Treatment of epidemic and pandemic influenza with neuraminidase and M2 proton channel inhibitors

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A small armentarium of anti-influenza drugs now exists, and includes the M2 blockers (amantadine and rimantadine) and the neuraminidase inhibitors (Relenza and Tamiflu). The neuraminidase inhibitors have certain advantages, including a broader spectrum of antiviral activity, including influenza A and B viruses. On the other hand, there is now much clinical experience with the M2 blockers, and these drugs are inexpensive. It is clear that influenza in different community groups needs to be managed in specific and targeted ways. For example, in the over-65-years and at-risk groups, vaccination will remain a mainstay of disease prevention. However, up to 40% of those in these groups may fail to receive vaccine, and therefore the antivirals can be used therapeutically, or, in defined circumstances, as prophylactics. At present, influenza is hardly managed in the community. The infrequent global outbreaks, pandemics, present further problems. The more extensive use of the two classes of antivirals, and also vaccines, in the important interpandemic years will provide a very significant investment in health benefits in the face of a new pandemic virus in an otherwise completely vulnerable population.

Keywords epidemic, influenza, inhibitors, neuraminidase, M2 proton channel, pandemic

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Influenza is a difficult target for antiviral chemotherapists; it is a genetically mobile virus that has two faces, the pandemic one and the epidemic one. It is our contention in this review that most scientific and clinical attention should be focused upon the epidemic face of influenza and that new antivirals should be more aggressively used in the years between pandemics. In the years that intervene between pandemics (1919–56, 1958–67, 1969–2001), the rates of hospitalization and premature death are far in excess of those during the pandemic years. As a single example, UK at the time of the millennium celebration witnessed nearly 20 000 deaths from influenza pneumonia and bronchitis (Figure 1). In turn, such a focus on the interpandemic period will lead to more ideas about how drugs can be used against influenza during a pandemic itself. In preparation for a pandemic, many countries are now considering the creation of stockpiles of the antiviral drugs reviewed below.

It is quite clear that there are economic and medical benefits in preventing hospitalization caused by influenza in the interpandemic periods [1]. Also, the number of productive days lost because of influenza each year is very significant. Therefore, it is not surprising, but also encouraging, given the attitudes of the past, that new anti-influenza drugs were developed towards the end of the last century and more are in the pipeline as we start a new millennium.

INHIBITORS OF THE VIRAL M2 PROTON PUMP OF INFLUENZA A VIRUS

To combat influenza, a drug is needed that reaches the respiratory tree and, particularly, the upper regions of the nasal and throat mucosa and trachea, where most infections are thought to begin and thereafter focus [2]. More rarely, the virus descends into the bronchi, bronchioles, and even the alveoli, and destroys the cellular lining of the lung. Thus, bronchopneumonia is the hallmark of

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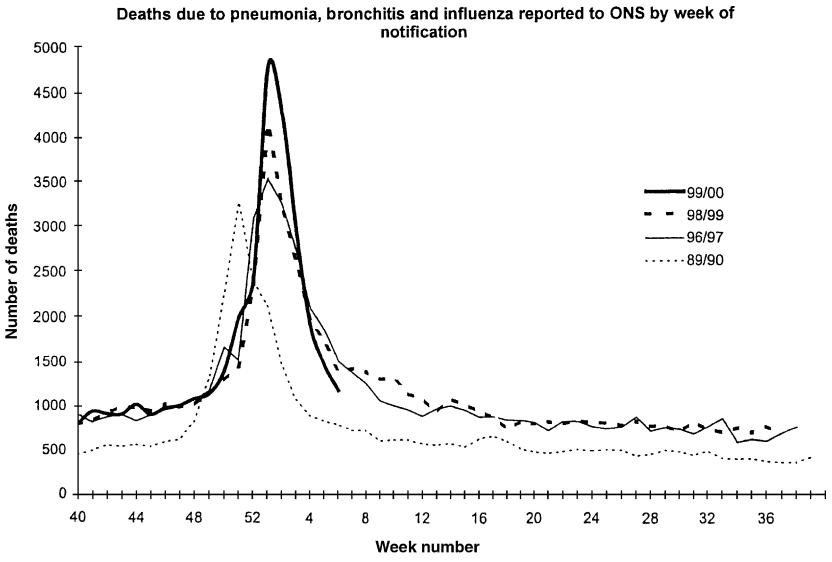

Figure 1 Mortality from influenza, pneumonia and bronchitis in the UK at the millennium 1999–2000.

a serious and life-threatening influenza virus infection, rather than solid consolidation, which more often follows superinfection of the lung with *Streptococcus pneumoniae* [2–4]. The discovery of amantadine (also called I-adamantanamine hydrochloride, Symmetrel or, more recently, Lysovir) enabled us to break through these pharmacologic barriers [5], allowing the molecule to be distributed preferentially in the airways. The drug has significant antiviral effects against all influenza A viruses in cell culture, including the most recent H5 viruses, in animal model infections in mice, and, most importantly, in humans. The method of action has been well characterized as blocking of the vital acidification function of the viral M2 channel [6]. Analysis of rare biopsy material established that the drug was concentrated, to higher levels than simple tissue distribution models predicted, in the upper respiratory tract [7]. However, the most significant discovery was that amantadine, and its molecular relative rimantadine, had prophylactic and therapeutic activity in human infection with influenza A H1N1, H2N2 and H3N2 viruses. At the start of this work nearly 40 years ago, amantadine was most studied in the UK, Europe and Japan, while rimantadine was investigated in very large trials in Russia, the USA and, to a lesser extent, eastern Europe. These extensive studies will be summarized briefly below and can be easily consulted [8]. The prophylactic activity, around 80–90%, is similar to that of the recent neuraminidase (NA) inhibitors [9,10], as is the therapeutic activity, reducing illness by approximately 1.5 days if the drug is used within 36–48 h of symptoms appearing. In fact, the scientific community did not accept that an anti-influenza drug could have any therapeutic activity until the first studies with amantadine proved otherwise.

