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Recent Progress in Anti-Influenza Chemotherapy

Shiro Shigeta

Department of Microbiology, School of Medicine, Fukushima Medical University, Fukushima, Japan

Abstract

Influenza virus infections in high risk individuals, such as infants, the elderly, and patients with cardiopulmonary disorders or immunocompromised states, cause severe manifestations which often result in fatalities. The emergence of a new antigen type of influenza A virus (H5N1) in Hong Kong during 1997 and 1998 threatened a possible pandemic of a new influenza infection.

The investigation for anti-influenza chemotherapies has progressed in the last decade whereas clinical trials of new compounds have been limited to amantadine, rimantadine and ribavirin. Fusion inhibitors which directly inhibit conformational change of haemagglutinin (HA), protease inhibitors which inhibit cleavage of HA to HA1 and HA2, RNA transcription inhibitors which inhibit cap formation of mRNA and antisense oligonucleotides targeted at mRNA of PB2 (a part of viral RNA polymerase) have been reported, in their development phases.

Recently, 2 neuraminidase (NA) inhibitors, zanamivir and oseltamivir (GS 4104), were used in clinical trials for the treatment of patients with influenza. Both agents showed promising results. A polyoxometalate, PM-523, inhibits fusion between the virus envelope and cell membrane and inhibits the penetration of the virus into cells. This compound has shown potent anti-influenza activity and synergistic inhibitory activity in combination with ribavirin or zanamivir *in vitro* and *in vivo*.

Resistant strains for zanamivir, oseltamivir or PM-523 have been isolated. The analysis of mutation points of these strains have contributed to the investigation of the antiviral mechanisms of action of these compounds and the mechanism of resistance of the mutants to these compounds.

1. Clinical Importance of Anti-Influenza Virus Chemotherapy

Influenza is a highly contagious acute respiratory illness. Epidemics of influenza are characterised by a sudden appearance in the winter season, worldwide spread and then a sudden disappearance. Numerous epidemics have been recorded from ancient times; among these, the 1918-1919 pandemic was particularly severe (the Spanish influenza). After the Spanish influenza pandemic, 3 major antigen shifts in the influenza A virus have been encountered, from H1N1 (the Puerto Rico virus) to H2N2 (the agent for the Asian influenza) and then to H3N2 (the Hong Kong virus). Since 1977 we have encountered epidemics by 2 major antigenic subtypes: H3N2 and H1N1 (the Russian virus).

Influenza viruses are divided into 3 types (A, B and C) based on the differences in their ribonucleoprotein (RNP) antigenicity. Influenza A virus is further subdivided into subtypes based on differences in the antigenicity of the haemagglutinin (HA) and neuraminidase (NA) components of the virus. The current nomenclature system for human influenza

virus considers the geographical location of first isolation, strain number, year of isolation and antigenic description of HA and NA, for example A/Ishikawa/7/82/(H3N2) or B/Singapore/222/79.

The clinical features of influenza in humans range from asymptomatic infection to primary viral pneumonia. The onset of influenza is usually abrupt, with headache, chills and a dry cough, rapidly followed by high fever, myalgia, malaise and anorexia. The fever often peaks to as high as 41°C within 24 hours, but more commonly the temperature ranges between 38° and 40°C. The fever usually begins to decline on the third day of the illness and the patient usually becomes afebrile by the sixth day.

When the virus infects children or individuals at high risk for complications (i.e. the elderly, patients with cardiopulmonary disease or immunocompromised patients), the clinical manifestations are more severe and the disease is sometimes fatal. In children, abrupt high fever is sometimes followed by CNS involvement, the manifestations of which can range from drowsiness and confusion to delirium and coma. This condition is termed influenza encephalitis or influenza encephalopathy. Influenza encephalitis [with inflammatory cells in the cerebrospinal fluid (CSF)] and influenza encephalopathy (without inflammatory cells in the CSF) sometimes manifest as convulsions.

In Hokkaido (an island located in the northern part of Japan), 53 cases of influenza encephalitis/encephalopathy were reported during epidemics of influenza between 1994 to 1998. Among the 31 cases in whom the diagnosis was confirmed virologically, the average age was 3.7 years and the ratio of males to females was 2 : 1 (table I). Influenza virus types A (H1N1), A (H3N2) and type B were isolated from the throat swabs of these patients. Mortality was reported to be 51.6%, and 19.4% of the patients exhibited some sequelae after recovery from the disease.[1] The appearance of CNS involvement after infection was rapid (2.8 days after the onset of fever) [table I]. The titres of inflammatory cytokines, such as interleukin-6 and tumour necrosis factor α, were high whereas the virus itself could not be isolated from the CSF. It is possible that these cytokines could be implicated in the neuropathology in these patients.

