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Zanamivir: from drug design to the clinic

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The development of the neuraminidase inhibitors has revolutionized the management options for influenza. Zanamivir was the first such inhibitor to be approved for the treatment of influenza in humans. It is delivered by inhalation to the respiratory tract, which is the site of viral replication, in order to ensure immediate antiviral activity. Early treatment with zanamivir in clinical trials rapidly reduced the severity and duration of influenza symptoms and associated complications. Furthermore, chemoprophylaxis with zanamivir was shown to be effective in the prevention of influenza illness. To date, there is no evidence for the emergence of clinically significant zanamivir-resistant isolates. In conclusion, zanamivir offers a useful complementary strategy to vaccination in the effective management of influenza.

Keywords: zanamivir; neuraminidase inhibitors; influenza

1. INTRODUCTION

Influenza is a highly contagious respiratory tract infection that is caused by influenza type A and B viruses. Virus-laden respiratory aerosols expelled during coughing and sneezing perpetuate infection throughout the community. The spectrum of influenza illness can vary widely from asymptomatic through to respiratory tract illness with systemic symptoms that affect a wide variety of organs. Typical early symptoms of uncomplicated influenza include an unproductive cough, sore throat and nasal congestion followed by the abrupt onset of systemic symptoms such as fever, malaise, chills, headache, myalgia, dizziness and loss of appetite.

Influenza can cause serious morbidity and increased mortality, particularly in the elderly or the immunocompromised and those with underlying diseases such as chronic obstructive lung disease, cardiovascular disease and diabetes. These individuals are deemed high risk because influenza may exacerbate their underlying condition or predispose them to secondary bacterial infections. Hospitalizations, particularly of the elderly, increase during influenza epidemics because of the high incidence of influenza-related complications such as pneumonia, acute bronchitis, chronic respiratory disease and congestive heart failure (McBean *et al.* 1993). Consequently, the demands on health care resources increase during these epidemics.

The effective management of influenza and its complications remains a major public health concern with an estimated 10% of the world's population infected annually in a typical influenza season. Although vaccination is the 'gold standard' for prophylaxis, many persons still become infected during annual epidemics. Until recently, the adjuncts to vaccination were limited to the use of the M2 inhibitors amantadine or rimantadine. However, their use has not gained wide acceptance due in part to their ineffectiveness against influenza B, the rapid emergence of drug-resistant variants (Belshe *et al.* 1989; Hayden *et al.* 1989; Hayden 1994) and their poor adverse event profile (Hayden *et al.* 1981, 1983). Furthermore, the effectiveness of amantadine or rimantadine in severe influenza or in patients at high risk of developing influenza-related complications is uncertain.

The development of the neuraminidase inhibitors, with antiviral activity against both influenza A and B, has revolutionized the management of influenza. The preclinical and clinical development of zanamivir (Relenza), the first neuraminidase inhibitor to be approved for influenza treatment, is reviewed in this manuscript; other neuraminidase inhibitors such as oseltamivir (Tamiflu) will not be discussed further.

2. ZANAMIVIR: A MOLECULAR PROFILE

Influenza A and B viruses are negative-strand RNA viruses with a genome segmented into eight sections that are independently encapsulated by viral nucleoproteins. There are two glycoproteins present on the surface of the virus, namely haemagglutinin (H) and neuraminidase (N). Both of these glycoproteins are important in determining the pathogenicity and infectivity of the virus. Annual epidemics are caused when these antigens undergo periodic mutation, resulting in antigenically novel influenza viruses. Minor changes in antigenic type (antigenic drift) that occur in both influenza A and B are responsible for seasonal influenza epidemics. Major changes in antigenic type (antigenic shift) only occur with influenza A because of its ability to undergo genetic reassortment. These changes are responsible for occasional influenza pandemics. Influenza A can be subtyped by H and N antigen types: H1N1, H2N2 and H3N2 are the influenza subtypes known to have caused disease in humans since 1918 (Zimmerman *et al.* 1997).

The replication cycle for influenza is shown in figure 1 and is briefly summarized below. For influenza virus to be infective, its haemagglutinin must first bind to sialic acid glycoconjugates, the putative receptors on the host cell for the virus. Binding of the haemagglutinin allows the virus to penetrate the plasma cell membrane, uncoat and enter