In commercial terms, and in view of the investment of some £300 million needed to develop any new drug, pharmaceutical companies need to be assured of a market. However, examination of the history of the underuse of amantadine and rimantadine illustrates the difficulty of introducing antivirals into the management of influenza in the community. Amantadine and rimantadine have been licensed as anti-influenza A drugs for four decades, but their application in the community has been bedevilled by two worries: emergence of drug resistance, and fear of toxicity. As regards toxicity, most earlier clinical studies employed a dosage of 200 mg/day of amantadine. Some 'jitteriness' was noted in about 10% of patients, particularly the elderly. Significantly, some studies [11] indicated that the dose could be halved to 100 mg daily, with continued anti-influenza A activity. At this drug level, toxicologic problems would not be expected, but the dosage can still be adjusted downwards for the frail elderly, who could be very underweight. We will return to this important low-dose study below.

The problem of drug resistance is more difficult to resolve, but is not unique to amantadine. Amantadine-resistant mutants can be generated among experimentally infected mice, but only after the use of very high concentrations of drug [12]. It is not known whether these viruses are less pathogenic or virulent than the wild-type virus, but the

low frequency of detection in the field does suggest this. Mutations which confer resistance to amantadine can be clearly identified in the viral M2 gene, and such viruses are cross-resistant to other M2 proton channel inhibitors.

CLINICAL THERAPEUTIC EFFECTS OF AMANTADINE

Clinical trials with amantadine or rimantadine have been carried out over four decades in at least eight countries, involving over 30 000 volunteers or patients, with over 14 500 receiving amantadine. The overall protection rate in prophylactic trials against illness caused by influenza A H2N2, H3N3 or H1N1 viruses has varied between 50% and 100%. In the majority of controlled therapeutic studies with amantadine or rimantadine, a reduction in the severity and duration of influenza-caused fever was reported in those groups receiving the drug, compared to the control groups, provided that treatment was begun within 2 days of the onset of the clinical symptoms.

Arguably, the most significant discovery for the future development of antivirals against respiratory viruses was that of the therapeutic effect of amantadine. In the initial studies, volunteers were artificially infected with influenza virus and then given amantadine or placebo. Symptoms, including objective parameters such as temperature, subsided more rapidly when amantadine was administered [8].

Perhaps not surprisingly, the use of the antiviral much later than 48 h after the diagnosis of clinical disease failed to abrogate symptoms of the disease. A similar situation has been documented with the new anti-NA drugs. It should be noted that, prior to these trials with amantadine, most virologists considered that, concomitant with respiratory symptomatology, virus replication would reach a peak and the therapeutic use of an inhibitor at this stage of the disease would be fruitless. Surprisingly, few studies have actually been performed on the quantity of influenza virus excreted in the lower respiratory tract of infected humans. Post-mortem examination of trachea specimens by immunofluorescence and cytology during the influenza A H2N2 pandemic did show that virus replication was patchy and that many cells remained uninfected [2,4]. This observation would give credence to the concept that therapeutic intervention with antiviral drugs would prevent the spread of virus further down the respiratory tract. Encouragingly, pharmacologic experiments, in both animals and humans, have shown a preferential accumulation of amantadine in respiratory tissue, including the lung [8]. Therefore, the scientific basis for therapy with M2 blockers appears firm.

In a typical example of clinical investigations at the time, two therapeutic studies were carried out in the winters of 1972–73 and 1973–74 in general practice in the UK. Both influenza A and influenza B were circulating. As the influenza A viruses during both winters had essentially the same sensitivity in vitro to amantadine, the authors considered it appropriate to combine the results from the two winters. The mean duration of fever in the drug-treated group was 51.4 h, and that in the controls was 73.4 h ($P < 0.05$). Symptoms cleared completely within 4 days in 53% of patients receiving amantadine, compared with only 23% of controls. Mean days in bed were 2.58 for the amantadine group and 3.44 for the placebo group ($P < 0.01$). In those patients with influenza B, there were no significant differences between active drug and placebo. The appearance of influenza B infection, confirmed only after the clinical recording was completed, illustrated the sensitivity of the trial design, for it had been demonstrated previously [5] that this virus was insensitive to amantadine. No adverse effects were noted in this trial, which employed 200 mg of amantadine each day.

CLINICAL STUDIES WITH LOW-DOSE (100 mg) AMANTADINE

Because of concern about the mild toxic effects of 200 mg amantadine per day, it is relevant to briefly review clinical trials where lower doses were used, and which showed that most adverse effects could be avoided while antiviral activity was retained.

