bioterrorism, the US National Institute of Allergy and Infectious Diseases (NIAID) made a major investment in furthering the development of new drugs to treat potential agents of bioterror in 2006. NIAID awarded six product development contracts totalling more than $212 million to advance the therapeutics against the most significant threats to the nation’s biodefence. One of the six contracts is related to influenza, ‘Development of DAS 181 (Fludase®) as a Broad Spectrum Therapeutic Agent for All Annual and Pandemic Variations of Influenza’. As described by NIAID, sialidase fusion protein (Fludase) is a broad-spectrum anti-influenza agent with the potential for prophylactic and therapeutic use, which targets the host receptor for influenza rather than the virus. The novel mechanism may overcome the problem of antiviral resistance, an issue with currently licensed antivirals. NIAID expect Fludase to be developed into a drug in 4 years. Theoretically, if successful, the world would not need to worry about the limited capacity of global vaccine manufacturing, worldwide shortage of anti-influenza drugs such as...
Influenza virus NA and secondary bacterial pneumonia in animal models

Influenza-related secondary bacterial pneumonia is an important cause of death in young children, the elderly and high-risk patients with chronic diseases during influenza epidemics. During the last three influenza pandemics (1918, 1957 and 1968), the excess mortality rates were mainly caused by secondary bacterial pneumonia rather than the acute influenza respiratory infection itself. The severity of secondary bacterial pneumonia during or after influenza infection is determined by a complex interaction among virus, bacteria and host. To study the relationship between influenza virus infection and secondary bacterial pneumonia, different animal models including mouse, chinchilla, cotton rat and ferret have been used, which has contributed to the understanding of the disease synergism.

van der Sluijs et al. established a mouse model to study post-influenza pneumococcal pneumonia and evaluated the role of IL-10 in host defence against Streptococcus pneumoniae (S. pneumoniae) after recovery from influenza infection. They demonstrated that mice who had recovered from influenza infection appeared to be highly susceptible to secondary bacterial pneumonia, as reflected by increased lethality after infection with S. pneumoniae (100% lethality in mice who had recovered from influenza infection by day 3). LeVine et al. demonstrated that prior exposure to influenza A decreases clearance of a secondary S. pneumoniae exposure from the lungs of BALB/cJ mice. Scanning and transmission electron microscopy revealed that the ciliated and secretory cells of the mouse tracheal epithelium had desquamated and the mucosa were coated with a continuous layer of basal cells by the fourth and sixth days after influenza virus infection. The adherence of S. pneumoniae to influenza virus-infected tracheae was significantly enhanced on day 6.

Studies by McCullers’s group using a mouse model of synergism between influenza virus and S. pneumoniae, have shown that viral influenza NA increases S. pneumoniae adherence in the lungs by cleaving sialic acid residues from the surface of host cells, exposing cryptic receptors for S. pneumoniae to adhere, and established that viral NA is an important factor in viral-bacterial synergism. The same group also created and characterized a set of recombinant influenza viruses with NA from representative strains from 1957 to 2004. They found that the specific level of their NA activity correlated with their ability to support secondary bacterial pneumonia in a mouse model (female BALB/c mice). They explained: ‘that generally higher levels of NA activity are found in modern H3N2 than in H1N1 viruses is consistent with the hypothesis that high levels of NA activity lead to higher mortality from secondary bacterial pneumonia’ and concluded: ‘our data provide direct evidence that NA activity in influenza viruses is a predictor of mortality from secondary bacterial pneumonia’.

Seki et al. published two papers in 2004 related to influenza virus and bacteria co-infection in mice. In the first paper, they demonstrated that all mice (male, CBA/J) infected with both influenza virus and S. pneumoniae died within 3 days of S. pneumoniae inoculation. Their results were consistent with those reported by van der Sluijs et al. and Peltola et al., which demonstrated that influenza virus infection contributes to secondary bacterial pneumonia and death in mice. In the second paper, Seki et al. reported that none of the control mice (without Pseudomonas aeruginosa infection) died even when co-infected with S. pneumoniae and influenza virus. Seki et al. realized that the results of their second report were in conflict to those of their first report and explained: ‘the doses of influenza virus and S. pneumoniae used in our study were probably insufficient to induce death of ddY mice, although when used at the same doses, both organisms caused death of other murine strains, such as BALB/c or CBA/J mice, respectively’.

Giebink et al. used the chinchilla model to study the interaction of influenza A virus with S. pneumoniae in the pathogenesis of experimental otitis media. Their studies suggest that influenza A virus infection contributes significantly to the pathogenesis of acute otitis media caused by S. pneumoniae. The cotton rat model has also been used by Braun et al. to study the relationship between influenza and bacterial super-infection. Braun et al. demonstrated that co-infection of cotton rats with both S. aureus and influenza A/Wuhan/359/95 (H3N2) caused significantly higher mortality, higher levels of bacteraemia and pulmonary bacterial load 4 days post-infection and worse pathology 7 days post-infection. Using the ferret model, Peltola et al. demonstrated that influenza viruses of any subtype increased bacterial colonization of the nasopharynx in young ferrets infected with influenza virus and then challenged with pneumococcus; 9 out of 10 ferrets infected with H3N2 subtype influenza A viruses developed either sinusitis or otitis media, whereas only 1 out of 11 ferrets infected with either an H1N1 influenza A virus or an influenza B virus did so. These data may partially explain why bacterial complication rates are higher during seasons when H3N2 viruses predominated.

