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Mini-review

# Antivirals for influenza: Historical perspectives and lessons learned

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

**Abstract**

The development of the currently available classes of antivirals, the M2 proton channel inhibitors and the neuraminidase inhibitors, provides valuable perspectives relevant to the field of antiviral chemotherapy in general and insights into aspects of viral pathogenesis and antiviral resistance relevant specifically to influenza. The efficacy observed with these antiviral drugs has proven the importance of these antiviral targets, as well as the principle that chemoprophylaxis and early treatment are possible in influenza infections with small molecular weight inhibitors.


*Keywords:* Influenza; Neuraminidase; Amantadine; Oseltamivir; Zanamivir; Pathogenesis; Antiviral resistance

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## 1. Introduction

The use of antiviral agents for influenza is receiving much greater attention in part because of the threat of avian influenza virus infections and because of stockpiling decisions for their application in pandemic response (Hayden, 2004, 2001) and perhaps containment (Ferguson et al., 2005; Longini et al., 2005). In general, the development of drugs for influenza has been slow and restricted in scope relative to that for other viral infections, in part because of lack of commercial opportunities. However, the development of the currently available classes of antivirals, the M2 proton channel inhibitors (amantadine and rimantadine) and the neuraminidase inhibitors (zanamivir and oseltamivir), provides valuable perspectives relevant to the field of antiviral chemotherapy in general and insights into aspects of viral pathogenesis and antiviral resistance relevant specifically to influenza. The efficacy observed with these antiviral drugs has served to establish the importance of these antiviral targets, as well as the principle that chemoprophylaxis and early treatment are possible in influenza infections with small molecular weight inhibitors

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[reviewed in Hayden and Aoki, 2005a,b; Moscona, 2005]. The following commentary considers selected aspects of these two antiviral classes with a focus on studies performed at the University of Virginia and by our collaborators at other institutions.

## 2. M2 inhibitors

Amantadine and its cogener rimantadine were identified by traditional biologic screening assays in the early 1960s and shown to be inhibitory for influenza A viruses in cell culture and animal models (Davies et al., 1964; Tsunoda et al., 1965). Amantadine was taken forward into clinical development in the United States, where it was initially approved for A/H2N2 infections in 1966 and subsequently all influenza A infections in 1976, whereas rimantadine was developed and utilized widely in the former Soviet Union before its approval in the United States in 1993 (Hayden and Aoki, 2005a). Controversy regarding the magnitude of amantadine's effectiveness (Sabin, 1967, 1978) impacted its uptake. Interestingly, the anti-Parkinsonian effects of amantadine were discovered serendipitously during a course of prophylaxis for influenza (Schwab et al., 1969).

### 2.1. Pathogenesis

In contrast to neuraminidase inhibitors (NAIs, see below), the ability of M2 inhibitor treatment to prevent influenza complications has not been rigorously proven in prospective studies. In experimentally infected volunteers, early rimantadine treatment reduced viral replication and illness but not objective measures relating to middle ear alterations, including otoscopic and middle ear pressure abnormalities (Doyle et al., 1998). These findings predicted an observed lack of efficacy of rimantadine in preventing otitis media in influenza-infected children (Hall et al., 1987), whereas oseltamivir treatment was subsequently found to reduce influenza-associated otitis media in children (Whitley et al., 2001).

Differences in the risk of central nervous system (CNS) complications contributed to the decision by Russian scientists to select rimantadine as the preferred drug. Subsequent studies in the United States confirmed the lower risk of CNS toxicity with rimantadine (Hayden et al., 1981) and established that this was related to differences in pharmacokinetics between the drugs (Hayden et al., 1983). These findings highlighted the fact that relatively subtle differences in chemical structure can result in important differences in human pharmacology and tolerance profiles.