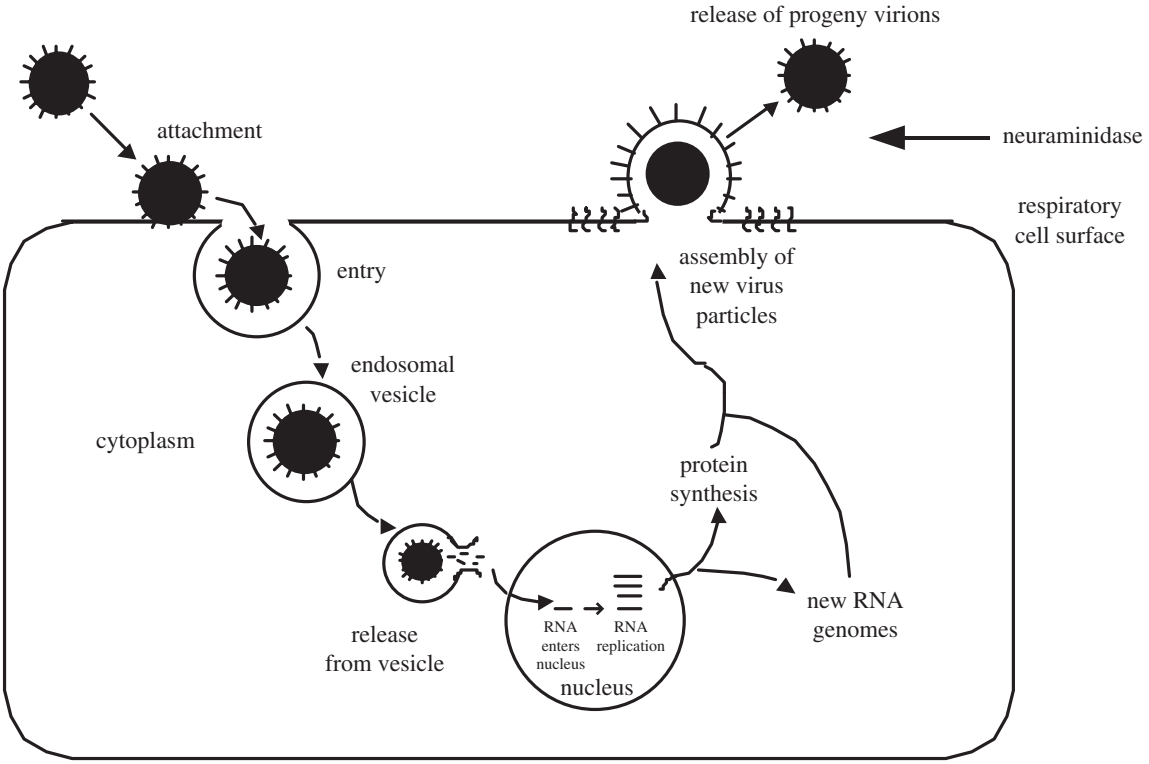

Figure 1. A schematic diagram of the influenza virus replication cycle.

the cytoplasm. Viral RNA strands replicate in the nucleus and new virus particles are produced. Neuraminidase is a glycohydrolase enzyme that is responsible for the cleavage of terminal sialic acid residues from carbohydrate moieties on the surface of host cells and the influenza virus envelope. Neuraminidase is responsible for the release of newly formed virus from the surface of infected cells and aids the motility of the virus through the mucous lining of the respiratory tract.

Neuraminidase inhibitors such as zanamivir are sialic acid analogues. They work by preventing the enzyme from cleaving sialic acid residues on the surface of the host cells and influenza viral envelope (Von Itzstein *et al.* 1993; Whittington & Bethell 1995). Viral haemagglutinin binds to uncleaved sialic acid residues, resulting in viral aggregation and a reduction in the amount of virus that is able to infect other cells.

Early attempts at inhibiting influenza neuraminidase with antivirals were largely unsuccessful because of their lack of specificity and potency and suboptimal routes of administration. A breakthrough came when neuraminidase was crystallized, thereby allowing the three-dimensional structure of the active site to be elucidated. Various sialic acid analogues were developed aided by computer-assisted modelling of the active site. This led chemists to identifying 4-guanidino-2,4-dideoxy-2,3-dehydro-*N*-acetylneuraminic acid (zanamivir) as a potent inhibitor of influenza neuraminidase.

3. PRE-CLINICAL EVALUATION

Zanamivir (figure 2) is a highly polar molecule and as such does not readily penetrate cell membranes. Direct topical delivery of zanamivir to the infection site is required for optimal antiviral activity.

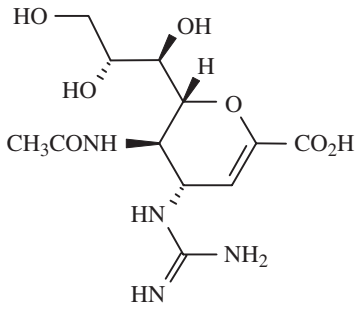

Figure 2. The chemical structure of zanamivir.

Neuraminidases (sialidases) are found in a wide variety of organisms and play an important role in a number of processes within mammalian cells (e.g. the unmasking of various glycoconjugates leading to the exposure of receptors). *In vitro* studies have shown that zanamivir is a specific influenza neuraminidase inhibitor with only partial activity for mammalian, bacterial and parainfluenza sialidases (Holzer *et al.* 1993; Von Itzstein *et al.* 1993).

(a) *Activity against the influenza virus* in vitro

The susceptibility of influenza viruses to zanamivir was studied *in vitro* using plaque reduction assays in Madin–Darby canine kidney cells (Woods *et al.* 1993). Zanamivir inhibited the growth of a wide range of influenza A and B viruses, with 50% inhibition concentrations (IC_{50}s) of plaque formation ranging from 0.005 to 0.014 μM for laboratory-passaged strains and from 0.02 to 16 μM for

clinical isolates (Woods *et al*. 1993). Direct testing of neuraminidase inhibition showed that zanamivir was a potent neuraminidase inhibitor of both influenza A and B viruses: the IC_{50}s were much lower and narrower in range (0.64–7.9 nM) compared with those observed in the plaque reduction assays. Furthermore, the antiviral activity was not affected by resistance to amantadine or rimantadine, nor by the passage history of the virus (Woods *et al*. 1993).