In the late 1960s [13], Smorodintsev et al. performed two trials, the first with 100 mg (38 cases) or 200 mg of amantadine as a single or divided dose (168 cases) or placebo (198 cases) daily for 11 days in healthy volunteers who were challenged with virus 24 h after starting treatment. One hundred and thirty-two of 146 (90.4%) of placebo subjects developed influenza. In three groups receiving amantadine (different strains of influenza A), the rates were 49%, 33%, and 35% ($P \leq 0.01$). Fever was reduced from 64% in volunteers receiving placebo to 25% in volunteers receiving amantadine ($P < 0.01$). In those subjects

developing influenza, dosing with amantadine resulted in milder symptoms and a shorter duration of illness (8.2% longer than 7 days versus 49% longer than 7 days on placebo). In this study, there were no significant antiviral differences between low-dose 100 mg and 200 mg amantadine daily.

Younkin et al. [14] treated influenza patients for 5 days with amantadine 100 mg (16 cases) or 200 mg (14 cases), or 3.25 g of aspirin (17 cases), daily. Although the aspirin treatment group defervesced more rapidly, by the second day the amantadine-100 mg recipients showed greater symptomatic improvements ($P < 0.01$). The 200-mg dose did not show such significance. Bothersome side effects resulted in discontinuation of therapy by 35% of patients on aspirin but only 3% of patients on amantadine. At 100 mg, the side effects reported were considered to be minimal, and consisted of dizziness, loss of concentration, or insomnia. The study also demonstrated that 100 mg/day amantadine had therapeutic efficacy equal to 200 mg/day.

Sears and Clements [15] induced influenza in 44 healthy volunteers; 22 received 100 mg of amantadine, and 22 placebo, once daily for 8 days, with intranasal viral challenge on day 4. Influenza illness was seen in two of 22 volunteers (9%) on amantadine versus nine of 22 (41%) on placebo ($P < 0.04$). With amantadine, the illness was mild and tended to consist only of mild transient rhinitis. Infection was seen in 77% of volunteers on amantadine versus 91% of volunteers on placebo. Virus shedding was reduced significantly by amantadine during ($P < 0.003$) and after ($P < 0.03$) treatment, with total days of virus isolation during treatment being 1.2 on amantadine and 3.5 on placebo. The amount of virus shed was halved by low-dose amantadine treatment.

Reuman et al. [11] carefully investigated the antiviral effects and also toxicity of low-dose (100 mg) amantadine, and compared the results with those of a group given 200 mg of the drug. In this direct virus challenge experiment, 100 mg of amantadine reduced the rate of illness from 58% to 15%, and the number of volunteers infected from 95% to 60% (Table 1).

Analysis of the three groups for central nervous system and gastroenteritis effects showed no increase in the low-dose (100 mg) drug group compared to the placebo group (Table 2).

Table 1 Protective effect of low-dose amantadine in 78 subjects challenged with influenza A virus

Treatment group	Number of subjects	Number infected	*P*-value	Number ill	*P*-value
Placebo	19	18 (95%)		11 (58%)	
50 mg/day amantadine	20	16 (80%)	0.187	4 (20%)	0.017
100 mg/day amantadine	20	12 (60%)	0.012	3 (15%)	0.005
200 mg/day amantadine	19	13 (68%)	0.045	2 (11%)	0.003

Influenza infection is defined as virus isolation, antibody response, or both. Influenza illness is defined as fever >37.8 °C and two influenza symptoms [11].

Table 2 Absence of toxicity of low-dose amantadine in community studies [11]

	Placebo	Amantadine 100 mg (low dose)	Amantadine 200 mg
Total no. of subjects at risk	159	159	158
Total no. of subjects with adverse experiences	49 (31%)	47 (30%)	71 (45%)[a]
Central nervous system	25 (15%)	23 (14%)	47 (30%)
Gastrointestinal	12 (8%)	12 (8%)	17 (11%)
Cardiovascular	1 (0.6%)	1 (0.6%)	1 (0.6%)
Whole body	24 (15%)	16 (10%)	21 13%)

[a]Significantly different compared to placebo ($P = 0.009$) and the 100-mg group ($P = 0.005$).

In conclusion, it would appear that amantadine or rimantadine should be considered very seriously in any modern clinical management strategy for influenza. They are not effective against influenza B, but are well tested antivirals that are relatively safe and very cheap.

THE NEURAMINIDASE INHIBITORS: A MODERN DEVELOPMENT OF AN ANTIVIRAL DRUG

Not until the early 1970s was the existence of a physically separate NA enzyme spike established [4]. The virus was known from earlier and classic studies to possess NA activity *per se*, but physical separation techniques, electron microscopy, reassortment genetics and serology by immunodouble diffusion established NA as a distinct mushroom-shaped protein. The molecule was crystallized by a scientist who, rather uniquely, had developed the techniques of scale-up protein chemistry in an academic laboratory [16]. Using cellulose acetate strips, he was able to separate NA and haemagglutinin (HA), and, indeed, performed peptide mapping on a viral protein for the first time. The NA crystals themselves were not easy to obtain in a high-quality form, but it was possible to perform X-ray crystallography from such crystals and to establish the position of antigenic epitopes and, importantly, the enzyme active site [17].