### 2.2. Antiviral resistance

Phenotypic resistance to amantadine was detected in cell culture studies shortly after its discovery (Cochran et al., 1965), and subsequent studies showed the ease of selecting resistant variants in vivo (Oxford and Logan, 1970). The study of the genetic basis of resistance, ultimately shown to be linked to single nucleotide changes and corresponding single amino acid substitutions in the trans-membrane of the M2 ion channel protein, was critical to understanding amantadine's mechanism of antiviral action (Hay, 1996). Pre-clinical studies established that emergence of pre-existing subpopulations of resistant variants occurred readily with selective drug pressure and that in vitro resistance was high-level and conferred loss of antiviral activity in vivo with cross-resistance to other compounds in the class. Clinical studies in the 1980s found that resistant variants emerged rapidly in rimantadine-treated children and adults (Hall et al., 1987; Hayden et al., 1991), that the resistant variants were as fit as drug-susceptible, wild-type virus in animal models (Abed et al., 2005; Sweet et al., 1991), and that they were transmissible from person-to-person under close contact conditions in households (Hayden et al., 1989) and nursing homes (Mast et al., 1991). Furthermore, these resistant variants were able to cause typical influenza illness in persons on chemoprophylaxis. Modeling studies predicted that resistant variants could disseminate widely in closed populations when the M2 inhibitors were used for treatment and prophylaxis (Stilianakis et al., 1998). Subsequent studies have found high frequencies of resistance emergence in immunocompromised hosts (Englund et al., 1998; Klimov et al., 1995) and hospitalized children (Shiraishi et al., 2003) treated with M2 inhibitors, as well as apparent de novo or primary resistance in an increasing proportion of human isolates (Bright et al., 2005) and one lineage of avian A/H5N1 viruses in Vietnam, Thailand, and Cambodia in 2004 (Hien et al., 2004). Recently a particular resistance mutation (Ser31Asn) has been detected in over 70% of community A/H3N2 isolates in China and Hong Kong in 2004–2005 (Bright et al., 2005) and in over 90% of such isolates in North America in late 2005 (Bright et al., 2006). Such findings have demonstrated the potential for global spread of M2 inhibitor-resistant influenza A in the absence of substantial selective drug pressure viruses and emphasized the need for alternative antiviral agents.

## 3. Neuraminidase inhibitors

In contrast to the M2 inhibitors, the development of inhibitors directed against influenza neuraminidase (NA) is one of stepwise scientific achievements over nearly six decades that culminated in a process of structure-based drug design leading to synthesis of selective, potent inhibitors (Table 1). Indeed, the development of NA inhibitors is one of the first examples of so-called rationale drug design. These drugs entered into clinical practice in 1999, and oral oseltamivir has quickly become the principle drug of choice for treating influenza and pandemic stockpiling (Hayden and Aoki, 2005b; Moscona, 2005).

### 3.1. Pre-clinical development

Following Hirst's description of a putative viral enzyme, F. MacFarlane Burnett and co-workers at the Walter and Eliza Hall Institute in Melbourne showed that the actions of the putative viral enzyme on erythrocytes were reproduced by an enzyme from *Vibrio cholerae*, which was termed receptor-destroying enzyme (RDE), and that the targets of both the viral enzyme and RDE included a variety of mucopolysaccharides (Burnet and Stone, 1947). In addition, various mucins, including crude human respiratory mucus, inhibited the hemagglutination prop-

Table 1
Milestones in development of neuraminidase inhibitors

| |
|---|
| Viral attachment to erythrocyte receptors (hemagglutination) altered by putative viral enzymatic activity (Hirst, 1942) |
| Receptor-destroying enzyme (RDE) from *Vibrio cholerae* culture filtrates causes loss of cellular receptors (Burnet and Stone, 1947) |
| Respiratory mucus inhibition of influenza hemagglutination action/infectivity reversible by exogenous RDE (Burnet et al., 1947; Burnet, 1948) |
| Topical RDE reduced influenza infection in mouse lung (Anderson et al., 1948) |
| Characterization of sialic acid-bearing receptors (Gottschalk, 1958) |
| Anti-NA antibody inhibits viral release from infected cells (Seto and Rott, 1966) |
| First neuraminidase inhibitor Neu5Ac2en described (Meindl and Tuppy, 1969) |
| Inhibition/inactivation of NA leads to aggregation of virus at cell surface (Palese et al., 1974; Palese and Compans, 1976) |
| Crystal structure of influenza A NA solved (Colman et al., 1983) |
| Crystal structure of NA complexed with its natural substrate sialic acid reported (Varghese et al., 1992) |
| Crystal structure of influenza B NA complexed with sialic acid reported (Burmeister et al., 1992) |
| First potent, selective NAI (zanamivir) described (von Itzstein et al., 1993) |
| First potent, orally bioavailable NAI (oseltamivir) described (Kim et al., 1997) |

erties of heat-inactivated virus (lacking intrinsic neuraminidase activity), and this inhibition could be overcome by addition of exogenous RDE. Furthermore, application of RDE to the tracheobronchial tree of mice inhibited influenza viral replication. This led the group to write "Theoretically there are several possible ways in which a prophylactic or therapeutic effect could be developed on the basis of these conceptions. (1) Cell receptors in the respiratory tract might be removed by regular inhalation of RDE or other destructive agent, (2) an effective "competitive poison" for the virus enzyme might be similarly administered which, when deposited on the mucous film lining the respiratory tract would render this an effective barrier against infection, both initial infection from without and the spreading surface infection of the mucosa which follows the initiation of infection." (Anderson et al., 1948). Over three decades after these seminal observations, the detailed crystal structure of influenza neuraminidase was reported in 1983 (Colman et al., 1983), and this in turn provided the foundation for the development of selective, potent inhibitors of its action.

