Targeting influenza virus neuraminidase – a new strategy for antiviral therapy

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Influenza virus infection causes substantial morbidity and mortality worldwide, but current options for control are limited. Although major advances have been made in antiviral therapy of infections such as herpes and HIV, a 'magic bullet' for influenza has proved elusive. The influenza neuraminidase enzyme is an attractive target for antiviral intervention, its active site is antigenically conserved in all clinically relevant strains and is critical to viral replication. The authors consider the subject of neuraminidase inhibition and discuss, in particular, the development of the oral agent Ro640796 (GS4104). The concept of neuraminidase inhibition is likely to lead to a major breakthrough in the control of influenza.

In recent years, major advances have been made in antiviral chemotherapy, most notably in the treatment of herpes virus, cytomegalovirus and human immunodeficiency virus infections. The core principle of antiviral drug intervention is the specific inhibition of the viral life cycle, which translates directly into an interruption of viral replication and a reduction of infectious particles.

Influenza virus infection presents a substantial medical, epidemiological and economic burden (see Boxes 1 and 2). The dramatic impact of morbidity and mortality associated with influenza has been recognized since at least the time of Elizabeth I of England. Excess mortality has been documented since 1889 and the infamous 1918 outbreak confirmed that influenza is truly 'one of the last major plagues'[1]. With an annual attack rate of 5–20%, outbreaks of influenza virus affect a considerable proportion of the population each year. Such influenza outbreaks translate into millions of work-days lost, hundreds of thousands of hospitalizations, tens of thousands of deaths and billions of dollars in healthcare costs. In pandemics, which occur every 10 to 40 years, the impact of influenza can be even higher: the 1918 pandemic influenza killed an estimated 20 million people worldwide[2]. The recent outbreak of a new highly pathogenic influenza strain (H5N1) in Hong Kong[3] highlights the continuous nature of the threat.

Current options for the control of influenza are limited. An inactivated influenza vaccine is currently available and is the mainstay of public health preventative measures against influenza in the high-risk population. However, influenza vaccines provide only limited protection because of antigenic variation of the influenza virus, and new vaccine components and revaccination are needed annually (see Box 3). Antivirals have been available since the early 1980s, but they are not widely used due to a limited spectrum of activity, their side-effect profiles and the rapid development of influenza viral resistance[4,5]. New therapeutic and prophylactic options for the control of influenza are therefore warranted. The discovery that the active site of influenza neuraminidase is highly conserved offers the potential for effective antiviral therapy and prophylaxis. This enzyme is essential for viral replication and its active site remains unchanged during genetic drifts and shifts, giving the potential for target drugs to be active against all

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Box 1. Epidemiology and surveillance

During each winter influenza infection spreads rapidly through the population, infecting an estimated 100 million people each year in the USA, Europe and Japan. Influenza outbreaks are usually of short duration (several weeks) and vary annually in intensity and between various types of influenza viruses.

In an average year one in ten adults is ill with influenza, but annual attack rates reported from clinics exceed 30% in the paediatric population. Morbidity is concentrated in school children and healthy adults, but the highest mortality rates due to influenza virus infection occur in individuals aged >65 years, with 10,000–40,000 influenza-related deaths per influenza season in the USA alone[40–42]. Influenza virus infection results in 25 million visits to physicians each year, millions of lost work days and hundreds of thousands of hospitalizations[43]. The annual economic burden associated with influenza constitutes an enormous societal cost, estimated at $12 billion per year in the USA alone[44].

Influenza virus pandemics occur unpredictably as a consequence of a genetic shift, approximately every 10–40 years, and affect up to 50% of the community. The 'Spanish flu' (A/H1N1) pandemic of 1918, the worst influenza outbreak this century, killed an estimated 20 million people worldwide[2]. The mortality in the USA associated with each of the recent pandemics of 1957 [A/Asia (H2N2)] and 1968 [A/Hong Kong (H3N2)] has been estimated to be more than 100,000 per pandemic period[45]. The recent outbreak of the new influenza strain H5N1 in Hong Kong illustrates the continuous threat of a new pandemic.

Information obtained from integrated regional and international surveillance activities help to monitor and diagnose influenza activity. Rapid diagnostic tests support surveillance activities, and can be particularly useful as a diagnostic tool outside of an influenza outbreak.

clinically relevant influenza strains. Influenza neuraminidase therefore became an attractive target for antiviral intervention and the focus for rational drug design. If used promptly after infection, drugs emerging from the concept of neuraminidase inhibition could limit the burden of disease by directly targeting the cause of influenza illness, the virus.

