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Influenza: vaccination and treatment

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ABSTRACT: Few conditions exert such an enormous toll of absenteeism, suffering, medical consultations, hospitalization, death and economic loss as influenza. Patients at high risk of complications and mortality include the elderly and those with pre-existing cardiopulmonary disease.

The outbreak in 1997 in Hong Kong, of avian H5N1 influenza in man, which resulted in six deaths among 18 hospitalized cases, and the recent isolation of H9N2 viruses from two children in Hong Kong, are reminders that preparation must be made for the next pandemic. Since the 1970s, efforts to control influenza have mostly focussed on the split product and surface antigen vaccines. These vaccines are of proven efficacy in healthy adults and are effective in elderly people with and without medical conditions putting them at high risk of complications and death following influenza infection.

However, vaccine coverage is patchy and often low, and outbreaks of influenza are not uncommon in well-immunized residents of nursing homes. New vaccines and methods of vaccine delivery are being developed in attempts to overcome the limitations of existing vaccines.

The antiviral drugs amantadine and rimantadine were developed in the 1960s, but have not been used widely due to their spectrum of activity, rapid emergence of resistance, and adverse effects associated with amantadine. The site of enzyme activity of the influenza neuraminidase is highly conserved between types, subtypes and strains of influenza and has emerged as the target of an exciting new class of antiviral agents that are effective both prophylactically and as therapy.
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Influenza is a contagious respiratory viral illness of global importance. Influenza viruses are unique among respiratory viruses with their segmented genome and great antigenic diversity. They have been classified into three distinct types: A, B and C. Influenza A is responsible for occasional pandemics involving millions of people worldwide and frequent, usually annual epidemics that are often associated with considerable morbidity and mortality. Outbreaks of influenza B are less frequent and are associated with a lower burden of illness overall. Influenza C is associated with sporadic and usually asymptomatic infection. At least three pandemics occurred during the last century. By far the worst was the pandemic of 1918–1920. Over a 10-month period, this "Spanish flu" killed an estimated 20–40 million people worldwide with many of the deaths occurring in young, previously healthy adults.

When symptomatic, influenza typically produces an acute febrile illness characterized by cough, headache and myalgia. The illness is generally debilitating, often causing several days of restricted activity including lost school or work days. The majority of morbidity and mortality occurs in the elderly with underlying chronic cardiorespiratory disease or diabetes mellitus, particularly those in residential care. Complications of acute influenza include viral and secondary bacterial pneumonias, exacerbations of pre-existing cardiopulmonary disease and, in children and infants, croup, otitis media, bronchiolitis and febrile convulsions [1, 2].

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During influenza outbreaks, hospital admission rates for respiratory illnesses increase, as does mortality from all causes [3].

Structure and classification

Influenza viruses are enveloped ribonucleic acid (RNA) viruses with a segmented genome belonging to the family of *Orthomyxoviridae*. Both influenza A and B viruses contain eight RNA segments, each individually encapsidated by the viral nucleoprotein, and possess two surface glycoproteins embedded into the membrane: the haemagglutinin (HA) and neuraminidase (NA), which are capable of eliciting antibody responses in humans. Influenza C viruses contain seven RNA segments. Haemagglutinin is involved with receptor binding and membrane fusion. The neuraminidase enzyme catalyses the cleavage of viral progeny from infected cells. It also prevents virus clumping so that each virion can function as an independent infectious unit. It facilitates cleavage of sugar residues on the HA, and by modifying HA carbohydrate side chains, may be implicated in virulence. It also facilitates movement of the virus through inhibitory mucopolysaccharides coating the respiratory tract epithelium, allowing cell to cell spread through the respiratory mucosa. A third surface protein, the M2 ion channel, is a tetrameric membrane channel important in the regulation of internal pH of the virion. By allowing the acidification necessary for the uncoating of viral ribonucleoprotein, the M2 ion channel plays a pivotal role in viral replication [4].

Strains of influenza are classified on the basis of their core proteins into types (*i.e.* A, B or C), by host species of origin (*e.g.* avian, porcine), geographic site of isolation, serial number, year of isolation and, for influenza A viruses, by subtypes of HA and NA antigens. A total of 15 HAs (H1 – H15) and nine NAs (N1 – N9) have been identified for influenza A within its natural avian reservoir. Only one novel HA has been discovered in the past 10 yrs, despite extensive surveillance of influenza in humans and animals, implying that the number of HA′s and NA′s in nature is finite. Despite the presence of 15 HAs in nature, only a few (H1, H2 and H3) have formed stable lineages in man during the last century implying host specificity. The HA of human influenza viruses binds preferentially to terminal sialic acid with an α2,6 linkage to galactose on cell surfaces. In contrast avian influenza viruses preferentially bind α2,3-galactose linkages. Human epithelial cells contain sialic acid α2,6-galactose linkages, but not sialic acid α2,3-galactose linkages. In contrast, avian intestinal epithelial cells (the principal site of replication in birds) contain sialic acid α2,3-galactose linkages, but not α2,6-galactose linkages. The pig trachea contains receptors for both avian and human influenza viruses and the domestic pig supports the growth of viruses of both human and avian origin. For this reason, it has been proposed that genetic reassortment between avian and human virus, leading to a novel strain, may occur in pigs and that pigs represent a "mixing bowl" for the evolution of human pandemic strains [5]. However, it is now recognized that avian viruses can replicate in humans.