Zanamivir also reduces virus yields in human respiratory epithelial cells (Hayden *et al*. 1994). The 90% effective inhibitory concentrations (EC_{90}) of virus yields for two influenza A viruses (A(H1N1) and A(H3N2)) at 24 and 48 h after inoculation were less than 0.01 $\mu g\,ml^{-1}$ at both time-points. Similarly, the EC_{90}s for influenza B (B/Hong Kong/5/72) at the same time-points were 0.54 and 0.25 $mg\,l^{-1}$, respectively. Furthermore, zanamivir concentrations up to 100 $\mu g\,ml^{-1}$ had no cytostatic effects on the outgrowth of cells from human respiratory epithelium explants (Hayden *et al*. 1994).

(b) *Activity against the influenza virus* in vivo

The *in vivo* activity of zanamivir against experimental influenza A and B (A/Singapore/1/57; B/Victoria/102/85) infections was examined in animal models (Ryan *et al*. 1994). Intranasal zanamivir treatment (0.01 or 0.4 $mg\,kg^{-1}$ per dose) given prophylactically plus twice daily over days 0 (day of viral inoculation) to 3 in mice infected with influenza A reduced mortality and viral titres in lung homogenates and improved lung consolidation scores over 10 days. No virus growth was observed in the lung homogenates after therapy was stopped. The administration of zanamivir via the oral or intraperitoneal route did not result in antiviral activity (Ryan *et al*. 1994).

Intranasal administration of zanamivir twice daily on the day before infection and continuing through to day 5 after infection in ferrets experimentally infected with influenza A or B (A/Mississippi/1/85; A/Singapore/1/57; B/Victoria/102/85) significantly reduced the area under the curve values (over days 1–9) for nasal titres and the incidence of significant pyrexia ($p < 0.05$). The effective dose required for reducing the area under the curve in the zanamivir group to 10% of that seen in the vehicle-treated control group was less than 0.06 $mg\,kg^{-1}$ for both influenza A strains and less than 0.75 $mg\,kg^{-1}$ for the influenza B strain. In comparison with amantadine, intranasal zanamivir administered 1 day before or after infection was 100–1000 times more active against both influenza A and B in reducing nasal viral titres and the incidence of pyrexia. In addition, zanamivir did not affect the serum antibody response to infection (Ryan *et al*. 1995).

(c) *Pre-clinical safety*

Extensive pre-clinical testing of zanamivir administered by the inhaled and intravenous route has shown no significant toxicity in a number of animal models (Dines *et al*. 1998). No toxicity was observed with up to 1 month of daily intravenous administration of 36 and 90 $mg\,kg^{-1}day^{-1}$ in dogs and rats, respectively. The plasma zanamivir exposures achieved with these doses were 259 and 345 times more than those anticipated for clinical use. The 90 $mg\,kg^{-1}day^{-1}$ intravenous dose of zanamivir was not teratogenic to rat and rabbit foetuses and had no adverse effects on the fertility, reproductive performance or physical development of offspring in the rat (Dines *et al*. 1998). In a two-lifetime oncogenicity study in rats and mice, the use of up to 20 times more inhaled zanamivir than that anticipated for clinical use did not produce any carcinogenic effects.

(d) *Drug interactions*

Studies of *in vitro* and *in vivo* models have shown that zanamivir has a low potential for interactions with other drugs (Daniel *et al*. 1999). Intravenous administration of zanamivir in dosages of 1, 9 and 90 $mg\,kg^{-1}\,day^{-1}$ for 5 weeks to male and female rats did not produce significant changes in the expression of hepatic cytochrome isoenzymes. Zanamivir concentrations of up to 500 μM in human liver microsomes had no effect on the metabolism of the cytochrome substrates bufuralol, chlorzoxazone, coumarin, ethoxyresorufin, mephenytoin, midazolam, phenacetin and tolbutamide. The binding of zanamivir to human, dog and rat blood cells and plasma proteins was low. Furthermore, the *in vitro* potency of zanamivir against influenza viruses in Madin–Darby canine kidney cells was not adversely affected by drugs likely to be used for treating the symptoms of influenza, e.g. aspirin, ibuprofen, paracetamol, phenylephrine, oxymetazoline, promethazine and co-amoxiclav (Daniel *et al*. 1999).

(e) *Pharmacokinetic studies*

Zanamivir is readily bioavailable after intravenous, intraperitoneal and intranasal but not oral administration in mice (Ryan *et al*. 1994). The elimination half-life ($t_{1/2}$) in mice after intravenous administration is *ca*. 10 min. Similarly, zanamivir has a monophasic $t_{1/2}$ of *ca*. 15 min in rats and 50 min in dogs after intravenous administration. Zanamivir is almost exclusively eliminated by renal clearance in these species (Daniel *et al*. 1996).