Much earlier, two chemists in Vienna [18] had synthesized inhibitors of influenza NA, but the molecules had failed to show any in vivo activity. However, with new knowledge of NA crystal structure and the precise localization of the normal sialic acid substrate in the active site [19], it was possible to design a sialic acid look-alike molecule that would fit even more tightly in the active site of the NA. This molecule, now known as zanamivir or Relenza, was a very active inhibitor of a range of influenza A and B viruses of all subtypes, including those of birds, animals and humans. More interestingly, given by aerosol or spray, the compound inhibited virus replication in influenza-infected mice, ferrets, and human volunteers.

The activity of the NA enzyme is essential for the replication of influenza A and B viruses, enabling the virus to negotiate nasal fluid packed with sialic acid proteins when it enters the body, and at the time of virus release from the infected cell. Although most of the NA 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 [20]. In particular, the 11 key amino acid residues that line the shallow pocket of the enzyme active site and interact directly with the substrate (sialic acid) are highly conserved in all strains of influenza A and B investigated, even the 1918 influenza virus. This finding is important for two reasons. First, drugs that mimic the natural substrate sialic acid and act as competitive inhibitors should have broad inhibitor activity. Second, the uniformity of the influenza NA 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 [21]. Once this important discovery had been made, NA inhibition became an attractive concept for antiviral intervention.

Other innovative compounds that incorporate a carbocyclic structure into the molecule have also been developed; this arrangement offers greater stability than that of earlier compounds, and facilitates modification of the molecule to optimize its properties. One carbocyclic compound is (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylopropoxy)-1-cyclohexane-1-carboxylic acid, also known as oseltamivir or Tamiflu [22,23]. This compound fits precisely into the three-dimensional structure of the NA active site to interact with conserved residues and competitively inhibit the enzyme. The incorporation of a lipophilic side-chain into this molecule exploits X-ray crystallographic evidence of a hydrophobic pocket in the NA active site, enhancing the affinity for the target. More recently, a third and fourth inhibitor have been synthesized [24–26].

REPRESENTATIVE CLINICAL TRIALS WITH THE NEURAMINIDASE INHIBITORS

The efficacies of zanamivir and oseltamivir have been demonstrated in the prevention (prophylaxis) and in the treatment of experimental influenza infection [27]. Hayden et al. reported the results of four randomized, double-blind, placebo-controlled trials that evaluated intranasal zanamivir (two to six times daily) in the prevention and treatment of experimental influenza A (H1N1) in artificially infected volunteers. Overall, the drug

prevented laboratory-proven infection and febrile illness in 82% and 95% of subjects, respectively (both $P < 0.001$ versus placebo). Early treatment of experimental infection with zanamivir in these studies reduced peak viral titers, the duration of viral shedding, the frequency of illness and other measures of illness compared with placebo.

Subsequent clinical studies in the community showed that administration of inhaled drug within 48 h of natural influenza A or B infection significantly reduced the duration of symptomatic illness by 1 day (4 days versus 5 days) compared with placebo. Importantly, data also indicated that zanamivir treatment reduces the impact of influenza virus infection on patients' productivity and health status and the number of contacts made with healthcare professionals [28–30].

To study the therapeutic effect of zanamivir, Monto et al. [30] analyzed the overall intent-to-treat (ITT) population, and showed that the drug reduced the median number of days to alleviation of clinically significant symptoms by 1 day compared with placebo (6 versus 7 days; Table 3).

This difference was statistically significant for both zanamivir treatments ($P = 0.012$ twice daily versus placebo, $P = 0.014$ four times daily versus placebo). The difference between the zanamivir and placebo groups was evident by inspection by day 2, and was maintained until the end of treatment. For patients who began treatment >30 h after onset of symptoms, the difference between the zanamivir and placebo groups, although still present, was reduced to 0.5–1 day; this difference was not statistically significant. Zanamivir reduced the time to symptom alleviation in both febrile and non-febrile patients, but had a greater effect on febrile patients. Zanamivir given twice daily reduced the median time to alleviation of symptoms by 0.75 days in the non-febrile group ($P = 0.049$) and by 1.5 days in the febrile group ($P = 0.049$). Similar differences were seen for zanamivir four times daily, compared with placebo.

Of the persons who were febrile on study entry, 348 (74%) were influenza positive by isolation and serology; only 57% of the total ITT population was influenza positive. This suggests that a febrile state early in the course of infection may be a good marker for distinguishing between influenza-like illnesses in the adult population. Similar benefits (not shown) regarding symptom alleviation were seen in the corresponding analyses for the influenza-positive population. A reduction of 1.5 days in the time to symptom alleviation was seen in both zanamivir groups for the total influenza-positive population, although the differences were not statistically significant.