### 3.2. Pathogenesis

The clinical studies of the NAIs has provided not only evidence of their efficacy [reviewed in Hayden and Aoki, 2005b; Moscona, 2005] but also informed our understanding of important aspects of influenza pathogenesis. Initial volunteers studies, in which susceptible adults were inoculated intranasally with virus, established that intranasal zanamivir by drops or coarse spray was protective against infection and to greater extent illness (Hayden et al., 1996). The protection against laboratory-documented infection with administration of the drug before experimental virus exposure provided suggestive evidence that viral NA was important in allowing initial infection of the respiratory epithelium in vivo, perhaps by preventing inactivation and clearance of virus by respiratory secretions, and that inhibition of NA activity could block initiation of infection. This has been documented recently in cell culture experiments (Matrosovich et al., 2004). In addition, the findings are indicative of the requirement for NA activity in promoting sustained viral replication in humans and reinforce the value of inhibiting this target.

However, naturally acquired influenza commonly involves the lower respiratory tract when clinically manifested. In addition, there has been uncertainty regarding the relative importance of different levels in the respiratory tract (nose, throat, tracheobronchial tree, distal airways, and alveoli) as the sites of both initial infection and subsequent replication. Consequently, the initial human trials with topical zanamivir were designed in part to address the importance of drug delivery to the nasopharynx and/or lower airways. Placebo-controlled, blinded studies in North America and Europe in the 1994–1995 season compared two methods of zanamivir administration in previously healthy adults with suspected acute influenza: oral inhalation of dry powder using a commercial Diskhaler device and intranasal sprays of an aqueous solution (Hayden et al., 1997). Among the 62% of participants who had influenza virus infection documented by laboratory studies (56% A/H3N2, 44% B), the time to alleviation of illness was significantly shorter for those receiving inhaled zanamivir alone or inhaled plus intranasal zanamivir compared to placebo. Of note, greater symptom relief was observed in those treated early (within 30 h of illness onset) and those with febrile illness, but no important difference was observed between the inhaled and inhale/intranasal groups. These findings, confirmed in subsequent studies, indicated the importance of early treatment in uncomplicated influenza, the value of using fever as a discriminator for diagnosis and management in adults, and the critical importance of drug delivery to the lower respiratory tract. In addition, they reinforced the principle that antiviral administration well after symptom onset can provide meaningful illness modification.

In this study, intranasal but not orally inhaled zanamivir reduced nasal viral titers and nasal symptoms. Subsequent work has shown that intranasal but not inhaled drug appears to reduce upper respiratory tract complications (Kaiser et al., 2000b) and that treatment with inhaled zanamivir, in contrast to oral oseltamivir (Longini et al., 2004), does not appear to diminish the likelihood of transmitting virus to close contacts in household-based studies (Halloran et al., 2006). Other studies testing the protective efficacy of topically applied antivirals against natural influenza found that orally inhaled but not intranasal zanamivir was highly effective for prophylaxis (Hayden et al., 2000; Kaiser et al., 2000a; Monto et al., 1999). Similarly, studies of intranasal interferon (IFN)-alfa2 found partial protection against experimental intranasal influenza virus challenge but no reductions in naturally acquired influenza illness (Douglas et al., 1986; Hayden et al., 1986). Such intervention studies indicate that antiviral administration to the nose is insufficient to protect against natural influenza and support the hypothesis that many influenza infections are acquired by aerosols that are deposited in the lower airways or perhaps pharynx.

Another aspect of human influenza that has been partially elucidated by studies of antivirals is the role of host pro-inflammatory cytokines and chemokines in symptom pathogenesis. In experimentally infected volunteers, the nasal levels of several mediators (IL-6, IFN-alfa, IFN-gamma, and TNF-alpha) increase following infection and correlate with both measures of illness and of viral replication (Fritz et al., 1999; Hayden et al., 1998). Administration of intravenous zanamivir (Calfee et al., 1999) starting before viral inoculation or oral oseltamivir initiated 28 h afterwards (Hayden et al., 1999) either prevented or quickly reduced viral replication, respectively, and were associated with corresponding reductions in both nasal mediators responses and illness measures. Such observations help to establish a fundamental linkage between influenza viral replication, host pro-inflammatory innate immune responses, and symptom production that can be blocked by effective antiviral intervention. Vigorous cytokine and chemokine responses have been observed in uncomplicated human influenza (Fritz et al., 1999; Hayden et al., 1998; Kaiser et al., 2001), and some evidence indicates that avian H5N1 infections in humans are associated with dys-regulated and perhaps excessive mediator responses (Peiris et al., 2004; The Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza, 2005). It remains to be proven that early antiviral treatment can mitigate these responses, but the limited available evidence points to effective control of replication as being essential.