Antigenic shift and drift

Pandemics and epidemics arise as a result of changes in the HA. Pandemic influenza is the outcome of "antigenic shift" which reflects a major change in the HA and possibly NA and occurs only with influenza A virus. Antigenic shift occurs as a result of genetic reassortment between the avian and human pools when a virus with a "new" HA emerges (*i.e.* a new subtype) which is serologically distinct from earlier viruses circulating in humans and could not have arisen from these viruses by mutation. There is little or no background immunity in the population to the new virus so it spreads rapidly, usually causing extensive morbidity and mortality. Interpandemic outbreaks are caused by viruses that have undergone minor antigenic changes or "drift". These strains are generated from the accumulation of random point mutations in the genome at sites coding for exposed sections of either HA or NA [6]. Both antigenic shift and drift pose problems for vaccine production.

It is inevitable that another pandemic will occur at some point in the future. During March – May 1997, an outbreak of avian H5N1 influenza in chickens in the New Territories of Hong Kong affected three chicken farms, with an overall mortality of 70% among the 6800 chickens. In May and November/December 1997, cases of influenza due to avian H5N1 viruses occurred in humans in Hong Kong, causing six deaths (33% mortality) among 18 hospital admissions [7]. Extensive analyses revealed that the human isolates were of avian origin and not been derived by reassortment. Clearly, had the virus spread more widely, it could have resulted in a pandemic causing many millions of deaths. Influenza H9N2 virus is widespread in poultry in Asia and has recently been recovered from two children with mild, self limiting illness [8]. These experiences highlight the need for vigilance, and surveillance of both animals and man for influenza, and effective vaccines and antiviral therapies.

Inactivated vaccines

Vaccination represents the mainstay for influenza prevention. Antibodies directed against the haemagglutinin and neuraminidase glycoproteins are associated with protection against infection and amelioration of illness. Three types of inactivated influenza vaccine are currently available in the world: whole virus, split product and purified surface antigen vaccines. All commercially produced, inactivated influenza vaccines are grown in eggs.

Early whole virion inactivated vaccines were associated with frequent local and systemic adverse effects. Although very pure, whole virion vaccines still produce febrile reactions and are unsuitable for use in young children. Accordingly, whole virion influenza vaccines are little used and are unlicensed in many

countries. Most influenza vaccines are supplied as "split" vaccines, produced from the disrupted highly-purified influenza virus, or as "surface antigen" vaccines containing predominantly purified haemagglutinin and neuraminidase. Split and surface antigen vaccines are as immunogenic as whole virion vaccines in primed individuals, but two doses are required in young children and also during pandemics.

Influenza vaccines evoke a strain-specific response with reduced levels of protection against viruses produced by antigenic drift. Influenza vaccines are ineffective against unrelated strains. Accordingly, vaccine composition for the northern hemisphere is reviewed in February by the World Health Organization (WHO) and for the southern hemisphere in September, and the antigenic makeup updated depending on surveillance of the current prevalent circulating subtypes to provide vaccines well matched antigenically to strains that are expected to cause epidemics. In the decade 1987–1997, no changes were made in recommendations for the A/H1N1 strain, nine changes were made for the A/H3N2 strain and four changes for the B strain. A good match between wildtype and vaccine strains was made in respect of 23 (77%) of 30 circulating strains, but when considered on an annual basis, the epidemic strains differed from the vaccine strains during 5 of 10 seasons.

Current inactivated vaccines are usually trivalent, containing 15 μg each of two influenza A subtypes (H1N1 and H3N2) and one influenza B strain. Following vaccination with influenza A, ~90% of normal subjects achieve serum haemagglutinin-inhibition (HAI) titres of >1:40, a level generally associated with protection of about 50% of the population [9]. Protection against laboratory confirmed influenza A illness of 70–95% in young healthy subjects can be achieved when there is a good match between vaccine and circulating strains [10]. Few trials have studied the efficacy of the influenza vaccine in the elderly. A randomized, controlled trial conducted in Australia in 1969 showed that the monovalent influenza vaccine was associated with a 62% reduction in laboratory-confirmed illness [11]. In Holland, during the 1991–1992 season when vaccine and epidemic strains were well matched, vaccination of elderly, mostly healthy subjects, was associated with an overall 58% reduction in clinical and laboratory-confirmed influenza [12] (fig. 1).

Because placebo-controlled trials of the influenza vaccine are considered unethical in the elderly and/or at risk populations, recent studies have focused on the effectiveness of the influenza vaccine in preventing hospitalization for "pneumonia and influenza", all respiratory conditions, and deaths. Whether conducted as cohort or case-control studies, numerous studies have demonstrated effectiveness, both during influenza A and B seasons. In noninstitutionalized elderly people, vaccination is associated with a 19–63% reduction in hospitalization for pneumonia and influenza, a 17–39% reduction in hospitalization for all respiratory conditions, and a 27–75% reduction in all causes of mortality [13] (fig. 2). Comparable levels of effectiveness have been demonstrated in high-, intermediate-, and low-risk individuals [17].

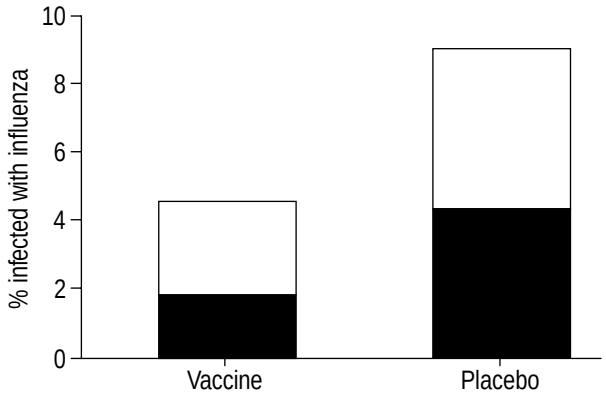

Fig. 1. – Efficacy of influenza vaccination (n=901) and placebo (n=889) in the elderly: a randomized double-blind, placebo-controlled trial. □ : subclinical influenza (relative risk (RR)=0.59 (95% confidence interval (95% CI)=0.36–0.96)); ■: symptomatic influenza (RR=0.42 (95% CI=0.23–0.74)). Adapted from [12].