Table I. Influenza encephalitis/encephalopathy in the Hokkaido area of Japan during epidemic seasons from 1994 to 1998[1]

Total number of cases[a]	31
Average age ± SD (years)	3.7 ± 2.9
Male : female	21 : 10
Dead/sequelae/complete recovery	16/6/9
Time from onset of disease to abnormal neurological sign (days ± SD)	2.8 ± 2.3
Number of patients (%) with:	
Unconsciousness	31 (100)
Fever	31 (100)
Convulsion	24 (77.4)
Vomiting	12 (38.7)

a Diagnosis confirmed virologically.
SD = standard deviation.

The reported mortality rate of influenza is not high, on average, throughout all age categories (1.0 per million), as revealed by the annual mortality report of 1997 in Japan. However, the mortality rate increases 7-, 16- and 44-fold among elderly individuals aged over 65 years, over 75 years and over 85 years, respectively.[2]

Much time has passed since the emergence of H3N2 in 1968 and H1N1 in 1977. A new antigenic shift of influenza A virus may occur in the near future. Influenza B virus also exhibits antigenic drift sequentially every few years. Over time, prophylaxis for both influenza A and B viruses becomes ineffective using current vaccines. In May 1997, a 3-year-old boy in Hong Kong died of respiratory failure caused by an influenza virus infection; an influenza A strain of H5N1 subtype was isolated from a sample of the bronchial aspirate. Between November 1997 and January 1998, 17 patients infected with the H5N1 subtype of the virus were detected, of whom 5 died. The influenza A H5N1 strain was isolated from chickens in several poultry farms and 1.5 million chickens were sacrificed in order to prevent an epidemic of the new antigenic virus among the human population. Direct person to person spread of this subtype has not yet been reported.[3,4] In a more recent report from the WHO, another avian influenza A strain (H9N2) was identified in 2 hospitalised

children in Hong Kong. The illness was not so servere as with the previously mentioned strain, and the patients recovered without any medical complications.[5]

From the highly contagious nature of the influenza virus and the relatively high mortality rate in children and the elderly, the necessity to treat patients with influenza virus infection using antiviral drugs cannot be overemphasised. This article reviews recent progress in anti-influenza chemotherapy. Prophylaxis of influenza virus infection with vaccination is not discussed.

2. Identifying Causative Viruses of Acute Respiratory Infections

Acute respiratory infection (ARI) includes several clinical diagnostic categories, such as rhinitis, pharyngitis, laryngitis (croup), tracheitis, bronchitis, bronchiolitis and pneumonia. However, terms such as 'common cold' or 'influenza-like disease' are also used to categorise ARI. Influenza virus does not only cause clinical 'influenza' or 'influenza-like disease' but also other ARIs such as, 'common cold' or 'catarrhal otitis media'. On the other hand, adenoviruses are commonly thought to be the causative agent of 'pharyngo-conjunctival fever', whereas they can sometimes cause influenza-like disease. Thus, it is rather difficult to determine causative viruses from clinical symptoms.[6,7]

There are many types of virus that cause ARI, including orthomyxoviruses, paramyxoviruses, picornaviruses, adenoviruses, coronaviruses and herpesviruses. Among these the most important viruses are orthomyxoviruses and paramyxoviruses which frequently cause acute lower respiratory infections in infants and children (table II). Recent progress in molecular and immunological techniques has made it possible to rapidly detect the genomic polynucleotides of viruses or virus antigens using polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA).

For rapid diagnosis of influenza virus infection, the influenza virus optical immunoassay (FLU OIA) test (Biota Holding Inc. Melbourne, Australia) is handy and convenient. This compact kit is devised to detect influenza A and B viral antigens from patients' specimens within 15 minutes. When the extracted specimen is placed on the thin film which contains immobilised enzyme-linked antibodies and the antibodies capture the antigen, the thickness of the film changes and the colour of enzyme reacted substrate appears blue to purple (thus called the optical immunoassay). Another diagnostic kit, Directigen FluA (Becton Dickinson Co., Ltd. Cockeysville, Maryland, USA) can detect influenza A antigen but not B antigen. It may be useful to use this kit before prescribing amantadine or rimantadine; these drugs are effective only for influenza A.