Studies using different animal models including mouse, chinchilla, cotton rat and ferret demonstrate that influenza virus infection contributes significantly to the secondary bacterial pneumonia, and the acute otitis media and NA activity in influenza viruses is a predictor of mortality from secondary bacterial pneumonia.

Will removing sialic acid receptors block influenza virus infection?

In order to start infection, influenza viruses must overcome obstacles in the human upper respiratory system to reach the cells of ciliated respiratory epithelium. The first step of influenza virus infection is recognition of sialic acid receptors on the surface of host cells by the viral HA protein. Theoretically, removing terminal sialic acid receptors from host cells should block infection by any existing or future strains of influenza virus.
viruses, because viruses could not enter the host cell without the receptor molecules bearing α2-3- and α2-6-linked sialic acids. However, the infection of desialylated cells has been reported recently, suggesting either the presence of sialic acid-independent receptors or a multi-stage process.28 Stray et al.28 reported: ‘exogenous sialidase quantitatively released all sialic acids from purified glycoproteins and glycolipids of MDCK cells and efficiently removes surface sialic acid from intact cells. . . . The ability of influenza A reassortant viruses to infect desialylated cells is shared by recent H3N2 clinical isolates, suggesting that this may be a general property of influenza A viruses. We propose that influenza virus infection can result from sialic acid-independent receptors, either directly or in a multistage process’. Stray et al.28 concluded: ‘any requirement for initial interactions with sialic acid to enrich the virus at the cell surface may be bypassed in the presence of large amount of virus’. In a 2004 paper, Chu and Whittaker29 showed that influenza virus undergoes efficient binding, fusion and replication in Lec1 cells, which are deficient in terminal N-linked glycosylation, but cannot initiate infection. They suggested: ‘influenza virus specifically requires N-linked glycoprotein for entry into cells, and that sialic acid, although acting as an efficient attachment factor, is not sufficient as an influenza virus receptor in vivo’. Thompson et al.30 characterized influenza A virus infection of an established in vitro model of human pseudostratified mucociliary airway epithelium (HAE). They concluded, based on their studies, ‘although a broad-range neuraminidase abolished infection of HAE by human parainfluenza virus type 3, this treatment did not significantly affect infection by influenza viruses’.30 It will be important to conduct further in vitro experimentation to determine how robust an infection by influenza virus can really be in cells under the conditions where surface sialic acids are removed by NA. It will also be important to identify sialic acid-independent receptors or co-receptors for influenza virus infection by using new technologies such as glycan microarray technologies.

Using Glycan Array containing 264 oligosaccharides, Kumar et al.31 recently investigated whether recent human H3N2 viruses specific for α2-6 sialyloligosaccharides show differential entry into cells that have varying proportions of α2-6 and α2-3 sialic acids, including human A549 and HeLa cells with high levels of α2-6 sialic acid and CHO cells that have only α2-3 sialic acid. Of the 264 natural and synthetic glycans, 76 have sialic acid; 54 with α2-3 linkages, 22 with α2-6 linkages, and 7 with α2-8 linkages. Kumar et al. found that ‘the virus enters all cell types tested and synthesizes viral nucleoprotein, localized in the nucleus, and haemagglutinin, transported to the cell surface, but infectious progeny viruses were released only from MDCK cells’. They concluded that absence of α2-6 sialic acid does not protect a cell from influenza infection.31

Much attention has focused on the role of α2-3- and α2-6-linked sialic acids as receptors and determinants of cell and host tropism for influenza viruses. A few publications mentioned above deserve more attention for future research, because results from these studies suggest that accessible surface sialic acid is dispensable for influenza virus infection, influenza virus specifically requires N-linked glycoprotein for entry into cells and absence of α2-6 sialic acid does not protect a cell from influenza infection. Consequently, removing sialic acid receptors from host cells with sialidase fusion protein may inhibit influenza virus infection, but will probably not block influenza virus infection completely. Based on the results of Chu and Whittaker,29 it is predictable that N-glycanase fused with a cell surface-anchoring sequence might be a more effective inhibitor of influenza virus infection than the sialidase fusion protein. Olofsson et al.32 proposed that the presence of α2-3-linked sialic acid receptor in human eyes explained the ocular tropism exhibited by zoonotic avian influenza A viruses such as H5N1 in Hong Kong in 1997, H7N7 in the Netherlands in 2003 and H7N2 in the USA in 2003. Therefore, future influenza viruses may still be able to initiate infection by entering human eyes, even though all the sialic acid receptors in the upper respiratory system could be removed completely by sialidase fusion protein treatment.