These estimates of clinical effectiveness are determined by the observed reductions in clinically-relevant but nonspecific outcomes (*i.e.* not laboratory-confirmed), such as influenza-like illnesses, pneumonia, hospitalization and deaths. Because other pathogens can cause these outcomes, estimates of vaccine effectiveness underestimate the true protection afforded by vaccination. Nonetheless, numerous outbreaks of influenza have occurred in well-immunized nursing home populations. Moreover, a study in the US found that 58% of patients admitted to hospital over the age of 65 yrs, who were culture-positive for influenza, had received influenza vaccination in the preceding few months [19]. Accordingly, attempts are being made to augment protection through the use of adjuvants such as MF59, virosomal delivery of antigen to mucosa-associated lymphoid tissue, and the coadministration of live and inactivated vaccines.

Safety of inactivated vaccines

Many millions of doses of split product and purified surface antigen vaccines are administered throughout the world each year and the overall rate of adverse reactions is low. Postimmunization local erythema and tenderness are well documented, but the incidence of systemic symptoms and fever is similar following placebo or vaccine [20, 21]. Severe cutaneous reactions (bullous pemphigoid and systemic vasculitis) have been described rarely, but their relation to vaccination is uncertain [22, 23]. Allergic reactions including anaphylaxis rarely occur following vaccination. Concern over vaccine safety among asthmatics and those with chronic airways disease has been raised because of a small number of adverse case reports [24]. Observational studies have found conflicting results with some postimmunization decreases in mean peak expiratory, flow increases in bronchial hyperreactivity and bronchodilator administration [25]. Other studies, including a large placebo-controlled, double-blinded crossover study have not found any exacerbations of asthma, change in peak flow or increased medication uses [26–30]. A recent review of the evidence

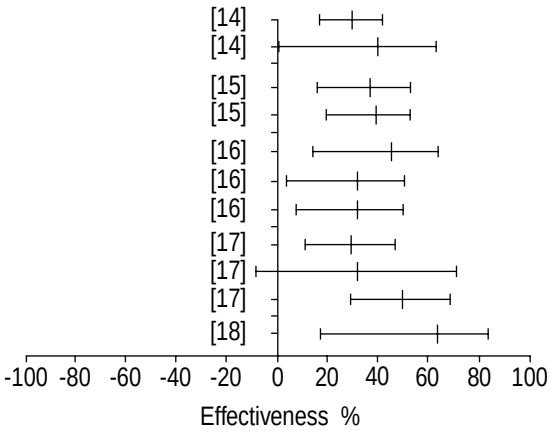

Fig. 2. – Influenza vaccine effectiveness in preventing pneumonia and influenza hospitalizations in the elderly. Data are presented as point estimates with 95% confidence intervals.

concluded that pulmonary function abnormalities may occur as a result of influenza vaccination, but the risks are very small and outweighed by the benefits of vaccination [31]. Guillain-Barré syndrome (GBS) was temporally associated with the 1976–1977 porcine influenza vaccination programme and the 1992–1993 and 1993–1994 seasons. An association between GBS and other vaccine programmes has not been established. Analysis revealed an attributable rate of vaccine-related GBS of one case per 100,000 vaccinations with A/NewJersey/76 vaccine. During 1992–1993 and 1993–1994, the overall relative risk of 1.7 (95% confidence intervals (CI) 1–2.8) for GBS was observed during the 6 weeks postvaccination [28]. This equates to one or two cases per million vaccinees. Persons with a history of GBS are at a higher risk of subsequently developing GBS than persons without such a history. Thus it seems prudent to avoid influenza vaccination in persons with a history of GBS, particularly those who are not at high risk for severe influenza complications or who developed GBS within six weeks of previous influenza vaccination [32].

Live cold-adapted reassortant vaccines

The highest annual attack rates for influenza occur in children and observations suggest that schoolchildren are central to the spread of influenza within the community [33, 34]. A 3-yr study of 3–5 yr old children found suboptimal antibody responses to inactivated influenza vaccine and little or no protection against infection [35]. A promising approach to the control of influenza is the use of live, cold-adapted (CA) reassortant vaccines. Attenuated CA strains suitable for use in children, are generated by genetic reassortment between a contemporary wild-type virus (which express current HA and NA antigens) and a CA donor of attenuation. Donors of attenuation are influenza A/Ann Arbor/6/60 (H2N2) and B/Ann Arbor/1/66. These donor strains have been extensively passaged at 25°C and 33°C (*i.e.* are cold-adapted), are temperature-sensitive (*i.e.* are unable to replicate at core body temperature), and attenuated. These properties are associated with polygenic mutations. CA reassortants have been evaluated in more than 10,000 subjects. They display high levels of phenotypic and genotypic stability and appear safe when administered to infants and children including those with asthma and cystic fibrosis. CA reassortants are not transmissible to close seronegative contacts, despite viral shedding for periods of up to 12 days [36–38].