3. Past Investigations of Anti-influenza Virus Chemotherapy

3.1 Amantadine and Rimantadine

Amantadine (amantadine hydrochloride) was the first anti-influenza drug approved for clinical use.[8] Amantadine is effective for influenza A virus, but not for influenza B and C virus infections. Amantadine has also been used as a drug for the treatment of Parkinson's disease for more than 30 years. Thus, the safety of amantadine for human use has been well established, although some neurological adverse effects, such as depression, difficulty in concentration and sleep disturbances, have been reported. Rimantadine hydrochloride is an analogue of amantadine hydrochloride (α-methyl 1-adamantanemethylamine hydrochloride) which has been shown to inhibit the replication of influenza

Table II. Ortho- and paramyxoviruses which cause acute respiratory infections in humans

Family	Subfamily	Genus
Orthomyxoviridae		Influenza virus A
		Influenza virus B
		Influenza virus C
Paramyxoviridae	Paramyxovirinae	
	Paramyxovirus	Human PIV-1,3
	Rubulavirus	Human PIV-2,4
	Morbillivirus	Measles virus
	Pneumovirinae	
	Pneumovirus	RSV

PIV = parainfluenza virus; **RSV** = respiratory syncytial virus.

A virus but not that of influenza B and C viruses.[9] This drug has been used in countries such as Russia.

Both drugs are absorbed well from the alimentary tract and the usual dosage is 100 to 200 mg/day. Rimantadine was reported to be more effective and safer than amantadine for the treatment of patients with influenza.[10] In a comparative study of amantadine and rimantadine (100mg twice daily for 6 weeks) prophylaxis of influenza A infection, Dolin et al.[11] reported that the withdrawal rate from medication was significantly higher in the amantadine group than in the rimantadine group (13 *vs* 6%).

During the course of chemotherapy for influenza A virus infection with amantadine, the development of resistance to this drug has been reported.[12] This discovery shed light on the unique mechanism of action of amantadine as an anti-influenza A virus drug and also on the function of the matrix protein (M2) of the virus. Amantadine and rimantadine inhibit virus replication by blocking the acidified ion channels formed by virion-associated M2 protein. The M2 ion channel activity is thought to facilitate the flow of ions from the lumen of the endosome into the virion interior. This ionic flow brings about the dissociation of the RNP and M1 (inner coat of RNP). The liberated RNP moves easily to the cellular nucleus. The genomic RNA (segment 7) of the influenza B virus encodes M1 and BM2 (the matrix of influenza B virus) proteins whose functions have not been determined yet. Segment 6 of the influenza B genomic RNA encodes NA and NB (the overlapping protein of neuraminidase gene) protein; NB protein is speculated to be equivalent to the M2 protein of influenza A virus. It is probable that amantadine and rimantadine do not 'fit' properly to the influenza B virus ion channel; this would explain the lack of channel activity inhibition.

3.2 Ribavirin

Ribavirin is an analogue of guanosine and is well known as an antiviral compound against several RNA viruses, including paramyxoviruses, bunyaviruses, flaviviruses and arenaviruses. Its anti-influenza virus activity is reported to be non-specific; it inhibits inosine monophosphate (IMP) dehydrogenase activity in cells, thereby decreasing the amount of xanthosine monophosphate and guanosine monophosphate, thus inhibiting viral RNA synthesis.[13] Ribavirin is efficacious in infections of influenza A and B viruses and respiratory syncytial (RS) virus but has only been approved by the US Food and Drug Administration (FDA) for use in the treatment of patients with RS virus pneumonia.[14] The usual formulation of ribavirin is an aerosol (20 mg/ml solution);[14] however, oral and intravenous infusions have also been reported to be efficacious.[15,16]

4. Recent Progress in Anti-Influenza Chemotherapy

Replication of the influenza virus in host cells proceeds in several steps: (i) adsorption of the virus to its receptor on the cellular membrane; (ii) penetration of the membrane, uncoating of the virus particle, transcription of mRNA, replication of viral genomic RNA, translation of the mRNA in the viral protein; then (iii) assembly of the viral particle and release of the virus from the cells. The initial steps of influenza virus replication start with a very unique interaction between the virus particle and the cellular membrane. Influenza virus acquires its infectivity after cleavage of the HA peptide in its envelope into HA1 and HA2 by the action of the host cell protease. Under conditions of low endosome pH, the cleaved HA molecule changes its stereoscopic conformation, and the N-terminus of the HA2 cleaved site comes in contact with the cellular membrane which fuses with the virus envelope. Simultaneously, influx of protons through the M2 ion channels occurs and results in uncoating of the M1 protein sheath as shown in figure 1. The liberated RNP then moves to the nucleus.