4. CLINICAL EVALUATION

(a) *Pharmacokinetics*

The kinetics of zanamivir were linear in healthy volunteers after single intravenous doses of up to 600 mg. There was no evidence of a modification in the kinetics with twice daily dosing. The elimination of zanamivir was a first-order process with a $t_{1/2}$ of *ca*. 2 h and the volume distribution (16 l) was similar to that of extracellular water (Cass *et al*. 2000*a*). Approximately 90% of zanamivir was excreted unchanged in the urine.

The mean oral bioavailability of 500 mg of zanamivir in healthy volunteers is 2% (range 1–5%). The maximal serum concentration (C_{max}) is reached in *ca*. 4 h. The $t_{1/2}$ was estimated to be 3.3 h, with absorption rather than elimination being the rate-determining step. After a single 16 mg intranasal dose of zanamivir, the C_{max} is reached in *ca*. 1.8 h. Again, the $t_{1/2}$ was estimated to be 3.4 h, representing the absorption phase rather than the elimination phase (Cass *et al*. 2000*a*).

The pharmacokinetics of inhaled zanamivir administered by a Diskhaler (GlaxoSmithKline, Ware, UK) as single doses of 5 and 10 mg and repeated doses of 10 mg four times daily were studied in healthy volunteers. The

Table 1. Summary of placebo-controlled trials of inhaled and intranasal zanamivir.

(The studies were randomized, double blind, placebo controlled and multicentre. Sixty-three per cent were confirmed as influenza positive in the Hayden *et al.* (1997) study and 57% were confirmed as influenza positive in the Monto *et al.* (1999*a*) study.)

study	design/population	treatment regimens	results/key findings
Hayden *et al.* (1997)	otherwise healthy patients of 13 years or older with influenza-like illness for less than 48 h	zanamivir 10 mg inhaled plus 6.4 mg intranasal twice daily for 5 days ($n=88$) zanamivir 10 mg inhaled plus intranasal placebo twice daily for 5 days ($n=85$) placebo by both routes ($n=89$)	reduction in the median time to alleviation of all major symptoms by 1 day for influenza-positive patients in both zanamivir groups compared with placebo ($p=0.05$)
Monto *et al.* (1999*a*)	otherwise healthy and high-risk patients of 13 years or older with influenza-like illness for less than 48 h	zanamivir 10 mg inhaled plus 6.4 mg intranasal twice daily for 5 days ($n=419$) zanamivir 10 mg inhaled plus intranasal placebo four times daily for 5 days ($n=415$) placebo by both routes twice or four times daily ($n=422$)	reduction in the median time to alleviation of all major symptoms by 1 day for influenza-positive patients in both zanamivir groups versus placebo ($p=0.012$ twice daily and $p=0.014$ four times daily)
Mastsumoto *et al.* (1999)	otherwise healthy patients of 16–65 years with influenza-like illness for less than 36 h	zanamivir 10 mg inhaled plus 6.4 mg intranasal twice daily for 5 days ($n=39$) zanamivir 10 mg inhaled plus intranasal placebo twice daily for 5 days ($n=37$) placebo by both routes ($n=40$)	the median time to alleviation of fever, headache and myalgia was reduced by 1 day for both zanamivir groups versus placebo ($p<0.01$)

C_{max} was generally reached within 1–2 h with no time-lag: the C_{max} values were 47 and 97 $\mu g l^{-1}$ after single 5 and 10 mg doses, respectively. The $t_{1/2}$s were estimated to be 5.05 and 4.14 h after single inhaled doses of 5 and 10 mg of zanamivir, respectively, with absorption rather than elimination being the rate-determining step phase (Cass *et al.* 2000*a*). Pharmacoscintigraphic evaluations showed that 13.2% of the inhaled dose of 10 mg zanamivir is deposited in the bronchi and lungs (Cass *et al.* 1999*a*). The local concentration of zanamivir deposited throughout the respiratory tract was estimated to be 10 μM (Cass *et al.* 1999*a*), which is far in excess of the IC_{50} values for zanamivir against viral neuraminidases, which are less than 10 nM (Woods *et al.* 1993). The concentrations of zanamivir in sputum exceeded the median IC_{50} up to 24 h after inhalation (Peng *et al.* 2000*a*).

The pharmacokinetic parameters observed in children older than 5 years were similar to those observed in adults given the same dose of zanamivir via the same routes of administration (Peng *et al.* 2000*b*).

The drug was well tolerated by all routes tested in the early volunteer studies of zanamivir. Although zanamivir is predominantly excreted unchanged in the urine, studies in patients with renal impairment suggest that no dosage modification is required with inhaled zanamivir in these patients because of it low systemic exposure (Cass *et al.* 1999*b*).