Live attenuated vaccines are well tolerated, with minor upper respiratory symptoms including rhinorrhoea or sore throat developing in 5–10% of adults and children, and ~5% of healthy young children experience fever >37.6°C following live CA vaccination [39–42]. Excess systemic complaints including myalgia, headache and lethargy occur in 2–5% of healthy, mostly adult subjects, but fever is unusual [43]. Pulmonary function measurements and airway reactivity to methacholine remain unchanged in young healthy adults infected with CA reassortant vaccine [42, 44]. CA vaccines are well tolerated by elderly nursing home residents and patients with asthma, chronic obstructive airways disease or cardiac conditions [45, 46].

By mimicking infection with the wild-type virus, the CA vaccine delivers a larger dose of antigen to bronchial lymphoid tissue than the inactivated vaccine and offers the advantage of a broader immunological response. This includes higher or equal serum antibody (immunoglobulin (Ig)G, IgA, IgM) to both the HA and NA, greater neutralizing antibody and local mucosal secretory IgA responses than parenteral vaccine [37, 39, 47–50], and a reduction of the amount and frequency of viral shedding in comparison with the inactivated vaccine following infection [44].

The protective efficacy of CA reassortant vaccines against artificial challenge or naturally-occurring infection has been demonstrated in a large number of studies [36, 37, 39–41, 45–51]. In general, the level of protection has been comparable to that observed with inactivated vaccines. In a 5-yr study of 5,210 healthy children and adults aged 1–65 yrs, bivalent CA vaccine was no better than inactivated vaccine in preventing culture-positive influenza or a four-fold rise in antibody titres during the influenza season [43]. The efficacy of the CA vaccine against directed strains may be similar to the efficacy at preventing influenza that is antigenically matched to the strain in the vaccine [52]. The efficacy of the CA vaccine in protecting against a significantly drifted influenza A (H3N2) epidemic strain was demonstrated recently in a 2-yr multicentre, double-blind, placebo-controlled trial [39]. During the first year of the study, 1,314 healthy children aged 15–71 months received a 2-dose regimen of trivalent CA reassortment vaccine. An additional 285 children received one dose of vaccine or placebo. Systemic haemagluttination inhibition (HAI) antibody responses developed in 92% and 88% of seronegative children after the first dose of H3N2 and influenza B vaccines, respectively and 96% had antibodies after the second dose. However, only 16% developed antibodies to the first dose of H1N1 vaccine and 61% after the second. In year 1, among those receiving a single dose of the CA vaccine, the efficacy of the vaccine in preventing culture-confirmed

influenza was 87% (95% CI 47–97) for influenza A (H3N2) and 91% (95% CI 45–99) for influenza B. The efficacy after two doses was 96% (95% CI 90–99) for influenza A (H3N2) and 91% (95%CI 78–96) for influenza B. Vaccinees had 30% fewer episodes of febrile otitis media. In year 2, 1,358 children aged 26–85 months, returned for revaccination. Unlike the first year, when the vaccine strains closely antigenically matched the circulating viruses (A/Wuhan/359/95 and B/Harbin) the outbreak during year 2 consisted largely of a variant, A/Sydney, not contained in the vaccine. Nonetheless, the vaccine was 86% efficacious, and illness in vaccine failures was milder with fewer days of fever than in placebo-recipients [52]. It has been suggested that mass vaccination of 70% of children with the CA influenza vaccine could provide substantial protection to the community at large [53].

Other vaccines and vaccine developments

The serum HAI antibody response to split and subunit influenza vaccines is generally lower in older people. This decrease in the immune response can be explained partly by variations of prevaccination HAI titre, chronic ill health and possibly nutritional status. Some individuals may respond poorly because of an age-related decline in immune function.

One approach to boost immunity in the elderly has been to co-administer intranasal CA reassortant live vaccine and conventional vaccine given by injection. The efficacy of this approach was evaluated over three years by a double-blind, placebo-controlled study in which 523 residents of nursing homes all received trivalent inactivated vaccine parenterally and were then randomized to receive either H3N2 CA vaccine or placebo [54]. The overall occurrence of influenza A was low. Laboratory-confirmed influenza outbreaks occurred in three homes over 3 yrs. In these homes, subjects who received the CA vaccine had significantly lower rates of laboratory-documented influenza (9/162 *versus* 24/169; 61% efficacy, 95% CI 18–82) [54]. Following simultaneous CA and inactivated vaccination in seronegative young volunteers, serum HAI responses reached a greater magnitude as opposed to the inactivated vaccine only, and were sustained over 3 months [47].

Various adjuvants have been used with influenza vaccines in an attempt to enhance immunogenicity, but this has typically been associated with increased reactogenicity. Influenza vaccine containing MF-59, an oil in water emulsion with squalene as the oil component and sorbitan trioleate (an oil soluble surfactant), was licensed in Italy in 1997 [55]. Comparative studies involving 4,100 subjects who were randomized to receive MF-59 adjuvanted trivalent vaccine or commercial comparator vaccines reveal significant increases of 5–8% of subjects responding with four-fold increases in HAI antibody to H3N2, H1N1 and influenza B antigens adjuvanted with MF-59, in comparison with conventional vaccines, and the postvaccination geometric mean titres were 13–31% higher (A. Podda, Chiron Vaccines, personal communication). MF-59 has a dramatic effect on the antibody responses of immunologically-naïve subjects. A small comparative study of two doses (7.5 μg–30 μg) given on days 0 and 21 of conventional subunit H5N3 vaccine and vaccine adjuvanted with MF-59 found that the numbers who attained HAI titres of $\geqslant$1:40 or neutralizing antibody titres $\geqslant$1:20 were significantly higher on day 42 after the adjuvanted vaccine. (HAI 42% *versus* 3%: neutralizing antibody 94% *versus* 22%) [52].