4.1 Fusion Inhibitor

A group working at the Bristol-Myers Squibb Research Institute (Wallingford, Colorado, USA) reported an inhibitor of the fusion between influenza A viral and cellular membranes, and designated it BMY-27 709. BMY-27 709 was reported to inhibit the replication of influenza virus A H1N1 and H2N2, but not that of H3N2 or influenza virus B.

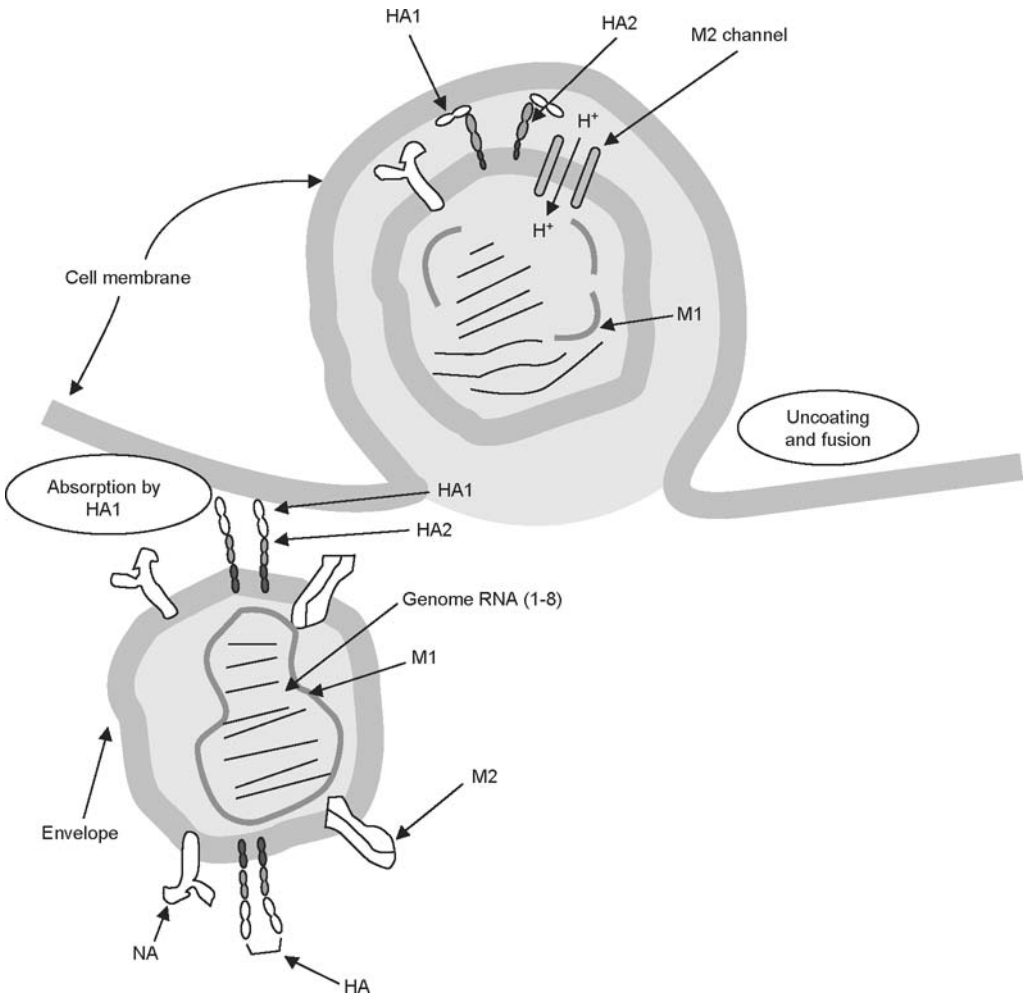


Fig. 1. Adsorption, uncoating and penetration of influenza virus at the cell membrane. **HA** = haemagglutinin; **M1** = inner coat of ribonucleotide protein; **M2** = matrix protein; **NA** = neuraminidase.