In rather similar treatment studies, the oral drug oseltamivir (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, the drug reduced the median area under the curve (AUC) of viral titer in nasal washes for all treatment groups, compared with placebo, producing a 100-fold reduction in viral load by 24 h and a 100-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 drug treatment group. There were significantly ameliorated clinical symptoms, with more rapid cessation of symptoms in the active drug treatment groups, the duration of symptoms

Table 3 Median number of days to alleviation of major symptoms in influenza-diagnosed patients given zanamivir

	Placebo		Zanamivir 2×/day				Zanamivir 4×/day			
Population group	**No.**	**Days**	**No.**	**Days**	***P***	**Difference[a]**	**No.**	**Days**	***P***	**Difference[a]**
All patients	422	7.0	419	6.0	0.012	1.0	415	6.0	0.014	1.0
symptom duration[b]										
≤30 h	249	6.5	242	5.5	0.015	1.0	240	5.0	0.001	1.5
>30 h	173	7.0	177	6.0	0.422	1.0	175	6.5	0.837	0.5
Fever[b] <37.8 °C	246	6.5	254	5.75	0.049	0.75	259	6.0	0.274	0.5
Fever[b] >37.8 °C	170	7.0	155	5.5	0.049	1.5	148	5.0	0.032	2.0
High-risk patients	68	7.8	48	6.3	0.137	1.5	42	5.0	0.042	2.75

[a]Difference from placebo (95% confidence interval).
[b]At study entry.
From Monto et al. [30].

Table 4 Effects of oseltamivir on duration and severity of illness [33]

	Influenza-infected participants			All treated participants		
	Placebo ($n = 129$)	Oseltamivir, 75 mg ($n = 124$)	Oseltamivir, 150 mg ($n = 121$)	Placebo ($n = 209$)	Oseltamivir, 75 mg ($n = 210$)	Oseltamivir, 150 mg ($n = 208$)
Illness duration, median (95% Cl), h	103.3	71.5	69.9	97.0	76.3	74.3
P-value		< 0.001	0.006		<0.004	0.004
Illness severity, median score	963	597	626	887	686	629
P-value		<0.001	<0.001		<0.001	<0.001
Return to normal health, median (95% Cl), h	178	132	154	178	134	155
P-value		<0.001	0.03		<0.001	0.03
Return to normal activity, median (95% Cl), h	225	157	180	230	173	202
P-value		0.02	0.05		0.01	0.10

being reduced by almost half compared with placebo, and reduced in severity [31,32].

In an important study in the community of oseltamivir [33], 629 healthy, non-immunized adults aged 18–65 years presenting within 36 h of onset and with a temperature of 38 °C or more plus at least one respiratory symptom and one constitutional symptom were enrolled (Table 4).

Individuals were randomized to one of three treatment groups: oseltamivir, 75 mg or oseltamivir, 150 mg twice daily for 5 days, or placebo. In total, 374 participants were confirmed to have influenza (60%). The duration of illness from the initiation of therapy was reduced by approximately 30% in the oseltamivir groups. In the 75-mg group, the median duration of illness was reduced to 3 days, compared to 4.3 days in the placebo group ($P < 0.001$), and in the 150-mg group, the duration was reduced to 2.9 days ($P < 0.001$). There was also a significant decrease in the symptom score AUC as a measure of the severity of illness. Oseltamivir-treated volunteers reported more rapid return to normal health and usual activities. Additionally, the incidence of secondary complications in subjects with influenza, predefined as pneumonia, bronchitis, sinusitis, and otitis media, was reduced from 15% in placebo recipients to 5–9% in the two oseltamivir-treated groups. Antibiotic prescriptions for these complications were reduced (Table 5).

In summary, both NA inhibitor drugs, Relenza and Tamiflu, show significant therapeutic antiviral activity in the community. The challenge now will be the reorganization of clinics and doctors' surgeries to allow rapid access of ill patients to the new drugs. All the recent trials have shown that reliance on a triad of symptoms (temperature, cough, and headache) can allow a doctor to diagnose influenza accurately, especially if virologic surveillance has identified influenza in the community.

Table 5 Effect of oseltamivir on the number of influenza-infected patients experiencing secondary complications [33]

	Study group		
Complication	Placebo ($n = 129$)	Oseltamivir, 75 mg ($n = 124$)	Oseltamivir, 150 mg ($n = 121$)
Otitis media	1	0	0
Sinusitis	11	6	4
Bronchitis	8	5	2
Pneumonia	1	0	0
Any secondary complication (%)	19 (15)	11 (9)[a]	6 (5)[a]
Antibiotic use (%)	14 (11)	8 (6)[b]	4 (3)[b]

[a]Combined oseltamivir results versus placebo (Fisher exact test), $P = 0.03$.
[b]Combined oseltamivir results versus placebo (Fisher exact test), $P = 0.05$.

DRUG RESISTANCE AND NEURAMINIDASE INHIBITORS: DRUG-RESISTANT MUTANTS HAVE REDUCED VIRULENCE

The most common mutation in the NA gene selected by exposure to NA inhibitors in vitro is an amino acid substitution of a lysine (K) for a conserved arginine (R) at position 292, in influenza N2 NA. The R292K mutation has been selected by oseltamivir carboxylate, zanamivir, and RWJ-270201, a third NA inhibitor. An H274Y mutation in N1 has also been selected in vitro by oseltamivir carboxylate, and in vivo in an HINI influenza virus challenge study with oseltamivir phosphate in healthy volunteers. The most common N2 mutation arising in vitro under the selective pressure of zanamivir has been that of the conserved Glu119 residue in the NA active site, causing a 100-fold reduction in the sensitivity of the enzyme to zanamivir. Importantly, virus carrying the zanamivir-selected mutation is attenuated in infectivity in mice and in pathogenicity in ferrets. There are no mutations selected by oseltamivir carboxylate at position 119 in vitro, but E119V has arisen clinically during oseltamivir phosphate treatment in just two patients infected with influenza A virus of the H3N2 subtype. No NA mutations giving rise to resistance have yet been generated by oseltamivir in influenza B NA in vitro, although there is some evidence that zanamivir in vivo has induced the development of a resistant mutation in influenza B NA in an immunocompromised child.