Targeting influenza neuraminidase

Influenza viruses belong to the orthomyxovirus family of viruses, and types A and B cause clinically relevant disease states. Figure 2 shows the structure of the influenza A virus. The major surface proteins, which project radially from the outer lipid bilayer, are haemagglutinin and neuraminidase. Haemagglutinin mediates the binding of the virion to sialic acid-containing receptors on the surface of target cells in the respiratory tract. It also mediates the fusion of the viral and cell membranes. This protein is the major target of neutralizing antibodies (and therefore influenza vaccines), but it is highly variable. The mushroom-shaped neuraminidase protein facilitates the release of newly formed virions from the host cell surface and prevents their aggregation by cleaving host terminal sialic acids[6–8] (Fig. 3). By inactivating mucins present within the respiratory tract (which inhibit haemagglutinin activity), neuraminidase also assists the movement of the virion towards the target cell[9]. The M2 tetrameric ion channel, of which only a few copies are present in each virion, is involved in the uncoating process during viral replication. The activity of the neuraminidase enzyme is essential for the replication of influenza A and B viruses[6]. Although most of the neuraminidase protein varies between influenza strains, X-ray crystallography and site-directed mutagenesis show that the amino acid sequence and three-dimensional structure of the enzyme's active site are conserved[10] (Fig. 4). In particular, the 11 key amino acid residues that line the shallow pocket of the active site and interact directly with the substrate (sialic acid) are highly conserved in all strains of influenza A and B investigated[11,12]. This finding is important for two reasons. Firstly, drugs that mimic the natural substrate sialic acid and act as competitive inhibitors should have broad activity. Secondly, the uniformity of the influenza neuraminidase active site underlines the importance of its three-dimensional structure for enzymatic function, and suggests that development of resistant strains could be hindered, as any change in this vital structure might reduce the viability of the virus. Once this important discovery had been made, neuraminidase inhibition became an attractive concept for antiviral intervention. Target drugs, unlike vaccines that protect only against certain influenza strains, would have the potential to show broad and persistent activity against all clinically relevant strains of influenza.

The development of sialic acid analogues to inhibit the neuraminidase active site is an excellent example of rational drug design[13–15] (Fig. 5). The first analogue, 2-deoxy-2,3-dehydro-*N*-acetyl neuraminic acid (DANA or Neu5Acen), was developed in 1969 (Ref. 16). While this compound inhibited neuraminidase, it lacked specificity for viral neuraminidases. The elucidation of the crystal

Box 2. Pathogenesis and disease manifestation

Influenza is very contagious and the virus is primarily spread from person to person by the aerosol route, via droplets formed during coughing and sneezing. Virus particles breathed in by the nose or mouth are likely to be deposited and initiate infection in the respiratory tract. However, influenza viruses can also enter the body through the mucous membranes of the eyes, nose or mouth. The virus replicates in epithelial cells throughout the upper and lower respiratory tracts and an infected person is contagious from 24–48 h before symptoms start, until about the 3rd or 4th day of illness. The pathogenesis of influenza infection suggests that antiviral treatment works best if taken early after the onset of symptoms (Fig. 1).

Compared with other respiratory infections, such as the common cold, influenza infection is much more severe. As the virus multiplies, the lining of the respiratory tract becomes inflamed and swollen. Onset of symptoms is often very sudden and symptoms are not confined to the respiratory tract. The victim at first usually complains of headache, chills and a dry cough. A fever follows soon after, which is usually in the 38–40°C range, but can be as high as 41°C, especially in children. Children are also more prone to gastrointestinal symptoms such as vomiting and abdominal pain[42]. Severe body aches occur, especially in the legs, arms and back. These severe systemic symptoms differentiate influenza from the ordinary common cold. The fever usually lasts about three days, and as this declines respiratory symptoms, such as a runny nose, sneezing and coughing, become more prominent. The acute respiratory symptoms last for five to seven days, but the cough and weakness can persist for up to two weeks.

In healthy adults and children, although influenza is a moderately severe illness, most people recover after one to two weeks with no apparent ill effects. However, influenza can have a serious impact on people with underlying health problems and also in the elderly, and these groups of people are more likely to require hospitalization than the general population. As the influenza virus replicates in the upper and lower respiratory tract, complications caused by primary and secondary (bacterial) infections in these sites are common[42,46]. Otitis media occurs in approximately 12% of children[47], and croup is also a common complication in this patient population. Pneumonia can be caused by the influenza virus itself, but as the natural body defences have been weakened by the influenza infection, also by secondary infections of bacteria. Pneumonia is particularly common in children and the elderly and has a mortality rate of 7–42%, depending on the infecting organism[40]. Less common complications include Reye's syndrome, Guillain-Barre syndrome, encephalopathy, myopathy and myocarditis[42,48].