An influenza A virus which was resistant to this compound was isolated by cultivating the virus in the presence of BYM-27 709. The resistant strain showed 2 major amino acid changes at positions Met313 of HA1 and Phe110 of HA2, both of which were located near the fusion peptide at the cleavage site. BMY-27 709 was shown to bind to cleaved HA and inhibit the low pH-induced conformational change of HA.[17]

4.2 Protease Inhibitors

The proteases that cleave HA to HA1 and HA2 are not of viral origin but of host cell origin. Several protease inhibitors have been shown to inhibit the growth of influenza virus A in Madin-Darby canine kidney (MDCK) cells. Camostat mesilate (fig. 2) and nafamostat are commercially available for the treatment of patients with acute pancreatitis and they protect intact tissues from proteolysis by

the leaked pancreatic proteases. Hosoya et al.[18,19] reported that these protease inhibitors inhibited the cleavage of HA and were highly inhibitory against replication of influenza A and B viruses in both MDCK cells and embryonated chicken eggs.

4.3 Endonuclease Inhibitors

As inhibitors of RNA replication and transcription, 2 novel inhibitors which target the endonuclease activity associated with virus-PB2 (a part of viral RNA polymerase) with a unique mechanism of action have been reported. Influenza viruses possess a unique system for the cap formation of its mRNA, utilising the cap of the host cell mRNA. PB2 may be responsible for endonuclease activity which cuts and transfers the 13 base pairs of the cap of the host mRNA to the viral mRNA. L-735 882 (Merck) and BMY-26 270 (Bristol-Myers Squibb) [fig. 2] were found to be specific inhibitors of this endonuclease activity and were reported to inhibit the replication of influenza virus in MDCK cells.[20,21]

In 1998 Abe et al.[22] reported that an antisense oligonucleotide thioate targeted the PB2 genome which was encapsulated with liposomes accumulated in the nuclei of virus-infected MDCK cells but not in those of uninfected cells. This antisense oligonucleotide thioate was designed to target the open reading frame codons of the PB2 genome mRNA and was effective for the inhibition of influenza virus A replication in MDCK cells. On the other hand, other antisense oligonucleotides which target PB1 and PA (also parts of viral RNA polymerase) near the open reading frame codons, were not effective for the treatment of influenza.[22] The antisense oligonucleotide for PB2 was encapsulated in liposomes and used for the treatment of mice infected with influenza A virus and was inhibitory for the growth of the virus in the lungs of mice.[23]

5. Polyoxometalate as a Candidate For a New Anti-Influenza Drug

Polyoxometalates are clusters of polyoxonic acid molecules and are composed of transition metal ions (tungsten, manganese, niobium, vanadium, etc.) and 6 oxygen atoms. They are classified based on the cluster shape and category and the number of nuclear metal ions. Polyoxometalates inhibit oxidation and reduction reactions *in vitro* and inhibit the replication of HIV, herpes simplex virus, RS virus and influenza viruses in tissue cultures. The antiviral activity of polyoxometalates against influenza virus and RS virus was shown to be highly dependent on the type of cluster and nuclear metal ions.[24,25]

We investigated the inhibitory effects of 86 polyoxometalates on ortho- and paramyxoviruses in a

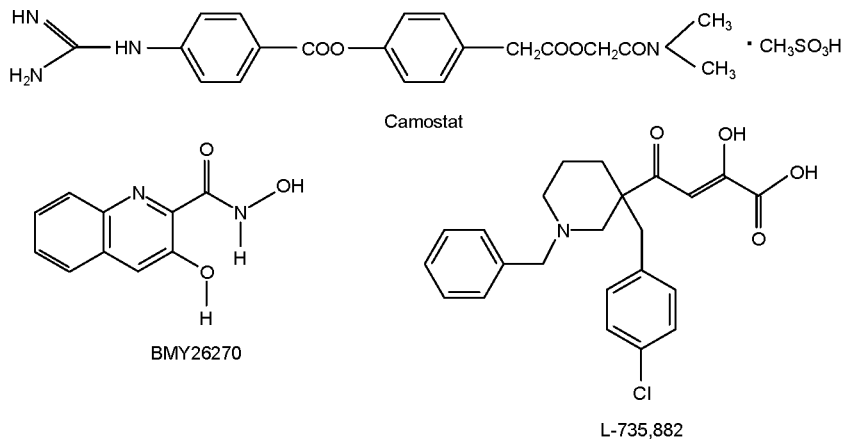


Fig. 2. Chemical structures of a protease inhibitor, camostat mesilate, and 2 endonuclease inhibitors, BMY-26 270 and L-735 882.