5. CLINICAL EFFICACY STUDIES

(a) *Experimental influenza infection*

Preliminary influenza challenge studies in volunteers showed that intranasal zanamivir can effectively protect against experimental infection with influenza A and B infection and can reduce viral titres when used for early therapy (Hayden *et al.* 1996*a*,*b*). Hayden *et al.* (1996*a*) administered intranasal zanamivir at doses of 3.6–16 mg two or six times daily beginning 4 h before inoculation (prophylaxis) or 1 or 2 days afterwards (early or delayed treatment) in susceptible adults inoculated with influenza A/Texas/91 (H1N1). For all dose groups combined, zanamivir prophylaxis was 82% effective in preventing laboratory-confirmed infection and 95% ($p<0.01$) effective in preventing febrile illness as compared to placebo. Early treatment reduced peak viral shedding by 2.0 $\log_{10}$ and the mean duration of viral shedding by 3 days compared with placebo ($p<0.05$ for comparisons). The frequency of febrile illness was concomitantly reduced by 85% ($p<0.05$). There was no difference in effectiveness between twice daily or six times daily dosing (Hayden *et al.* 1996*a*).

Intranasal zanamivir (3.2 or 6.4 mg twice daily or 6.4 mg four times daily) had an overall combined protective efficacy of 60% against viral shedding and 32% against infection in similar prophylaxis studies against experimental influenza B/Yamagata/88 (Hayden *et al.* 1996*b*). Lower overall symptom scores were observed in volunteers receiving zanamivir as compared with placebo (Hayden *et al.* 1996*b*).

No resistant variants were isolated in either the treatment or prophylaxis challenge studies.

(b) *Natural influenza infection*

Patients in naturally acquired influenza studies were recruited when local surveillance confirmed that influenza

was circulating in the community and on the basis of the presence of fever/feverishness and at least two of the following symptoms: headache, myalgia, cough or sore throat. Treatment was started within 36 or 48 h of symptom onset. Influenza diagnosis was confirmed by any of the following laboratory tests: virus isolation, a fourfold rise in anti-haemagglutinin antibody titre, viral antigen detection or a polymerase chain reaction assay.

The primary endpoint was the median time to the alleviation of influenza-like illness. Patients rated their symptoms twice daily (including headache, sore throat, feverishness, muscle and joint aches and pains and cough) using a four-point scale for severity (none, mild, moderate and severe). Alleviation was defined as no fever (temperature $< 37.8\,^{\circ}\mathrm{C}$ or feverishness score of none) and other influenza symptoms (headache, muscle/joint aches and pains, cough and sore throat) recorded as none or mild or absent in the patients' diary cards, which were maintained for 24 h. Secondary endpoints included the time to return to normal activities, the use of relief medication and the incidence of complications requiring antibiotic use.

The optimal route of administration and dose of zanamivir in the treatment of naturally acquired influenza was explored in a number of studies (Hayden *et al.* 1997; Matsumoto *et al.* 1999; Monto *et al.* 1999*a*). Their details are summarized in table 1. These studies showed that intranasal and/or inhaled zanamivir significantly shortened the time to the alleviation of the symptoms of influenza by 1 day in otherwise healthy adults (table 1). No difference was observed in the median time to the alleviation of influenza symptoms between inhaled or intranasal zanamivir. These studies helped to establish that a 10 mg twice daily-inhaled dose of zanamivir for 5 days is an optimum treatment regimen for influenza infection.

Subsequent treatment efficacy studies recruiting much larger patient numbers investigated this regimen. The details of these studies investigating the efficacy and safety of 10 mg of inhaled zanamivir twice daily for 5 days are summarized in table 2. Overall, these studies have shown that zanamivir significantly shortens the time to the alleviation of the symptoms of influenza by 1–2.5 days ($p < 0.05$) in otherwise healthy adults, paediatrics and at-risk patients. Importantly, influenza-positive patients (comprising 49–78% of patients recruited to studies) treated with zanamivir had significant reductions in the severity of their symptom scores. The symptoms included headache, sore throat, feverishness, muscle and joint aches and pains, cough, weakness and loss of appetite. Zanamivir also reduced the incidence of influenza-related complications by 16–31% as compared with placebo. As a result, patients treated with zanamivir returned to normal activities 1–2 days earlier than patients who received placebo (table 2).

Hedrick *et al.* (2000) evaluated the efficacy and safety of inhaled zanamivir in 471 children aged from 5 to 12 years with influenza-like symptoms of less than 36 h duration. Three hundred and forty-six (73%) of these children had laboratory-confirmed influenza. Zanamivir significantly reduced the median time to the alleviation of their symptoms by 1.25 days (from 5.25 to 4.0 days) ($p < 0.001$) in children with confirmed influenza as compared with placebo. This treatment benefit increased significantly to 1.5 days (from 6.5 to 5.0 days) ($p < 0.001$) with the additional requirement that no relief medication was being used at the time of alleviation. Patients treated with zanamivir also used less relief medication and returned to normal activities 1 day sooner than those receiving placebo. Fewer complications or associated antibiotic use in the zanamivir group relative to the placebo group were observed, which did reach significance (Hedrick *et al.* 2000).

Asthmatics and chronic obstructive pulmonary disease (COPD) patients represent a special group that is at greater risk of developing influenza-related complications because their airways are already compromised. Murphy *et al.* (2000) evaluated the efficacy and safety of zanamivir in 525 asthma or COPD patients of 12 years or over with influenza-like symptoms of 36 h duration. Three hundred and thirteen of these (60%) had laboratory-confirmed influenza. Patients treated with zanamivir had a significant reduction in the median time to the alleviation of their influenza symptoms of 1.5 days as compared with placebo (from 7.0 to 5.5 days) ($p = 0.009$). In addition, zanamivir significantly reduced the mean overall influenza assessment score as compared with placebo ($p = 0.004$) over days 1–5 of treatment. A lower incidence of complications requiring antibiotics and a change in respiratory medication was observed (58% lower) ($p = 0.064$) (Murphy *et al.* 2000).