The incidence of resistant virus has been shown to be low, and resistant virus, where it does occur, is detected only transiently, arising late in infection and being cleared normally. In one clinical study in which 385 patients were treated for naturally acquired influenza infection [33], only one patient was found to shed virus that carried NA with reduced sensitivity to oseltamivir. This patient received 75 mg of oseltamivir phosphate twice daily. The virus isolated from a post-treatment nasal swab carried NA with the R292K mutation.

The overall fitness (infectivity, replicative ability, and pathogenicity) of the virus carrying the R292K mutation in the NA gene was reduced in both mouse and ferret models of influenza. The compromised nature of the virus with R292K NA is consistent with the clinical course of influenza illness in the patients found to carry R292K influenza virus. These patients' symptom scores were generally indistinguishable from those of patients with wild-type virus, and the patients continued to recover normally, following emergence of resistant virus at time points late in infection. Thus there was no evidence for enhanced pathogenicity of the resistant viruses in humans, and no consequences for the patient of viral R292K NA mutations occurring during the course of infection.

The other NA mutations that have arisen with oseltamivir treatment of naturally acquired influenza infection, again with low frequency, are H274Y (one patient) and E119V (two patients). As for patients carrying R292K virus, there were no changes in symptom score and recovery for the patients when H274Y and E119V were present in the viral NA.

MANAGEMENT OF EPIDEMIC INFLUENZA IN THE VARIOUS SUSCEPTIBLE POPULATION GROUPS

The recent clinical studies with the NA inhibitors described above have confirmed the earlier findings for amantadine and rimantadine that rapid intervention with antivirals can either ameliorate symptoms in an already infected person or prevent the spread of influenza in family groups. But how are the new drugs actually to be used, and, more importantly, how can the totality of live and inactivated vaccines, amantadine and the NA inhibitors be best applied to combat both pandemic and epidemic influenza? There are large differences in the costs of these preventatives and therapies, as well as in clinical experience, and in effectiveness against viral subtypes (Table 6).

It would now appear that different clinical management approaches will apply to various subgroups of patients (Table 7). We will briefly review the different community sectors.

The at-risk group in epidemic years

There is a scientific and medical consensus that inactivated influenza vaccines should continue to be the cornerstone of influenza management in the elderly and at-risk groups. Indeed, for the winter 2000/2001 in the UK, 10 million doses of vaccine were administered to the 7% of the population who are at risk of serious complications during an influenza infection. Other European countries and the USA have immunized higher numbers of

Table 6 Licensed antivirals and vaccines for use against influenza

Antiviral or vaccine	Clinical experience	Cost of course (approximate)	Side effects	Drug resistance
Amantadine	30 years	£5	Rare if dosage reduced	Detected but have no growth advantage
Rimantadine	30 years	£5	Rare	Detected but have no growth advantage
Zanamivir	5 years	£25	Rare	Detected but have a growth and virulence disadvantage
Oseltamivir	2 years	£25 (estimate)	Rare	Detected but have a growth and virulence disadvantage
Subunit HA/NA vaccine	20 years	£5	Rare	NA
Live attenuated cold-adapted (ca) vaccines	20 years	£10	Rare	NA

Table 7 The clinical management of influenza in epidemic years with antivirals and vaccines

	Vaccines		Antiviral	
Group	Live attenuated	Inactivated	M2 blockers	Neuraminidase inhibitors
At risk population*	No	Yes	Yes	Yes
Children as a group of super-spreaders	Yes	No	Yes	No
Young and other active members of society†	No	No	Yes	Yes
Hospital staff and patients	No	Yes	Yes	Yes
Elderly persons' homes, staff and patients	No	Yes	Yes	Yes

Note that the differentiation of influenza A from influenza B is required for the use of amantadine/rimantadine.
*Approximately 7% of the population.
†<65 years.

vulnerable patients. This at-risk group is made up of everyone over the age of 65 years, and persons of any age who are diabetic or who have chronic heart, kidney or respiratory disease. However, it is acknowledged that influenza vaccine is not 100% protective against disease, and certainly not against infection. Therefore, each year a proportion of previously vaccinated at-risk persons will become infected with influenza and be vulnerable to severe respiratory complications. Also, perhaps 30–40% of the at-risk group will not receive vaccine. These persons are excellent candidates for chemotherapy with either amantadine, the new NA inhibitors, or both.

Children—the virus spreaders in epidemic and pandemic years

There is substantial evidence that children constitute a key group in the spread of influenza and, indeed, other respiratory viruses. There has been renewed interest in the application of live attenuated influenza vaccines to prevent virus spread among children, and thereby to ameliorate spread from children to adults, including the vulnerable elderly. A re-evaluation of vaccination policy in Japan during the 1970s and 1980s, which used inactivated influenza vaccine in children, has shown that the strategy may have reduced hospitalization and death in the elderly.