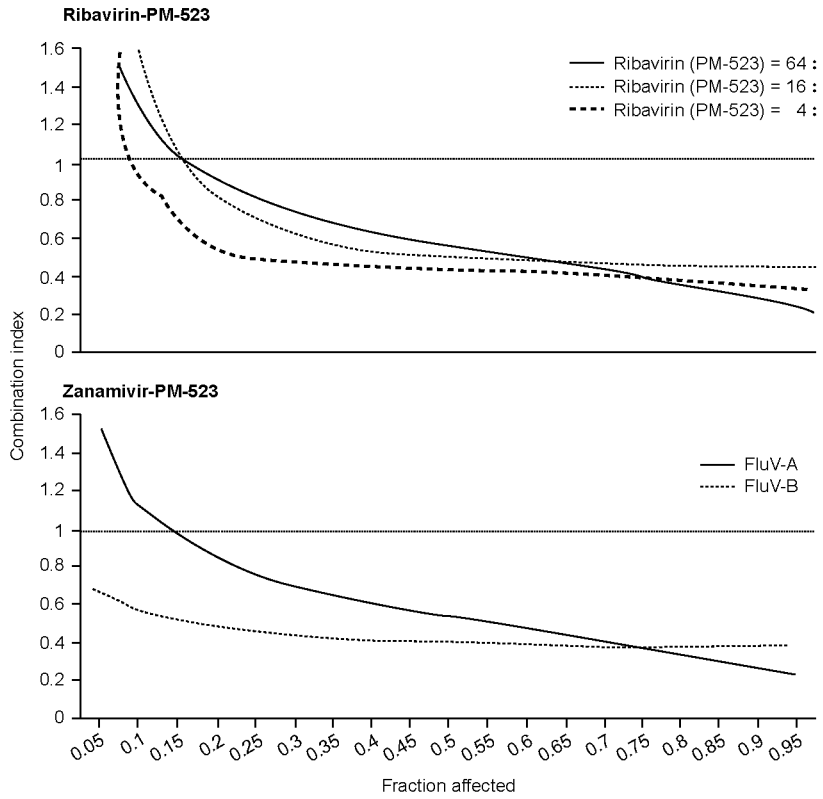


Fig. 3. Combination indices of ribavirin and PM-523, and zanamivir and PM-523. The combination index is presented for the inhibition of influenza virus type A (H1N1) multiplication in Madin-Darby canine kidney (MDCK) cells (**top**; ribavirin/PM-523) and for influenza virus type A (H3N2) and type B multiplication in MDCK cells (**bottom**; zanamivir/PM-523). The combination ratio of zanamivir and PM-523 was 1 : 16. The combination index was analysed with respect to the fraction affected under a mutually nonexclusive assumption using a computer program by Chou and Chou.[28,29] **FluV-A** = influenza A virus; **FluV-B** = influenza B virus.

tissue culture system. A titanium-containing Keggin-type polyoxotungstate (designated as PM-523) emerged as the most potent and selective antimyxovirus compound.[26] PM-523 and ribavirin mixtures showed a synergistic effect at ratios of 1 : 4 to 1 : 64.[27] The combination index was calculated by the formula from Chou[28] and was less than 0.6 (>1.0 is estimated as antagonistic, 1.0 as additive, <1.0 as synergistic) [fig. 3].

PM-523 was investigated for its anti-influenza A virus activity following administration as an aerosol to mice infected with a lethal dose of the virus (PR8/H1N1). Following exposure to PM-523 4.8 mmol/L for 2 hours twice daily for 4 days, 50% of the infected mice survived, whereas all the infected and untreated mice died by day 9. When we used a mixture of PM-523 and ribavirin at a ratio of 1 : 16 (PM-523 2.4 mmol/L and ribavirin 40 mmol/L) to treat the infected mice, a synergistic and improved therapeutic effect was achieved compared with the individual use of either compound; 80% of the mice survived to day 9 with the combined use of these compounds, in contrast to all the mice dying by day 9 with use of the individual compounds.[27]

We demonstrated that PM-523 inhibited fusion between the influenza A virus envelope and the

cellular membrane, whereas it did not inhibit the adsorption of the virus onto the cell membrane.[26] Thus, the mechanism of anti-influenza virus activity is quite different from that of ribavirin and zanamivir, which inhibit RNA replication and the NA activity of the influenza virus, respectively. PM-523 and zanamivir also showed a synergistic inhibitory effect against influenza virus *in vitro*; the synergism was more prominent against influenza B virus than against the A virus (Mori S et al., personal communication).