Liposomes are lipid membrane particles that can serve as delivery systems for vaccine antigens and immunostimulators. Another type of liposome referred to as a virosome is created by inserting virus fusion proteins (the HA for influenza) into a liposome bilayer. A virosomal influenza vaccine has been licensed in Switzerland. Two small randomized trials have compared the immunogenicity of virosomal influenza vaccine with that of whole and subunit vaccines in the elderly. Improved antibody responses were noted with the virosomal formulation, which for some comparisons reached statistical significance [57, 58].

Because of the large number of embryonated hens' eggs required for the production of current commercially-available vaccines, advanced planning must start nearly a year before vaccination. The interval between identification of a new pandemic strain and outbreaks may be insufficient to produce vaccine using current technology. Moreover, in the event of a pandemic, vaccine production is unlikely to match global needs. For these and other reasons, the WHO has recognized an urgent need to develop cell culture techniques for influenza vaccine production. Vaccines prepared using Madin-Darby-Canine-Kidney (MDCK) and Vero cells (derived from human embryonic lung fibroblasts) have been given limited clinical trials. These cell culture systems offer the advantage of increasing vaccine production at short notice to meet unexpected demand or supplemental vaccine can be produced if a new antigenic variant is detected.

An additional alternative to egg-derived antigen involves the use of haemagglutinins expressed in insect cells by recombinant baculoviruses. Potential difficulties with these vaccines include the use of uncleaved rather than cleaved haemagglutinin and differences in glycosylation in insect as opposed to mammalian cells. None the less, the responses of adults to doses of 15–45 μg of recombinant H1 and H3 antigens is similar to the responses to licensed vaccines. There is a dose-related response with serum HAI and neutralizing antibody titres equal or significantly higher using 90–135 μg haemagglutinin than with subunit inactivated vaccine containing 15 μg of egg-derived HA [59–61]. Another recent approach has been the development of nucleic acid vaccines. Deoxyribonucleic acid (DNA) sequences encoding the gene for the antigenic protein of interest can be integrated into bacterial plasmids, grown, purified and then inoculated into the host. After being injected, the plasmid enters a host cell, remaining in the nucleus as an episome. Expression of the plasmid DNA produces the antigenic protein that, in turn, hopefully produces

an immune response. A protein produced by a plasmid-transfected cell is more likely to be folded in its natural configuration favouring neutralizing antibody formation and is more likely to be displayed on the surface by human leukocyte antigen (HLA) class I molecules inducing cytotoxic T cell responses [61, 63]. Experiments in mice have shown that influenza HA-specific IgG and IgA can be induced by both intramuscular and intranasal administrations of plasmid containing HA sequences, and further development is awaited with interest [64].

One of the problems associated with the use of current inactivated vaccines is the need to give repeated annual injections. The mucosal delivery of influenza vaccine would avoid the use of syringes and improve compliance. Relatively few antigens evoke humoral and secretory antibody responses when in contact with mucosal surfaces. Cholera toxin (CT) and *Escherichia coli* heat labile enterotoxin (LT) are potent mucosal immunogens and adjuvants in animal models. As a consequence of these properties, non-toxic LT mutants that retain adjuvant activity are being developed and clinical trials of such an adjuvanted vaccine are likely to proceed in the near future. Workers in Switzerland have recently evaluated a trivalent intranasal virosomal influenza vaccine with and without *E. coli* heat labile enterotoxin. Two spray inoculations given with an interval of 1 week induced a humoral response which was comparable to that with a single parenteral vaccination of virosomal vaccine containing the same total of influenza HA content [65]. In addition, a significantly higher induction of virus-specific IgA was noted in the saliva after two intranasal applications.

Vaccine recommendations

National authorities of many countries in Europe and North America recommend influenza vaccination for those >65 yrs of age; residents of nursing homes and other chronic care facilities that house persons of any age who have chronic medical conditions; adults and children with chronic cardiopulmonary conditions including asthma, and those with chronic medical conditions including metabolic disease and diabetes, renal dysfunction and immunosuppression [66]. Despite these guidelines, vaccine uptake rates remain variable and generally poor with $<20\%$ asthmatics vaccinated in the UK [67]. There may also be medical, social and economic benefits of preventing influenza in young children and the working population especially healthcare workers [68–70].

Antiviral therapy

M2 ion channel inhibitors

The adamantanes, amantadine and rimantadine were discovered in the 1960s to inhibit strains of influenza A. They are C-10 tricyclic primary amines that inhibit viral uncoating by their effects on the M2 ion channel. They inhibit human H1N1, H2N2, and H3N2 subtypes of influenza A. Nonhuman subtypes of influenza A are also sensitive, indicating that future human pandemic strains will also be susceptible. At therapeutic doses, they have no effect on influenza B or other respiratory viral pathogens [67]. Amantadine is well absorbed with concentrations in nasal mucus approximately half those in plasma. Amantadine is excreted renally and can cause minor central nervous system (CNS) adverse effects such as insomnia, dizziness and lowered seizure threshold, particularly in the elderly and those with impaired renal function [72]. It is contraindicated in severe renal impairment and patients with epilepsy, and should be used in caution in elderly patients in whom renal function is likely to be impaired, *i.e.* many of those in the higher risk groups for influenza. Amantadine is extensively metabolized by the liver ($\sim 65\%$) and kidney (20%) and is also excreted as unchanged drug by the kidney. Rimantadine concentrations in nasal mucus are ~ 1.5 times greater than in plasma. Comparative studies indicate that rimantadine is better tolerated than amantadine at equivalent doses.