Amantadine at reduced dosage can be used for therapy or prophylaxis in children. However, at present there are only limited clinical data for the NA inhibitors in children.

The working population

Although there have been studies in the past of the use of vaccine, and also rimantadine, in large cohorts of factory workers, more often influenza has not been managed clinically in this important group. Generally, the advice has been not to bother the doctor, to self-administer paracetamol, and to stay in bed. But each influenza epidemic brings a

toll in hospital admissions and deaths among this unprotected group in the population. Approximately half of the deaths rated in Figure 1 were of persons in younger age groups. In addition, there is significant economic disruption in this community group.

The NA inhibitors could form an ideal group of drugs for large-scale therapeutic use in this group, although, of course, cost is a factor. With evidence of influenza A in the community, rather than influenza B, amantadine or rimantadine could also be extremely useful drugs, especially for prophylaxis.

Hospital patients and staff

Influenza outbreaks place secondary care services under particular pressure for acute admission. In studies in the USA, during some winters as many as 140% of excess admissions were attributed to influenza. No nurse or doctor suffering from chicken pox, measles, hemorrhagic fever or hepatitis would be found helping patients in a modern hospital. Unfortunately, at least up to the present time, influenza has been viewed differently, and there is no doubt that hospital staff sometimes carry on working with this infection. Obviously, influenza is not being managed correctly under these circumstances, because the virus could be introduced into the ward of a hospital.

A few studies have examined the effect of vaccination of hospital staff and detected some reduction in influenza in wards where a high percentage of staff were vaccinated. However, hospitals do suffer from high staff turnover, making vaccination policies difficult to maintain. Chemoprophylaxis or therapy with amantadine and/or the new NA inhibitors could now play an important role.

THE THREAT OF PANDEMIC INFLUENZA AND THE USE OF ANTIVIRALS

Influenza is a unique virus in having two epidemiologic forms, epidemics and pandemics, and management of community illness will be different in each case. It is quite clear that effective utilization of antiviral drugs to combat a pandemic virus will depend upon the prior widespread use of the drugs during interpandemic years. By definition, most persons would be vulnerable to infection with a new pandemic virus, although during epidemic years those over 65 years of age are most vulnerable to complications. It is not reasonable to deduce that antivirals can be stored during interpandemic years and only used in a pandemic. The best approach would be consistent and detailed clinical use of antivirals and vaccines to prevent the year-to-year medical and economic impact of influenza. In this manner, physicians and nurses would become familiar with influenza as a unique disease entity, and, at the time of a pandemic, considerable clinical expertise in the use of antivirals would have accumulated. The WHO has requested each member state to produce a pandemic plan, but few governments have responded to date. We have recently published a focus alert document to highlight concern about this lack of preparation [34]. European countries are now considering a strategy of stockpiling anti-influenza drugs; this, alongside more clinical use each year, could be a significant investment in future community care in Europe.

The essential objectives of a pandemic plan are to alert scientific, medical and political groups, and to reduce the morbidity and mortality from influenza illness, and thereby to increase the ability of a community to cope with large numbers of people who are ill, at home and in hospital, and dying, and to ensure that essential services are maintained [35].

The date of the next influenza pandemic is unknown. Intervals between previous pandemics have varied from 11 to 42 years, with no recognizable pattern. Thus, previous pandemics have been caused by influenza A viruses in 1889, 1918, 1957, and 1968 [36]. Re-emergence of an H2 or N7 or H5 component has been anticipated by some as the most likely event leading to a pandemic, and 15 hemagglutinins exist in nature, particularly in bird reservoirs.

In two pandemics (1957 and 1968), new influenza viruses emerged in the Far East, whereas in two earlier pandemics (1889 and 1918), the virus arose in Europe [36], in Russia and France respectively. In all cases, the new pandemic virus spread along trade and transportation routes. The two most recent pandemic strains have spread worldwide in about 6–12 months, although successive waves of illness may occur over a longer period. The 1918 pandemic was recognized in France in the early months of 1916, and by April 1918 was widespread in western Europe. During the spring and summer, large numbers of people were

affected with relatively mild disease. The high mortality occurred in a later wave in the autumn [36]. It does appear that the evolution of a pandemic is complex.

Pandemic influenza may appear at any time of the year, not necessarily during the 'normal' influenza season (November to March in the northern hemisphere). In most pandemics, activity can be expected to last 6–8 weeks, although in 1968–69 lower levels of activity continued for 3–4 months. This relatively short period of the outbreak, as in epidemic years, allows prophylactic strategies with antivirals to be implemented.

ESTIMATES OF INCIDENCE OF ILLNESS IN A PANDEMIC

In 1918, about 23% of the UK population developed influenza, in the 1957 Asian influenza pandemic an estimated 17% of the population suffered from influenza illness; and in 1969 the Hong Kong virus produced illness in 8% of the adult population. It could be safely predicted that each clinical case would be accompanied by a non-clinical case of influenza.

In normal years, although most influenza infection occurs in children, the serious morbidity and mortality occurs almost entirely among elderly people with underlying chronic disease. A different pattern may emerge in a pandemic, as it did in 1918. The 1918–19 pandemic affected mainly healthy young adults, and seemed to spare those at the extremes of life. Similarly, in 1957, the brunt was suffered by schoolchildren and young adults. Therefore, it is clear that clinical management of influenza with antivirals will differ in a pandemic year compared to the interpandemic period.