PM-523 did not show any cytotoxic effect on MDCK, HEp-2 (a human epiglottis carcinoma cell line) and Vero (a cell line from green monkey kidney cells) cells at 400 µmol/L. PM-523 inhibits both orthomyxo- and paramyxovirus replication at concentrations in the range of 1.3 to 7.0 µmol/L. The broad spectra of inhibitory activity of PM-523 and other polyoxotungstates are listed in table III. All the polyoxometalates examined had inhibitory activity against influenza A virus, RS virus and parainfluenza 2 virus replication. The common chemical features of these polyoxometalates are:

- they are all Keggin-type polyoxotungstates
- PM-518, PM-520, PM-523 have titanium atoms and PM-43, PM-47, PM-1001 have vanadium atoms in addition to tungsten.

In order to analyse the antifusion activity of PM-523 for the influenza virus, strains of influenza virus A resistant to PM-523 were isolated after sequential passages of the wild strain of A/Ishikawa/H3N2 in PM-523–containing cultures of MDCK cells. The resistant strains were analysed for amino acid substitutions of HA after cDNA amplification of the HA genome. All 5 strains which were resistant to PM-523 possessed mutations in HA1 at positions Lys189 and Ile204 compared with the sequence of the wild-type (sensitive) strain. These point mutations were located in the globular head of HA1 and the interface edges of the trimer molecules. The trimers open their heads in response to the conformational change of HA at low pH. Thus, we assumed that PM-523 binds to the interface edges of HA trimers and inhibits the opening of HA1 trimers, consequently inhibiting fusion of the viral envelope to the cellular membrane by HA2 hydrophobic amino acids at the edge of the cleavage site (unpublished observations) [fig. 4].

In another experiment, PM-523 obviously inhibited cell fusion between influenza virus-infected MDCK cells and uninfected cells but did not inhibit the fusion between the resistant virus–infected cells and uninfected cells (unpublished observations).

The mechanism of anti-influenza virus activity of PM-523 is unique and entirely different from that of amantadine, ribavirin or NA inhibitors. The synergistic anti-influenza virus activity of this compound with ribavirin or zamamivir offers promise for the use of PM-523 as a therapeutic drug for influenza virus infection. In addition, PM-523 and its congeners have broad antiviral activities against enveloped RNA viruses and may be developed as broad-spectrum drugs for ARI caused by ortho- and paramyxoviruses.

Table III. Antimyxovirus activities of several polyoxotungstates[26]

Compound	Chemical formula	EC_{50} ± SD (µmol/L)		
		FluV-A	RSV	PIV-2
PM-43	$K_5[SiVW_{11}O_{40}]$	8.4 ± 6.5	1.6 ± 0.4	>100
PM-47	$K_7[BVW_{11}O_{40}]$	11.5 ± 0.6	29 ± 15.6	67 ± 32.9
PM-518	$[PriNH_2]_7[PTi_2W_{10}O_{40}]$	62.3 ± 26.5	25.6 ± 8.1	53.2 ± 39.2
PM-520	$[PriNH_2]_5[PTiW_{11}O_{40}]$	45.2 ± 25.8	0.74 ± 0.58	3.2 ± 2.4
PM-523	$[PriNH_3]_6H[PTi_2W_{10}O_{38}(0_2)_2]H_2O$	3.5 ± 1.7	1.27 ± 0.46	2.5 ± 1.2
PM-1001	$[K_6V_3O_3(SbW_9O_{33})_2]$	1.3 ± 0.7	<0.16	1.1 ± 0.9
Ribavirin		9.6 ± 2.6	3.9 ± 3.1	14.0 ± 4.8

EC_{50} = drug concentration producing half of the maximum effect; **PIV** = parainfluenza virus; **FluV-A** = influenza A virus; **PIV-2** = parainfluenza virus type 2; **RSV** = respiratory syncytial virus; **SD** = standard deviation.