Concerns of sialidase fusion protein as a drug to combat seasonal and pandemic influenza

An increased incidence of complicated pneumonia associated with S. pneumoniae infection and a clinical history of a recent flu-like illness has recently been observed in otherwise healthy children, which suggests that an antecedent influenza infection predisposes the otherwise healthy patient to subsequent bacterial super-infection and more severe disease.33,34 The clinical features and complications of 35 patients hospitalized with influenza admitted to a large metropolitan referral hospital in Washington, DC, from December 1999 to February 2000 were described by Oliveira et al.35 Their studies showed that hospitalization of patients with influenza pneumonia occurred in both previously healthy and immunocompromised patients and had a high mortality rate. Clinical features, illness types of pneumonia and outcome in 84 patients in Japan with influenza pneumonia were examined by Takayanagi et al.36. They found that the overall mortality rate was 9.5% among all 84 patients with influenza pneumonia. Therefore, preparations for the next influenza pandemic should consider the possibility that many deaths will be caused by secondary bacterial pneumonia.

The purpose of generating the sialidase fusion protein was to make the target cells inaccessible to influenza viruses so that no influenza viruses could infect host cells in the human respiratory tract.13 The advantages of using sialidase fusion protein as an alternative approach to prevent and treat influenza are that it targets the host receptors for influenza rather than the virus and it is a broad-spectrum anti-influenza agent with the potential for prophylactic and therapeutic use. If successful, sialidase fusion protein should be able to inhibit all strains and subtypes of influenza, including H5N1. Theoretically, the world would never have to worry about the potential threat of any future seasonal and pandemic influenza if the sialidase fusion protein could be successfully developed into a drug and approved by the FDA.

Since sialidase fusion protein treatment is based on the activity of NA, it may have potential risks of causing a higher incidence of secondary pneumonia and death during clinical trials in humans. Results from animal studies have demonstrated that the action of NA promotes adherence and invasion of S. pneumoniae to host cells treated with NA. Bhatia and Kast17 recently proposed two hypothetical functions of influenza NA: NA may remove sialic acid in IgA’s hinge region and from the surface of either gamma-delta T cells or mucosa-residing B cells, which together could cause disruption of the mucosa-inA axis, creating localized partial immunosuppression state, enhancing both influenza infection and secondary bacterial pneumonia. To avoid unnecessary damages to human volunteers,
investigators should keep in mind the potential risks of causing a higher incidence of secondary pneumonia when they design protocols for human studies and conduct clinical trials for the experimental drug, sialidase fusion protein.

Studies using existing animal models such as mouse, chinchilla, cotton rat and ferret have demonstrated that influenza virus infection contributes significantly to the secondary bacterial pneumonia, and NA activity in influenza viruses is a predictor of mortality from secondary bacterial pneumonia. Therefore, it will be very important to conduct preclinical studies in existing animal models such as mouse and ferret to validate or disprove the safety concerns of potential secondary bacterial pneumonia in animals treated with the recombinant sialidase fusion protein. Such studies should include observations of animals for clinical signs of secondary bacterial pneumonia and other adverse clinical effects at different time points after treatment with different doses of the recombinant sialidase fusion protein and infection with S. pneumoniae. After randomization, animals should be assigned to different treatment groups and one vehicle control group for preclinical studies performed according to Standard Operating Procedures in compliance with FDA Good Laboratory Practice Regulations.

Using in vitro solution tests and ferret model preclinical studies, Rennie et al. recently investigated whether a low pH nasal gel composition could be used as a readily available, safe and effective influenza therapy to inactivate influenza virus. They tested a range of prototype nasal spray formulations, which were all pH 3.5, buffered, aqueous solutions, based on L-pyroglutamic acid with variable secondary acids: ascorbic acid, citric acid, phytic acid and succinic acid. Their virus inactivation studies showed that influenza viruses [A/Sydney/5/97 (H3N2), A/Hong Kong/8/68 (H3N2) and the avian reassortment virus, A/Washington/897/80 A Mallard/New York/6750/78 (H3N2)] are rapidly inactivated by 1 min contact with acid buffered solutions at pH 3.5; the titres of influenza viruses were reduced by 3–6 log cycles. In the similar studies using sialidase fusion protein in the ferret model, Malakhov et al. showed that the titres of influenza viruses were reduced by 0.1–2 log cycles. Rennie et al. concluded: ‘if human influenza benefits were proven, the non-drug nature of the approach means that it might be more readily available to the population at an early stage of infection than current therapies’. By comparing the results of Rennie et al. to those of Malakhov et al., one would probably conclude that low pH gel intranasal sprays are as effective as the recombinant sialidase fusion protein in inhibiting influenza virus infection. In addition, it will be more cost-effective and safe to use the low pH nasal sprays than the recombinant sialidase fusion protein, assuming both approaches can provide the same benefits in inactivating influenza viruses for the prophylaxis and treatment of early influenza in humans.

Hopefully, both recombinant sialidase fusion protein and low pH gel intranasal sprays will be tested and proved to be safe and effective in humans against different strains of influenza viruses before the next pandemic influenza. Both approaches will have advantages of being readily available and applied topically as intranasal sprays or inhalants.

**Transparency declarations**

H. Z. has shares in Z-BioMed, which is involved in the area of influenza research.

**References**

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