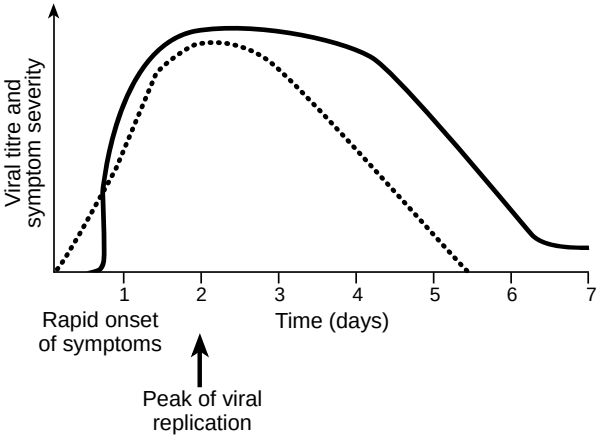

Figure 1. *Association between viral titre (dashed line) and symptom severity (solid line). This demonstrates the need for early antiviral intervention.*

structure of influenza neuraminidase in 1983 (Ref. 10) was a key turning point, allowing the rational design of more potent and specific inhibitors. Modifications to Neu5Acen were made using computerized analysis of the enzyme-substrate transition-state complex and were found to greatly increase affinity for the active site[17]. This led to the development of 4-guanidino Neu5Acen (GG167 or zanamivir), which shows potent and selective inhibition of neuraminidase *in vitro*[18–20].

More recently, innovative compounds that incorporate a carbocyclic structure into the molecule have been developed[15,21], the arrangement offers greater chemical stability than earlier compounds and facilitates modification of the molecule to optimize its properties. The most promising carbocyclic compound is (3R, 4R, 5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexane-1-carboxylic acid, also known as Ro640802 (GS4071)[21]. This compound precisely fits into the three-dimensional structure of the neuraminidase active site to interact with antigenically conserved residues and competitively inhibit the enzyme (Fig. 6). The incorporation of a lipophilic side chain in this molecule exploits X-ray crystallographic evidence of a hydrophobic pocket in the neuraminidase active site, enhancing the affinity for the target.

Box 3. Virus variability

Influenza viruses are divided in types A, B and C, with only types A and B causing clinically relevant disease. Influenza A viruses can infect horses, pigs, seals and a large variety of birds as well as humans[42], while type B infects humans only. Type A viruses are further subdivided on the basis of their two surface antigens, haemagglutinin (H) and neuraminidase (N). The three strains of influenza A virus which commonly affect humans are H1N1, H2N2 and H3N2. Very recently an influenza virus, H5N1, was isolated from humans, which had originated in chickens in Hong Kong.

Influenza viruses undergo significant variation in their surface proteins. Infection with, or vaccination against, one subtype of virus confers little or no protection against a different one, and long-lasting immunity cannot therefore be achieved. Influenza B viruses have more antigenic stability than influenza A, but antigenic variation does occur. Antigenic variation results in major influenza epidemics, as new variants of influenza virus occur. The characteristics of the currently-circulating strains form the basis for selecting those strains to be included in each year's influenza vaccine.

Two types of variation occur[42]. Antigenic drift is the steady accumulation of point mutations that result in amino acid changes in the antigenic sites of the haemagglutinin and/or neuraminidase proteins. These changes reduce antibody binding, thereby reducing pre-existing host immunity within the population and facilitating the spread of epidemic influenza. Antigenic shift is a dramatic and more abrupt change, occurring in the haemagglutinin and/or neuraminidase surface proteins of influenza A viruses. Such changes occur as a result of the replacement of an entire viral gene segment with one from an animal (e.g. avian) influenza virus. As the population has little or no immunity to these new strains, they cause pandemics associated with unusually high morbidity and mortality. In addition to antigenic drift and shift, reintroduction of an older strain of virus is a potential cause of outbreaks.

Unlike haemagglutinin, the structure and amino acid sequence of the active site of the neuraminidase surface protein is conserved across antigenically diverse strains, even though the rest of the protein may vary[10] (Fig. 4). This makes neuraminidase an attractive target for influenza control.