Both drugs are effective for the treatment of acute influenza A infection, if commenced within 24 h of illness onset, reducing fever and symptoms by 1–2 days and allowing earlier return to work [71, 73–75]. Chemoprophylaxis taken daily during influenza season reduces infection rates by 50–90% over the outbreak period [72, 76–78]. Administration of a drug as prophylaxis and treatment to nursing home residents during outbreaks of influenza within the facility may interrupt transmission but has led to the emergence and transmission of drug-resistant strains. Postexposure prophylaxis of household contacts prevents influenza A illness by $\sim 70\%$, but drug resistance may emerge rapidly and render prophylaxis ineffective if the index case is also treated simultaneously [79]. The genetic basis for resistance appears to be single amino acid substitutions at positions 26, 27, 30, 31 or 34 in the transmembrane portion of the M2 ion channel [80]. Amantadine-resistant viruses are resistant to rimantadine and *vice-versa*.

Neuraminidase inhibitors

The enzyme neuraminidase (NA) (sialidase) is a tetramer composed of a cytoplasmic tail, transmembrane domain, stalk region and globular head. Sialic acid is an integral part of the influenza virus receptor. The active site of NA lies within the head and is a highly conserved region among influenza A and B viruses [81]. Because of its essential role in viral replication, NA represents a potential target for antiviral therapy. By analysing its crystallographic structure and interaction with sialic acid, selective reverse competitive inhibitors have been developed [82]. There are two licensed drugs: zanamivir and oseltamivir and a third, RWJ-270201 is in Phase II clinical studies. All three compounds are highly active against a broad range of influenza A of human and avian origin and B viruses, including all nine NA subtypes in the avian reservoir. They tend to be less active *in vitro* against influenza B than influenza A

neuraminidase. All three compounds inhibit amantadine and rimantadine resistant strains of influenza A.

Zanamivir has poor oral bioavailability and consequently has been administered topically. The licensed formulation is administered as a dry-powder aerosol. This may pose practical difficulties in the very young, frail or confused patients, although 74% of nursing home residents in one study had no difficulty with compliance [83]. In healthy volunteers, a mean of 13% (range 4–21%) of the 10 mg dose from the Diskhaler™ device is deposited in the bronchi and lungs and 78% is deposited in the oropharnynx [84]. Deposition studies have not been conducted in subjects with influenza or patients with obstructive airways disease. Nonetheless, the data from the lung deposition studies suggest that the concentration of zanamivir throughout the airways is far in excess of the median inhibitory concentration (IC50) values for zanamivir against the viral neuraminidases. Zanamivir remains detectable in sputum for as long as 24 h after dosing [85]. After oral administration, a median of 10–20% of the dose is systemically absorbed. Approximately 90% of intravenously administered zanamivir is excreted unchanged in the urine. Given the wide safety margin of zanamivir the possible increased exposure to zanamivir in patients with renal failure does not indicate a reduction in dosage during administration by oral inhalation. Oseltamivir ethyl ester prodrug is well absorbed with bioavailability in excess of 80% and metabolized to the active oseltamivir carboxylate. Animal studies suggest good distribution throughout the respiratory tract.

Clinical efficacy

Both zanamivir and oseltamivir have been found to be effective in prevention and treatment of experimental influenza A infection in healthy volunteers [86–88]. Intravenous zanamivir was found to significantly reduce the cytokine and chemokine proinflammatory response seen in influenza infection [89].

Acute treatment in naturally acquired influenza

Treatment studies are summarized in table 1 and discussed here.

Zanamivir. A double-blind, placebo controlled study of aerosolized zanamivir, with or without intranasal delivery, was conducted during the 1994–1995 season in North America and Europe [90]. Four-hundred and seventeen adults presenting within 48 h of onset of influenza-like symptoms were enrolled, with 262 (63%) having confirmed influenza infection. Overall, there was a reduction of 1 day (20%) in the median duration of time to alleviation of symptoms. Among those infected patients who were febrile at enrolment ($>38^{\circ}C$) and began treatment within 30 h of onset of symptoms, there was a 40% reduction in the median time to alleviation of symptoms from 4–7 days. Patients who were afebrile at time of enrolment and those who began treatment beyond 30 h after onset of symptoms had no significant benefit. The antiviral spectrum and clinical effectiveness of zanamivir included both influenza A and B virus infections. The Southern Hemisphere group recruited 455 adults into a double-blind, placebo-controlled trial presenting within 36 h of onset of influenza-like symptoms in Australia, New Zealand and South Africa [91]. Seventy-one per cent of the patients had laboratory confirmed influenza infection (67% influenza A and 33% influenza B). Self-administered inhaled zanamivir was used for a period of 5 days following enrolment. There was a mean reduction of 1.5 days in the median time to alleviation of symptoms for influenza-positive patients and a reduction of 2 days for those febrile ($>37.8^{\circ}C$) at the time of treatment. For afebrile patients, no benefit was observed and there was no difference between influenza A and B virus infections.