Lalezari *et al.* (2001) published a retrospective pooled analysis of data from high-risk patients ($n = 321$) recruited to randomized, placebo-controlled zanamivir studies that were completed before or during the 1998–1999 influenza season. The high-risk population included patients with chronic respiratory disease, cardiovascular disease and the elderly (older than 65 years). Zanamivir significantly reduced the time to the alleviation of influenza symptoms in these patients by 2.5 days as compared with placebo (from 7.5 to 5.0 days) ($p = 0.015$). Zanamivir-treated, high-risk patients returned to normal activities significantly earlier (3 days) ($p = 0.022$) and had an 11% reduction in their median total symptom scores over days 1–5 of treatment ($p = 0.039$) as compared with placebo. Importantly, zanamivir treatment reduced the incidence of complications requiring antibiotic use by 43% ($p = 0.045$) as compared with placebo (Lalezari *et al.* 2001).

A pooled analysis of randomized, placebo-controlled studies that investigated the treatment of influenza with 10 mg of inhaled zanamivir twice daily was performed by Monto *et al.* (1999*b*) in order to evaluate its efficacy more precisely. This analysis showed that zanamivir significantly reduced the median time to the alleviation of symptoms by 1 day as compared with placebo (5.0 versus 6 days) ($p = 0.001$). A significant treatment benefit of 1.5 days was seen in the time to the alleviation of symptoms in febrile, influenza-positive patients (5.0 versus 6.5 days) ($p < 0.001$). Greater treatment benefits of up to 3 days (5.0 versus 8.0 days) ($p < 0.001$) were seen in patients who had severe symptoms compared with a 1 day (4.5 versus 5.5 days) ($p < 0.001$) benefit in patients whose symptoms were not severe. Patients who were considered to be at high risk of developing influenza-related complications had a 2.5 days reduction (5.5 versus 8.0 days)

Table 2. Summary of placebo-controlled trials of inhaled zanamivir.

(The studies were randomized, double blind and placebo controlled.)

study	design/population	treatment	reduction in days to alleviation of symptoms in patients with influenza	comments
The Management of Influenza in the Southern Hemisphere Trialists Study Group (1998)	otherwise healthy and high-risk patients of 12 years or older with influenza-like illness for less than 36 h	zanamivir 10 mg inhaled for 5 days ($n=227$) placebo ($n=228$)	1.5 (4.5 versus 6.0) ($p=0.004$)	reduction in the median time to alleviation of symptoms by 2.5 days in the high-risk group ($p=0.048$) versus placebo: high-risk patients on zanamivir had a 32% reduction in the incidence of complications versus placebo ($p=0.004$)
Makela *et al.* (2000)	otherwise healthy and high-risk patients of 12 years or older with influenza-like illness for less than 48 h	zanamivir 10 mg inhaled for 5 days ($n=174$) placebo ($n=182$)	2.5 (5.0 versus 7.5) ($p<0.001$)	zanamivir was associated with a 23% reduction in the incidence of complications versus placebo ($p=0.037$)
Lalezari *et al.* (1999)	otherwise healthy and high-risk patients of 12 years or older with influenza-like illness for less than 48 h	zanamivir 10 mg inhaled for 5 days placebo	1.0 (5.0 versus 6.0) ($p=0.045$)	zanamivir was associated with a 31% reduction in the incidence of complications versus placebo ($p=0.049$)
Murphy *et al.* (2000)	asthma and/or COPD patients of 12 years or older with influenza-like illness for less than 36 h	zanamivir 10 mg inhaled for 5 days ($n=262$) placebo ($n=263$)	1.5 (5.5 versus 7.0) ($p=0.009$)	the patients' asthma and/or COPD status did not affect the efficacy of zanamivir: zanamivir did not adversely affect pulmonary function as determined by FEV_1 and PEFR measurements
Hedrick *et al.* (2000)	otherwise healthy and high-risk children of 5–12 years with influenza-like illness for less than 36 h	zanamivir 10 mg inhaled for 5 days ($n=224$)	placebo ($n=247$)	1.25 (4.0 versus 5.25) ($p<0.001$) children treated with zanamivir used less relief medication than those treated with placebo ($p=0.016$)
Hayden *et al.* (2000)	families with at least one child 5–17 years old and family members with influenza-like illness for less than 36 h	zanamivir 10 mg inhaled for 5 days ($n=158$) placebo ($n=163$)	2.5 (5.0 versus 7.5) ($p=0.01$)	zanamivir was 79% effective in protecting against influenza ($p<0.001$)

($p=0.114$) in the time to the alleviation of their symptoms with zanamivir treatment as compared with placebo. The incidence of complications and antibiotic use for complications (e.g. sinusitis, otitis media and bronchitis) was significantly reduced in zanamivir-treated patients. There was an overall reduction of 28% in antibiotic use (13 versus 18% for zanamivir and placebo, respectively) ($p=0.006$) (Monto *et al.* 1999*b*).