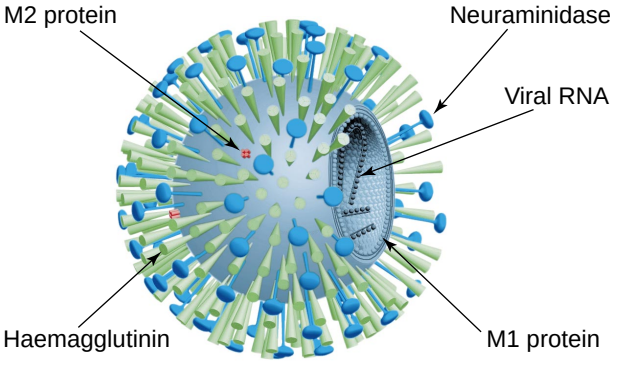

Figure 2. *Structure of the influenza A virion.*

Proving the concept: *in vitro* activity and early clinical studies

Both Ro640802 and GG167 potently inhibit neuraminidase activity at low nanomolar concentrations in all human influenza A and B strains tested (Table 1)[22,23]. Ro640802 also inhibits neuraminidase activity in various avian influenza strains[23,24]. Ro640802 is not cytotoxic in canine kidney cell culture assays, even at concentrations as high as 1 mM. As predicted during drug design, Ro640802 is highly selective for influenza neuraminidase, having little or no inhibitory activity against neuraminidases from human, bacterial or other viral sources[22].

The clinical efficacy of GG167 has been demonstrated in the prevention and treatment of experimental influenza infection. Hayden *et al.*[25] reported the results of four randomized, double-blind, placebo-controlled trials that evaluated intranasal GG167 (3.6–16 mg two to six times daily) in the prevention and treatment of experimental influenza A (H1N1) infection in volunteers. Overall, GG167 prevented laboratory-proved infection and febrile illness in 82% and 95% of subjects, respectively (both $p<0.001$ vs. placebo).

Early treatment of experimental infection with GG167 in these studies reduced peak viral titres, the duration of viral shedding, the frequency of illness and other measures of illness compared with placebo[25]. Subsequent clinical studies showed that administration of inhaled GG167 10 mg (with or without concomitant GG167 6.4 mg administered by intranasal spray) within 48 h of natural influenza A or B infection significantly reduced the duration of symptomatic illness by one day (four days vs. five days) compared with placebo[26]. Preliminary data suggest that GG167 treatment also reduces the impact of influenza virus infection on patients' productivity and health status and the number of contacts made with healthcare professionals[27]. GG167 is currently undergoing Phase III trials, and is at the pre-registration stage in Australia.

GG167 has low oral bioavailability and must be administered by the inhalation or intranasal routes[28].