Table 1. – Summary of trials in treatment of naturally acquired or experimentally induced influenza infection

Reference	Therapy and duration	Patients (proved influenza)	Age yrs	Time to treatment*	Reduction in days to relief of symptoms
[90]	Zanamivir inhaled 10 mg *b.i.d.* for 5 days	417 (63%)	>13	<48 h	1 day (2 days if <30 h and >38°C)
[91]	Zanamivir inhaled 10 mg *b.i.d* for 5 days	455 (71%) 76 'high risk'	>12	<36 h	1.5 days (high risk group 2.5 days and reduced complication and antibiotic use)
[92]	Zanamivir inhaled 10 mg *b.i.d* for 5 days	777 (66%) 109 'high risk'	⩾12	<48 h <36 h	1 day 1.5 days
[93]	Zanamivir inhaled 10 mg *b.i.d* for 5 days	356 (78%)	>12	<48 h	2.5 days (reduced antibiotic use and complication rates)
[94]	Oseltamivir 75 or 150 mg *b.i.d* for 5 days	629 (60%)	18–65	<36 h and 38°C	1.4 days. (returned to normal activity 2–3 days earlier; reduction in sinusitis by 50%)
[95]	Oseltamivir 75 or 150 mg *b.i.d* for 5 days	726 (66%)	18–65	<36 h <24 h	1.2–1.5 days 1.9 days (Oseltamivir: better symptom scores and less viral shedding)

*: Time after onset of symptoms to receipt of drug.

A small subgroup of high risk patients (n = 76), primarily those with asthma, >65 yrs of age or with underlying metabolic or cardiac diseases, was included. This group had a longer duration of illness than the general population of the study with median time to alleviation of symptoms of 8 days as compared with 6 days in the placebo "low risk" population. There was a median reduction of 2.5 days to alleviation of symptoms in the high risk subgroup when treated with zanamivir. There was a 33% reduction (p = 0.004) in complications such as bronchitis and sinusitis and 25% reduction (p = 0.025) in antibiotic use in those high risk patients receiving zanamivir [91]. A further double-blinded, placebo-controlled study of zanamivir recruited a total of 777 patients including 109 high risk patients within 48 h of onset of influenza-like symptoms [92]. Sixty-six per cent of patients enrolled had laboratory confirmed influenza. Zanamivir showed a clinically and statistically significant benefit with a 1-day reduction in the time to alleviation of symptoms in the healthy group and a 1.5-day reduction in the high risk group if administered within 36 h of onset of symptoms. Overall, there was a 31% reduction (p = 0.049) in the complications of influenza and faster alleviation of fever [92]. Another placebo controlled trial of 356 patients (78% confirmed influenza infection) found that inhaled zanamivir received within 48 h of onset of symptoms provided a 2.5-day reduction in time to alleviation of symptoms from 7.5 – 5 days [93]. Pooled analysis of studies totalling 2,235 patients treated with inhaled zanamivir found an overall 5% reduction from 18 to 13% (p = 0.006) of antibiotic prescriptions in influenza-positive patients [96].

Oseltamivir. Similar benefits have been seen with oseltamivir when used within 36 h of onset of symptoms. A double-blind, placebo-controlled trial over the 1998 season recruited 629 healthy adults presenting with influenza-like symptoms of which 374 (60%) were proved influenza-positive [94]. These patients were randomized to placebo or 75 mg *b.i.d.* or 150 mg *b.i.d.* of oseltamivir. For those with fever >38°C and presenting within 36 h of onset of symptoms, the median duration of time to alleviation of symptoms was 1.5 days (30% reduction), severity of illness as judged by patient score cards was reduced by 38% and there was earlier return to normal activities by 2 – 3 days. There was a reduction in incidence of bronchitis and sinusitis from 15 to 7% (p = 0.03) complicating influenza infected patients in those receiving oseltamivir. No dose difference between the oseltamivir treatment arms was noted [94]. A larger double-blind, placebo-controlled study of 719 adult patients in Canada, Europe and China tested the efficacy of oseltamivir 75 or 150 mg *b.i.d.* for 5 days within 36 h of onset of symptoms [95]. Four hundred and seventy-five patients (66%) were confirmed to have influenza infection. The duration of illness was reduced by 29 h (25% p = 0.02) and 35 h (30% p = 0.01) with 75 and 150 mg *b.i.d.*, respectively. If treatment was initiated within 24 h of onset of symptoms, duration of illness was shortened by 43 h (37% p = 0.02) and 47 h (40% p = 0.01) with 75 and 150 mg, respectively, compared with placebo. Patients who received oseltamivir had significantly lower viral titres in nose and throat swabs without impairing humoral (haemagglutinin inhibition) immune response to infection [95].

Prevention

A summary of seasonal and postcontact prophylaxis studies is shown in table 2. A 4-week study of 1,107 subjects over the 1997 (H3N2) influenza season concluded that 10 mg inhaled zanamivir once per day was 84% efficacious (95% CI 39% – 83%) in preventing febrile laboratory-confirmed influenza infection [97]. No significant adverse effects were associated with drug prophylaxis.