The zanamivir clinical studies were not designed for assessing its efficacy for each influenza subtype separately. However, most studies did show significant benefits for patients with influenza A subtypes, but only one study by Hedrick *et al.* (2000) (in paediatrics) showed significant efficacy in both influenza A and B subtypes: 1 day ($p=0.049$) and 2 day ($p<0.001$) reductions in the median time to the alleviation of influenza symptoms with zanamivir for patients with confirmed influenza A and B, respectively (Hedrick *et al.* 2000). The majority of studies did not recruit enough patients to be sufficiently powered for demonstrating significant efficacy in the influenza B group separately.

A pooled efficacy analysis of data from patients with influenza B who were recruited to the zanamivir trials before or during the 1998–1999 influenza season showed a reduction of 2 days (4 versus 6.0 days) ($p<0.001$) in the median time to the alleviation of symptoms in the zanamivir-treated group as compared with placebo (Osterhaus *et al.* 2000). There was a 1.5 day reduction (5.5 versus 7.0 days) ($p<0.001$) in the time to the alleviation of influenza symptoms for patients with confirmed influenza A who received zanamivir as compared with those on placebo. The median time to the alleviation of symptoms was identical for both influenza A and B illnesses, suggesting a similar disease burden between the two subtypes. There was a 19% reduction ($p=0.356$) in the incidence of complications requiring antibiotic use in patients with influenza B as compared with those receiving placebo. The incidence of complications

requiring antibiotic use in patients with confirmed influenza A was significantly reduced by 28% ($p = 0.007$) (Osterhaus *et al.* 2000).

(c) *Prophylaxis against influenza*

The use of zanamivir can be extended to chemoprophylaxis. The efficacy of zanamivir in preventing naturally acquired influenza was evaluated in healthy adults (Monto *et al.* 1999*c*) and healthy families (Hayden *et al.* 2000). The study in healthy adults recruited 1107 volunteers who were randomized to zanamivir ($n = 553$) or placebo ($n = 554$) before the 1997 North American influenza season. Volunteers received 10 mg of inhaled zanamivir or placebo once daily for 4 weeks at the start of the influenza outbreak. The incidence of laboratory-confirmed influenza in the zanamivir group was 2% as compared with 6% for the placebo group, which is a significant relative risk reduction of 67% with zanamivir ($p < 0.001$). Zanamivir was 84% effective in preventing confirmed influenza infection with fever ($p = 0.001$).

A total of 799 families were randomized to either 10 mg of inhaled zanamivir once daily for 10 days or placebo in a trial of the prevention of influenza in families. Once influenza was documented in the community, all members of a family in which one member contracted an influenza-like illness began to take the study drug. The index case and family members who subsequently developed influenza-like illness were treated with either 10 mg of inhaled zanamivir twice daily for 5 days or placebo, depending on the prophylactic regimen to which the family was randomized. The proportion of families with at least one initially healthy contact in whom influenza developed was lower in the zanamivir group than in the placebo group (4 versus 19%) ($p < 0.001$); this difference represented a 79% reduction in the proportion of families with a least one affected contact. Although this study was not powered for showing a significant difference between influenza A and B, zanamivir prophylaxis appeared to be effective against both influenza A and B. The median duration of symptoms among the index cases with confirmed influenza was 2.5 days shorter in the zanamivir group as compared with placebo (5.0 versus 7.5 days) ($p = 0.01$) (Hayden *et al.* 2000).

Zanamivir was compared with rimantadine for chemoprophylaxis against influenza outbreaks in a highly vaccinated elderly population in long-term care facilities in a recent prospective, double-blind, randomized study (Gravenstein *et al.* 2000). Three hundred and seventy-five patients were randomized over three influenza seasons (some randomized more than once over the three seasons) to zanamivir (10 mg) or rimantadine (100 mg) once daily for 14 days. Seven out of two hundred and twenty-six (3.1%) patients randomized to zanamivir developed symptomatic laboratory-confirmed clinical influenza during prophylaxis for influenza A outbreaks as compared to 18 out of 231 (7.8%) randomized to rimantadine. The approximate relative risk of 0.39 represents a significant 61% (95% confidence interval, 12–82%) ($p = 0.038$) additional protection from influenza illness over rimantadine. Rimantadine resistance was prevalent in 38% of the isolates tested. No zanamivir resistance was reported in any of the isolates (Gravenstein *et al.* 2000).

(d) *Resistance to zanamivir*

There is no evidence of the emergence of zanamivir-resistant influenza viruses in a clinical trial programme in more than 4000 patients who have received zanamivir as prophylaxis or treatment for acute influenza infection. A single case of zanamivir resistance was reported for influenza B isolated for a severely immunocompromised child (Gubareva *et al.* 1998). A mutation in the neuraminidase active site led to a 1000-fold reduction in *in vitro* enzyme sensitivity to zanamivir. The mutant virus was less virulent than the parent when tested in ferrets, but had a growth preference in zanamivir-treated animals.