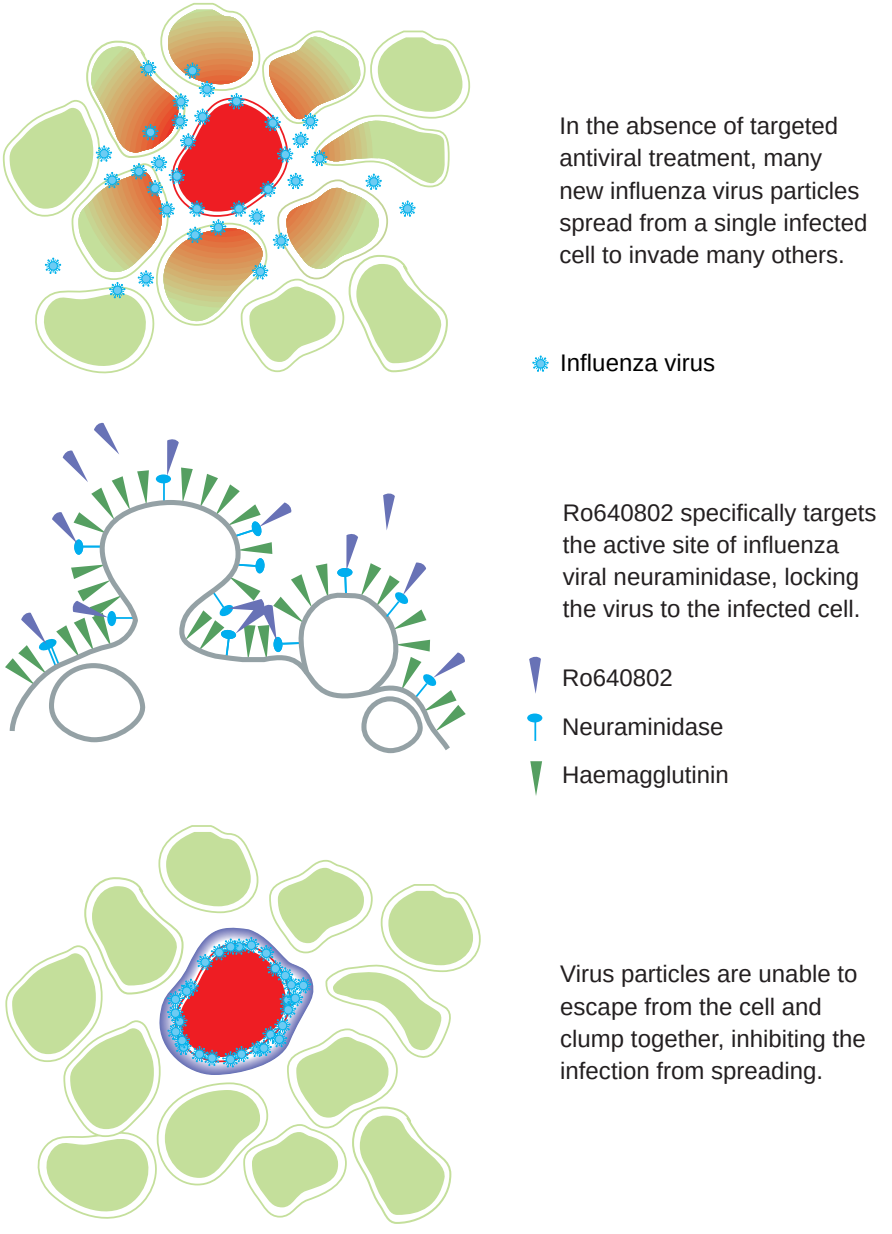

Figure 3. *One mechanism of action of influenza virus neuraminidase inhibitors to reduce spread of infection. Terminal sialic acid residues remain uncleaved on the infected cell, resulting in clumping of the influenza virus around the infected cell.*

Ro640796: an orally available neuraminidase inhibitor prodrug

A strategy for convenient administration and reliable drug delivery to the various sites of influenza infection is the development of orally administered neuraminidase inhibitors. Although Ro640802 is more lipophilic than GG167, its oral bioavailability is similarly low[29], and so an ethyl ester prodrug of Ro640802, known as Ro640796, was developed to improve oral bioavailability. This compound undergoes rapid enzymatic conversion to the active parent drug following gastrointestinal absorption (Fig. 5) causing high and sustained plasma concentrations of Ro640802 (the active drug) in all animals tested[29].

Whole body autoradiography in rats showed that radiolabelled Ro640802 is systemically distributed, with a half-life of approximately 5 h in most tissues. Systemic distribution of Ro640802 concentrations in the lung were approximately twice those in plasma at 6 h post-dose, and 30-fold higher at 24 h post-dose[30]. Importantly, distribution of Ro640802 into brain tissue was minimal, indicating a low potential for CNS adverse effects[30].

Comparison of concentration–time profiles of Ro640802 in bronchoalveolar lavage fluid (BALF) and plasma showed that peak concentrations were similar[31], however, the elimination half-life in BALF was over fourfold longer than that in plasma. This suggests that the local antiviral effect of Ro640796 may be more prolonged than its plasma levels would predict. Pulmonary changes associated with infection may further increase Ro640802 penetration into lung tissue[31].

Antiviral activity of oral Ro640796 in animal models

Mouse pneumonia model

When experimentally infected with influenza virus, mice develop pneumonia and exhibit a high mortality[32,33]. Oral administration of Ro640796 produced dose-dependent protective effects against various influenza viruses in this model[22]. A dose of 1 mg kg^{-1} day^{-1} significantly reduced mortality from influenza A/NWS/33 (H1N1) affording 100% protection. A 10 mg kg^{-1} day^{-1} dose produced similar effects against