### 3.3. Antiviral resistance

Primary resistance to the neuraminidase inhibitors among clinical isolates has not been described in enzyme inhibition assays (McKimm-Breschkin et al., 2003), and these agents are active against all of the nine neuraminidase subtypes recognized in avian influenza viruses. In vitro selection of variants with neuraminidase resistance mutations typically requires prolonged passage, and these variants usually show reduced enzyme activity or stability and infectiousness in animal models compared to wild-type susceptible viruses (Carr et al., 2002; Le et al., 2005; Yen et al., 2005). Some oseltamivir-resistant variants detected in the clinic (Arg292Lys in N2) but not others (His274Tyr in Nl, Glu119Val in N2) show reduced transmissibility in ferrets (Herlocher et al., 2004). Zanamivir retains full activity against most but not all oseltamivir-resistant variants (Mishin et al., 2005).

Treatment with NAIs is associated with a variable frequency of resistance emergence (Jackson et al., 2000). To date only one instance of zanamivir resistance in an immunocompromised host has been documented (Gubareva et al., 1998), and no resistance has been found in immunocompetent persons receiving zanamivir (Barnett et al., 2000; Hayden et al., 2000). The frequency of recovering resistant variants is higher with oseltamivir therapy, particularly in children (5–18%) than adults (<1%) (Jackson et al., 2000; Kiso et al., 2004; Whitley et al., 2001). However, clinical variants are generally detected late in therapy and are not associated with clinical deterioration, except in immunocompromised patients (Ison et al., 2006) and possibly A/H5N1-infected persons (de Jong et al., 2005). In contrast to the experience with M2 inhibitors, either inhaled zanamivir or oral oseltamivir used for combined treatment and post-exposure prophylaxis in families is highly effective and not associated with resistance emergence (Hayden et al., 2000, 2004). Most NA mutations conferring resistance to NAIs appear to alter the fitness of influenza viruses and their transmissibility, which suggests that resistance will be much less likely to be a threat during drug use in seasonal (Ferguson et al., 2003; NISN, 2005) or pandemic influenza, but continued surveillance is essential (Zambon and Hayden, 2001).

### 3.4. Antiviral combinations

The frequent emergence of antiviral resistance during oseltamivir treatment and its association with clinical failure in immunocompromised hosts (Ison et al., 2006) and H5N1-infected patients (de Jong et al., 2005) has highlighted the need for alternative therapies and consideration of using antiviral combinations. The concept of using two or more antiviral for influenza to enhance antiviral effects and perhaps reduce resistance emergence is decades old (Hayden, 1986; Hayden et al., 1980; Lavrov et al., 1968). Recent studies have shown that combinations of M2 inhibitors and NAIs show enhanced antiviral activity in vitro (Govorkova et al., 2004) and in animal models (Leneva et al., 2001) for amantadine-susceptible viruses. Such combinations have received limited study in human influenza (Ison et al., 2003), and further controlled studies of promising combinations that might enhance efficacy and reduce resistance emergence, including dual NAIs or perhaps an NAI with a transcriptase inhibitor like ribavirin, that has activity against both influenza A and B viruses, warrant investigation.

## 4. Summary

The antiviral and clinical activities observed with M2 inhibitors and NAIs have established the importance of these antiviral targets and the principle that antiviral prophylaxis and treatment are effective in human influenza infections. Studies of these antivirals in experimentally induced and naturally occurring influenza have provided useful insights into illness pathogenesis. These include the linkage between viral replication, pro-inflammatory cytokine responses, and symptoms and the finding that nasal antiviral administration is insufficient to protect against natural influenza, an observation indicating that influenza infections are initiated often in the lower airways or perhaps pharynx. The recent widespread circulation of M2 inhibitor-resistant influenza A(H3N2) viruses highlights the importance of antiviral resistance emergence. Resistant variants have also been detected in oseltamivir-treated patients, sometimes in association with apparent clinical failure in immunocompromised hosts or A(H5Nl)-infected patients. The reduced fitness of most such variants indicates different public health implications than with M2 inhibitors, although continued antiviral susceptibility monitoring is essential. Studies of antiviral combinations that might enhance therapeutic efficacy and reduce resistance emergence are needed.

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