MORTALITY AND MORBIDITY IN A PANDEMIC YEAR

Of course, the 1918 pandemic dominates the records of infectious disease during the 20th century, and indeed for the previous five centuries. The worst-case scenarios of a new pandemic indicate that, with the current massive world population, rapidity of transport, and persons afflicted with HIV, mortality in a new pandemic could approach that of 1918 [37]. In 1957, when the illness was, on the whole, milder compared to the other pandemics of the 20th century, more than 30 000 deaths occurred in England and Wales. Estimates ranged from 1.3 to 3.5 deaths/1000 cases, and two-thirds of the deaths were of people aged over 55 years.

In a pandemic, the number of new general practice consultations for influenza-like illness can be expected to exceed 500/100 000 population per week during a pandemic; a medical practice of 10 000 patients would therefore expect to see at least 50 new patients per week. Pandemics also have a marked effect on hospital admissions. During September and October 1957, between 25 000 and 30 000 more cases of acute respiratory infection were admitted to hospitals in England and Wales than would have been expected at that time of year.

THE IMPACT OF PANDEMICS ON THE ECONOMY

Not unexpectedly, pandemics have a serious effect on the economy. In 1957 in the UK, new sickness benefit claims by those working and aged 15–64 years increased by 2.5 million (of 17.5 million insured). Among the uninsured, an additional 1.5 million absences were estimated. Of the insured population, 8–10% were estimated to have lost 3 working days at home during the epidemic. In the Hong Kong pandemic of 1968, just over 1 million excess sickness claims were received in England over 5 months.

THE KEY ELEMENTS IN REDUCING THE MORBIDITY AND MORTALITY FROM PANDEMIC INFLUENZA

The morbidity and mortality of influenza can be reduced by prophylaxis and vaccination, and by best practice in the management of cases, including drug therapy. However, it must be acknowledged that prevention of influenza by immunization and/or the use of antiviral agents will be possible only to a limited extent, unless health authorities agree to store a quantity of each antiviral drug and also to use these drugs more proactively during the interpandemic years. In any case, vaccine is likely to be in short supply. Vaccine production takes time and is subject to various rate-limiting factors, and demand will be high worldwide. Vaccine will have to be distributed equitably and administered to predetermined priority groups. Also, it is not so certain that antiviral drugs

will be available in any quantity unless the drugs are used more during interpandemic years. In this case, production and storage of drugs will have reached quite high levels, and possibly only a three-to-four-fold increase will be required to cope with a pandemic. The UK pandemic plan acknowledges that the need to keep health and other essential services running will mean that, if vaccine and antiviral drug supplies are limited, certain groups in the community may need to take precedence over the risk groups normally recommended for vaccination during interpandemic years.

CONCLUSIONS

There is no doubting the impact of the Great Influenza Pandemic in 1918 and the subsequent pandemics in 1957 and 1968, and there is much to be learnt from a study of the genome of that virus: what is the virulence gene constellation, and what is the biology of a global pathogenic virus? These important questions led in the 20th century to four exhumations of influenza victims in Alaska [38], Spitsbergen [39] and, more recently, in the UK (J. S. Oxford and R. Daniels, unpublished data) to retrieve and study the genetic composition of this virus and to correlate virulence with particular genes. Nevertheless, these pandemic events are rare and need to be considered carefully in the complete context of antivirals and influenza. As we have seen in the current review, antivirals will have an important position in the combined human arsenal against virus-induced pneumonia, alongside vaccines and antibiotics for secondary bacterial complications. However, this review has also argued that success during a pandemic equates not to the sudden discovery in government warehouses of a stock of antivirals, but rather to the step-by-step use of these same drugs in the year-to-year conflict with this virus in epidemics. Recently, concern about influenza, along with tuberculosis and other public-health factors, has been sidelined in many countries, but it is quite clear that public-health issues are becoming increasingly important in a fast-moving and increasingly international society, where truly 'no man is an island'. Medical science is often visionary, but in the case of influenza this has unfortunately not been the case.

Those implementing medical care systems, often rather complacent about influenza and tending to muddle along with a label of 'flu and the common cold', will need to change to accommodate these new therapeutic agents against influenza. At present, the health systems in Europe militate against the successful deployment of a therapy in a disease where irreversible cellular damage happens within hours of the symptoms appearing. The recent proposals from NICE in the UK have suggested that practice nurses be empowered to issue prescriptions for zanamivir, and this could be a major step towards the speedy application of drugs. Ultimately, the drugs will need to be registered as 'over the counter' before the window of use of 36–48 h post-symptom onset can be exploited fully by sufferers. There has been a concern about drug resistance, but this will occur with every drug against RNA viruses. Fortunately, viruses resistant to the NA inhibitors are less virulent than wild-type viruses, while the virulence of those resistant to amantadine is either equivalent to or less than that of wild-type viruses.

Both the new NA blockers and the more comprehensively researched M2 channel blockers are powerful inhibitors of influenza virus replication, and can prevent death and serious disease in animal models. There is every reason to suppose that these compounds, used intelligently with or without vaccines, will have a major impact on the threatening disease of influenza as we know it today.

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