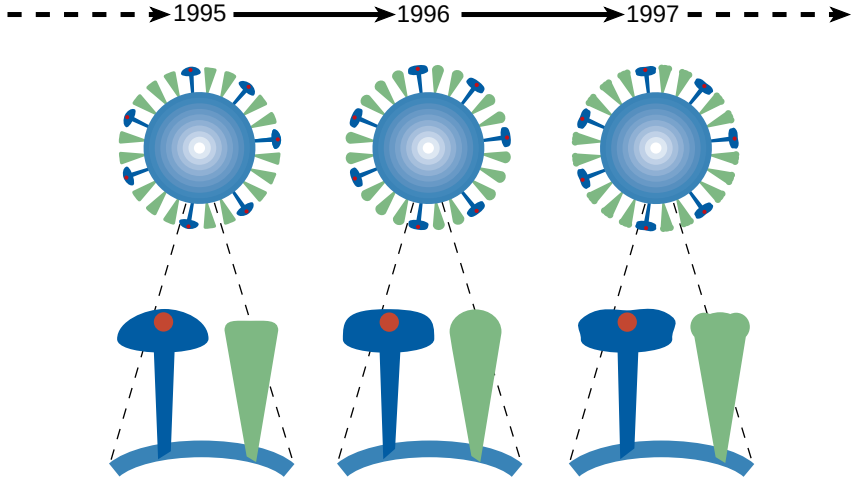

Figure 4. *Influenza viruses change their antigenic structure from year to year. However, the active site of influenza neuraminidase is highly conserved in all clinically-relevant strains, making it an ideal target for antiviral intervention.*

influenza A/Victoria/3/75 (H3N2) and influenza B/Hong Kong/5/72. These effects were associated with substantial reductions in lung viral titres. Interestingly, Ro640796 (10 mg kg^{-1} day^{-1}) increased survival following an 85% lethal dose of influenza A/NWS/33 (H1N1) when its administration was delayed for as long as 60 h after inoculation of virus[34].

Ferret model

In contrast to mice, ferrets infected with influenza show similar symptoms to those seen clinically in humans (i.e. fever, nasal signs and lethargy), the infection being primarily limited to the upper respiratory tract. Oral Ro640796 doses of 5 mg kg^{-1} and 25 mg kg^{-1} were administered to ferrets twice daily for three days beginning 2 h after inoculation with influenza A England/939/69 (H3N2)[22]. Both the 5 mg kg^{-1} and 25 mg kg^{-1} doses reduced the febrile response to infection, decreasing the area under the curve of temperature increase over time by 58% and 93%, respectively. Ro640796 also prevented the appearance of nasal signs and lethargy, reduced peak viral titres and decreased the local inflammatory response to infection (as measured by the number of inflammatory cells in

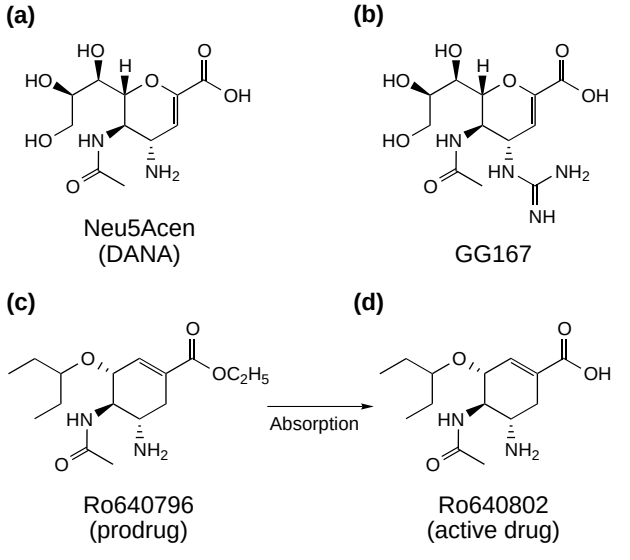

Figure 5. *Sialic acid analogue neuraminidase inhibitors: (a) Neu5Acen (DANA); (b) GG167 (Zanamivir); (c) Ro640796 (prodrug); (d) Ro640802 (active drug). Ro64096 is converted to Ro640802 following oral absorption.*

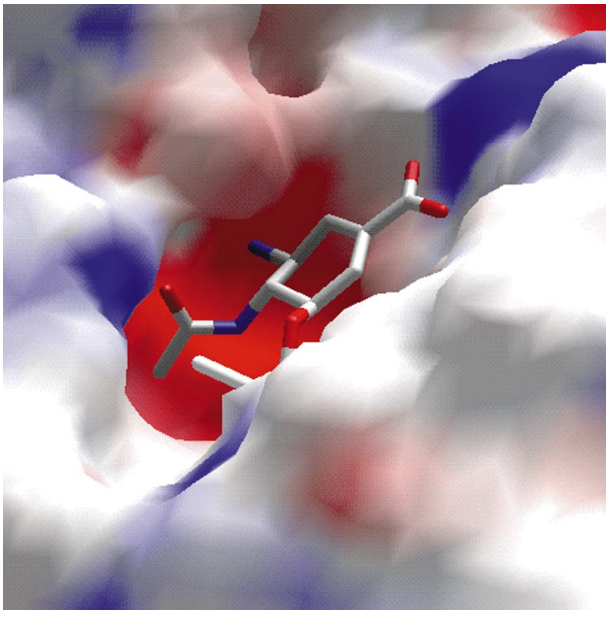

Figure 6. *The target-designed Ro640802 molecule nestling within the active site cleft in the neuraminidase globular head.*