A large study of 1,559 adults found that 75 mg oseltamivir, given orally either once or twice daily over 6 weeks of the winter 1997 – 1998, revealed a 74% reduction in laboratory proven cases of influenza [98]. In the area where the influenza attack rate was highest, oseltamivir was 82% efficacious in preventing clinical influenza infection. Both once-daily and twice-daily administration of oseltamivir were effective against influenza infection. There was a higher rate of gastrointestinal side effects in those receiving oseltamivir, although the frequency of drug discontinuation was similar in both treatment and placebo groups [98]. In a study of 548 mainly prevaccinated frail elderly nursing home residents, once daily oseltamivir provided further protective efficacy of 92% against influenza infection and a reduction in

Table 2. – Summary of seasonal and post contact prophylaxis with neuraminidase inhibitors

Reference	Prophylaxis treatment	Duration	Subjects n	Age yrs	Protective efficacy and comments
[97]	Zanamivir 10 mg inhaled *b.i.d*	4-week seasonal over outbreak	1107	18 – 69	67% against laboratory-confirmed influenza infection, 84% against clinical illness.
[98]	Oseltamivir 75 mg once daily or *b.i.d*	6/52 seasonal study	1559	18 – 65	74% against laboratory-confirmed influenza infection, no difference between *once daily and b.i.d.*, no emergence of resistance.
[99]	Oseltamivir 75 mg once daily	6/52 seasonal study in nursing home	548	Mean of 82	92% in prevaccinated group (80%) reduction in complications.
[100]	Oseltamivir 75 mg once daily	Household contacts 5/7	377 households	Healthy adults	82%

complications [99]. A postcontact prevention study of once daily oseltamivir for 5 days in healthy family members of 377 households reduced the likelihood of contacts developing influenza virus infection by 82% [100].

Use in special groups

There are limited data of neuraminidase inhibitors in the elderly and patients with high risk conditions including underlying cardiorespiratory disease. A small number of high risk patients included in two treatment studies of zanamivir were shown to have significant symptom benefit and reductions in complications and prescribed antibiotic treatment [91, 92]. Seasonal oseltamivir prophylaxis in elderly nursing home residents did not cause adverse events and reduced risk of influenza by 92% [99]. Results from studies of NA inhibitors in the immunocompromised, infants, and the frail elderly are awaited. No animal tetratogenicity has been reported but neuraminidase inhibitors pass through the placenta and also into breast milk and their use is best avoided in pregnancy unless there is life-threathening infection for the mother.

Resistance

Influenza viruses with reduced susceptibility to NA inhibitors have been isolated following sequential tissue culture passage of the virus in the presence of drugs. Two mechanisms of resistance have been identified. Firstly, resistant viruses may involve multiple mutations clustered close to or around the binding site of the viral haemagglutinin with its sialic acid receptors, thereby reducing efficiency of virus binding and decreasing dependence on NA function. Resistance may also arise from single amino acid substitutions in the highly conserved active site of neuraminidase in the central cleft of the global head [101]. There are differences in cross-resistance, *e.g.* substitution at position 119 leads to zanamivir resistance but maintained susceptibility to oseltamivir and RWJ-270201 [102]. NA mutant variants replicate in cell culture, but have reduced virulence in animal models [103]. Emergence of clinical resistance requires further study but appears rare. A study of over 600 samples collected from oseltamivir-treated patients found five resistant variants by genotypic and neuraminidase assays, but these patients did not have a poorer clinical outcome [104]. Attempts to select *in vitro* influenza B mutants resistant to NA inhibitors have failed. However a zanamivir resistant influenza B virus was recovered from a bone-marrow transplant recipient following two weeks of treatment [105].

Adverse effects

Most clinical trials of zanamivir and oseltamivir were performed in healthy volunteers and the drugs have low acute toxicity. In zanamivir treatment and prevention studies, minor adverse events such as nausea, diarrhoea, headache and cough occurred in $<3\%$ of recipients, and the frequency of such events was similar between placebo and drug arms [90–93, 97]. There have been a number of post marketing reports of transient acute bronchospasm and reductions in spirometric lung volumes in some patients [106]. However, more serious deteriorations in respiratory function have been reported in some patients with pre-existing respiratory disease [107].

Oseltamivir has been well tolerated with no serious adverse effects reported in clinical treatment or prophylaxis trials. It is associated with mild and transient gastrointestinal disturbance during the first two days of administration. In clinical trials, dropout rates were low and similar between treatment and placebo arms [95, 96, 98–100].

Current status

Neuraminidase inhibitors offer advances in treatment and chemoprophylaxis over M2 inhibitors due to their broader antiviral spectrum, proven efficacy with reduction of symptoms and complications, less potential for emergence of clinically resistant strains and for combating emerging influenza strains such as avian H5 and H9 for which vaccination is unavailable [108]. There may also be benefits in terms of delaying the emergence of drug resistance by decreasing unnecessary prescriptions [91, 94]. There is however, a need to assess direct comparative studies between the drugs available, and to consider the effect of combination treatment.

Several concerns have been raised regarding the prescription of NA inhibitors. Recommendations for their use in symptomatic patients of all age groups could theoretically overwhelm primary care physicians during outbreaks of influenza and other pathogens such as respiratory syncytial virus (RSV). Moreover, the lack of a simple, inexpensive, rapid, sensitive and specific "bedside" test for identifying influenza could result in considerable unnecessary and inappropriate use. In addition, unexpected adverse effects such as possible exacerbations of pre-existing respiratory disease are always a potential problem with new therapies as they become more widely used. Delivery issues and compliance with intranasal administration in the frail elderly and young children may be problematic. Although no clinical resistance appeared in trial settings, careful surveillance is required, particularly in populations in whom delivery and compliance are suboptimal.

From a healthcare provider′s perspective, the principal issue is whether use of NA inhibitors, in comparison with other healthcare interventions, is financially worthwhile in all symptomatic patients, in specific groups (*e.g.* high risk groups), or none at all. A formal health-economic study is needed to address these concerns [109].

It remains to be seen if the neuraminidase inhibitors fulfil their clinical potential. It should not be forgotten that "prevention is better than cure" and that the appropriate use of vaccines remains as priority.

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