Mutant influenza viruses have been generated *in vitro* by multiple passaging under the selection pressure of increasing zanamivir concentrations (Gubareva *et al.* 1996; McKimm-Breschkin 2000). The majority of mutants isolated after *in vitro* passaging with zanamivir have concomitant haemagglutinin mutations. These *in vitro* mutants have reduced neuraminidase dependence as a result of the reduced affinity of haemagglutinin for cellular receptors (McKimm-Breschkin 2000). However, the clinical significance of haemagglutinin mutations leading to resistance to zanamivir is unknown.

(e) *Safety of zanamivir*

Zanamivir has a safety profile comparable with placebo when given in both the treatment and prophylaxis of influenza-like illness (Freund *et al.* 1999). The most common adverse events were consistent with the signs and symptoms of influenza illness (nasal symptoms, diarrhoea, nausea, headache, bronchitis, cough and sinusitis) and all occurred at frequencies less than 3% during treatment in both the zanamivir and placebo groups (Freund *et al.* 1999). Overall, the incidence of drug-related adverse events during treatment was 13% in the zanamivir group and 11% in placebo recipients. No clinically significant abnormalities in haematology and clinical chemistry results were observed between zanamivir and placebo groups in clinical trials.

There have been rare reports of patients with a previous history of respiratory disease (asthma and/or COPD) and very rare reports of patients without a previous history of respiratory disease who experienced acute bronchospasm and/or serious decline in respiratory function following the use of zanamivir in post-marketing surveillance. Patients with asthma and/or COPD are not precluded from treatment with zanamivir, but it is recommended that these patients should have a fast-acting bronchodilator available and should discontinue zanamivir if they experience bronchospasm and/or a decline in respiratory function.

A clinical study involving 13 uninfected patients with mild to moderate asthma was performed in order to evaluate the effect of repeated zanamivir inhalation on pulmonary function and airway responsiveness. One of the 13 patients had a clinically significant drop in their forced expiratory volume 1 s (FEV_1) after inhalation with zanamivir (but not with lactose placebo), which resolved spontaneously in *ca.* 1 h. No overall changes in methacholine-induced airway reactivity were observed during 14 days of zanamivir exposure (Cass *et al.* 2000*b*). Cass *et al.* (2000*b*) concluded from their study that

zanamivir does not significantly affect pulmonary function and air responsiveness in patients with asthma.

Inhaled zanamivir was shown to be well tolerated with an adverse event profile similar to inhaled lactose placebo in a study of zanamivir for the treatment of influenza in patients with asthma and/or COPD. There was no evidence to suggest that zanamivir had an adverse effect on pulmonary function. However, modest improvements in respiratory function, as measured by morning and evening peak expiratory flow rates (PEFR), were observed with zanamivir during the acute stage of illness when pulmonary function is at its most compromised. There was no difference in the number of patients who experienced a decline from baseline in FEV_1 and PEFR of more than 20% at anytime post-treatment between the zanamivir and placebo groups. Larger declines in FEV_1 of more than 40% from baseline were uncommon in patients without laboratory-confirmed influenza ($<1\%$ in each treatment group); it is well known that influenza itself can cause such deteriorations (Murphy *et al.* 2000).

(f) *Patients' perceptions of zanamivir*

The efficacy and tolerability of zanamivir treatment has been well established in clinical trials. Overall, the greater than 90% compliance found with the inhaled treatment regimen across all clinical studies suggests that the topical administration and the zanamivir delivery device Diskhaler are acceptable.

Two surveys carried out in clinical practice in Australia (Silagy & Watts 2000) and the USA (Johnson *et al.* 2000) assessed patients' perceptions of zanamivir treatment. Symptom relief was reported by 71–77% of patients within 2 days of initiating treatment (Johnson *et al.* 2000; Silagy & Watts 2000) and 65–67% of patients resumed normal activities within 3 days after initiating treatment. Seventy-two per cent of clinicians with influenza in the USA study reported symptom relief within 2 days of zanamivir treatment. The Diskhaler was considered 'easy' or 'somewhat' easy to use by more than 90% of patients. Compliance with the treatment was high with 76% reporting using zanamivir for 4–5 days (Silagy & Watts 2000). When patients were asked to give their overall impression of treatment, 71–87% were satisfied or very satisfied (Johnson *et al.* 2000; Silagy & Watts 2000). Ninety-four per cent of the patients in the US survey said that they would recommend zanamivir to a friend if they contracted influenza.

6. CONCLUSION

Zanamivir is a potent and selective inhibitor of both influenza A and B virus neuraminidase. Its delivery to the site of viral replication by the Diskhaler ensures immediate antiviral activity and a rapid reduction in the severity of symptoms. Clinical studies have shown that early treatment with zanamivir significantly shortens the duration of influenza illness and helps patients return to their normal activities sooner. In addition, zanamivir reduces the incidence of complications requiring antibiotic treatment as compared with placebo. Inhaled zanamivir is well tolerated with a safety profile similar to inhaled lactose placebo. Patient surveys suggest that the Diskhaler is acceptable in routine clinical practice. There is no evidence to date for the emergence of clinically significant drug-resistant isolates. In conclusion, zanamivir offers a useful complementary strategy to vaccination in the effective management of influenza.

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