Table 1. Inhibitory activity of Ro640802 and GG167 on the neuraminidase activity of human influenza A and B strains[22,23]

Virus	IC_{50} (nM)[a]	
	Ro640802	GG167
Laboratory strains		
A/WS/33 (H1N1)	1.0	0.7
A/Victoria/3/75 (H3N2)	0.5	1.7
A/Port Chalmers/1/73 (H3N2)	0.3	1.1
B/Mass/3/66	0.8	1.7
B/Hong Kong/5/72	1.7	1.0
Clinical isolates		
A/Texas/36/91 (H1N1)	0.5	0.3
A/Texas/36/91-like (H1N1)	0.4	0.5
A/Taiwan/1/86-like (H1N1)	1.3	0.5
A/Johannesburg/33/94 (H3N2)	0.8	4.6
A/Victoria/7/87-like (H3N2)	0.7	2.6
A/Shangdong/09/93-like (H3N2)	0.2	0.7
A/Virginia/305/95 (H3N2)	0.1	0.6
B/Harbin/07/94	2.0	2.1
B/Beijing/184/93-like	2.6	1.2
B/Victoria/2/87-like	2.6	1.4

[a] IC_{50} = concentration required to produce a 50% reduction in the neuraminidase activity.

nasal washes). Oral Ro640796 has produced similar effects against influenza A Sydney/97 (H3N2), influenza A England/95 (H1N1) and influenza B Argentina/97 viruses[35].

The distribution of Ro640796 to the various sites of infection was investigated by whole body autoradiography in ferrets[36]. While greatest exposure was to the liver and kidney, concentrations in the lung were high, and exposure in this organ was greater than five times that of blood. There was also good penetration to the middle ear and nasal mucosa.

No drug-related toxicity with Ro640796 was observed in these studies in either mice or ferrets, nor in toxicological studies in rats, even after administration of 800 mg kg^{-1} day^{-1} for 14 days[22].

Oral administration of Ro640796 in man provides active drug levels and is well tolerated

The pharmacokinetic profile of oral Ro640796 has been investigated in a series of double-blind, placebo-controlled studies in healthy volunteers. Following administration of single Ro640796 doses (20–1000 mg), the maximum plasma concentration and area under the plasma concentration vs. time curve (AUC) for Ro640802 increased proportionately with dose[23,37]. Peak plasma concentrations of Ro640802 occurred 2.5 to 6 h after administration of Ro640796. The decline in plasma Ro640802 concentrations was slower than that observed for Ro640796, the mean terminal elimination half-life ranging from 6.8 to 9.3 h.

No accumulation of Ro640802 was observed following multiple oral doses of Ro640796 (50–500 mg twice daily). Plasma concentrations of Ro640802 were significantly higher and longer lasting than those of the prodrug and greater than concentrations that have proved active against influenza A and B viruses *in vitro* and *in vivo*. The pharmacokinetics of Ro640796 were similar in healthy elderly volunteers and younger subjects, and little inter-subject variability was seen. The elimination half-life of Ro640802 in healthy elderly volunteers was also similar to that observed in younger subjects. These data suggest that no dosage reduction is required in healthy elderly patients.

Ro640796 was well tolerated at doses up to 1000 mg, given either as a single dose or as 500 mg twice daily for seven days. There were no clinically relevant changes in vital signs or laboratory values. Mild nausea and vomiting were seen in some patients at the highest dose used (1000 mg). No serious adverse events were observed.

Ro640796 is effective in experimental influenza infection in volunteers

Two double-blind, placebo-controlled, randomized studies have evaluated the antiviral activity, clinical efficacy and tolerability of Ro640796 in the prevention and early treatment of experimental influenza virus infection[38,39]. Susceptible, healthy adults were inoculated with influenza A/Texas/36/91 (H1N1).

In the prophylaxis study, oral Ro640796 100 mg once daily (n = 11) or twice daily (n = 12) was initiated 26 h before inoculation and continued for five days. Both Ro640796 regimens proved significantly superior to placebo, preventing viral recovery and influenza-associated illness in all participants.

In the treatment study, oral Ro640796 (20, 100 or 200 mg twice daily or 200 mg once daily) was initiated 28 h after inoculation with influenza virus. In patients with proven infection, oral Ro640796 reduced the median AUC of viral titre in nasal washes for all treatment groups compared with placebo, demonstrating a 100-fold reduction in viral load by 24 h and a 1000-fold reduction by 36 h after treatment. The median duration of influenza virus shedding was reduced from 107 h in the placebo group to 58 h in the oral Ro640796

Box 4. Current options for the control of influenza infection

Influenza prevention (or reduction in severity) centres upon the administration of inactivated vaccine, which is given six to eight weeks before the start of the influenza season. Intact (whole) virus, split virus and subunit vaccines containing two strains of influenza A and one strain of influenza B are available, all types producing a similar serological response[49]. Each year the composition of the vaccine is based on those influenza strains expected to appear the following winter. Vaccination is recommended for use in populations at high risk of complications, such as the elderly and those with chronic pulmonary or cardiac disease[41].

When the vaccine and epidemic strains are well-matched, high vaccination rates in nursing homes and other chronic care settings induce herd immunity and can reduce the risk of outbreaks. In addition, vaccination can reduce the rates of hospitalization and death due to influenza and its complications[44]. However, the vaccine has several limitations[41,42,50]. Antigenic drift in the haemagglutinin antigen and limited immunological response after vaccination necessitate the annual reformulation of the vaccine and annual revaccination. In addition, vaccines have variable efficacy (70–90% in adults aged <60 years) depending on the accuracy of the match with circulating viral strains as well as the age and susceptibility of the vaccinee to infection. Efficacy is lower particularly in young children and the elderly, the two populations at an increased risk of complications, with protection rates of 30–70% in those aged ≥60 years.

Vaccines are administered by intramuscular injection, which has logistical and economic disadvantages, especially as it has to be repeated on an annual basis. This route of administration can also be unpopular with vaccinees, particularly children. New vaccination approaches, such as the development of live virus vaccines for intranasal administration, may improve the future control of influenza[50].

There are two antiviral agents with activity against influenza viruses. Amantadine and its derivative rimantadine (not licensed in Europe) have been available since the early 1980s and inhibit viral replication by inactivating the viral M2 protein ion channel[51]. These drugs are only active against influenza A, and resistance to them develops rapidly, both *in vitro* and *in vivo*. The resistant virus variants are transmissible and pathogenic. Their use is further limited by their side-effect profile: adverse events associated with amantadine and rimantadine involve CNS and gastrointestinal disturbances. CNS effects are more common with amantadine (which is also licensed for the treatment of Parkinson's disease) than rimantadine[52,53].

The most common treatments taken for influenza infection are OTC medications, which give partial symptomatic relief of symptoms. Paracetamol and aspirin are commonly used, but aspirin should not be taken by children under 12 years as it has been linked with the development of Reye's syndrome[54]. However, as OTC compounds are not targeting the cause of the disease, the influenza virus, their potential to relieve symptoms is limited.

treatment group. Oral Ro640796 also significantly ameliorated clinical symptoms, with more rapid cessation of symptoms in the active drug treatment groups, reducing the duration of symptoms by almost half compared with placebo, and also reducing their severity. Oral Ro640796 was generally well tolerated in these studies. Transient, mild-to-moderate nausea reported by some subjects receiving Ro640796 at the 200 mg doses could be prevented by administering the drug after food. No dose-limiting toxicity or significant changes in laboratory parameters were observed.

Ro640796 is currently undergoing extensive Phase III clinical trials, the results of which will be available later this year.

Conclusions

Influenza virus infection causes substantial morbidity and mortality worldwide and creates an enormous economic burden[55]. Current options for the control of influenza virus infection are limited (see Box 4). The neuraminidase enzyme offers an attractive target for antiviral intervention, not least in view of its antigenic conservation, and it is likely that neuraminidase inhibitors will show broad and persistent activity against all clinically relevant strains of influenza. Two neuraminidase inhibitors have proved this concept in preclinical and clinical trials and are currently in Phase III development for the treatment of influenza infection. The prodrug concept of Ro640796 allows convenient oral dosing and provides active drug levels of Ro640802 to the various sites of viral replication. Neuraminidase inhibition is likely to emerge as an important new concept in influenza treatment, and by providing additional agents to the anti-influenza armamentarium, could be the long-awaited 'magic bullet'.

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