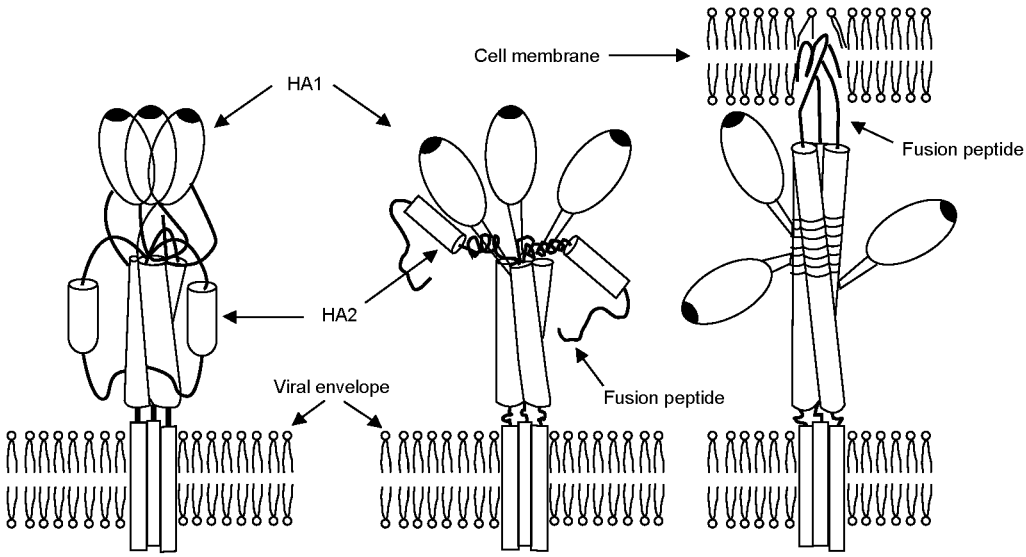


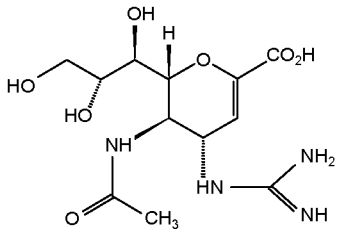
Fig. 4. The conformational change of haemagglutinin (HA) 1 at low pH. PM-523 is assumed to bind to the interface edges of HA trimers and inhibit opening of the HA trimers following the conformational change of HA1.

6. Clinical Use of Neuraminidase Inhibitors

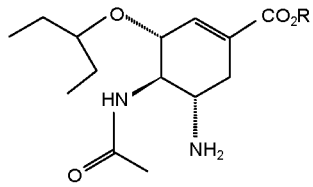
6.1 Zanamivir

In 1993, von Itzstein et al.[30] designed an influenza virus NA inhibitor (fig. 5) and reported its anti-influenza virus activity in tissue culture cells and animals.[30] This compound was later designated as zanamivir (or GG 167) and used as an aerosol for the treatment and prophylaxis of patients with influenza A and B virus infections. Data from double-blind clinical trials of zanamivir carried out during 1994 and 1995 demonstrated that the mean duration of symptoms in patients with virologically confirmed influenza infection was reduced from 6.3 days in the placebo group (n = 89) to 5.4 days in the treated group (n = 85) [p = 0.05]. Among the patients who were febrile and began treatment within 30 hours after the onset of symptoms, alleviation of the major symptoms occurred at least 2 days earlier in the treated group compared with the placebo group.[31]

A randomised double-blind clinical trial of zanamivir for the treatment of influenza A and B infections was carried out by the Management of Influenza in the Southern Hemisphere Trialists (MIST) study group during the winter season of 1997/1998.[32] 321 patients were randomly assigned to inhaled zanamivir 10mg or placebo twice daily for 5 days. Zanamivir significantly shortened the time to alleviation of symptoms compared with placebo (5.0 *vs* 6.5 days; p = 0.004). Patients who were febrile (temperature >37.8°C) and virologically positive for the influenza virus responded (alleviation of clinical symptoms) 2.0 days earlier in the zanamivir group than in the placebo group. High-risk patients (those with respiratory, endocrine, metabolic or cardiovascular disorders, immunocompromised patients or patients aged 65 years or older) responded a median of 2.5 days earlier when treated with zanamivir than when treated with placebo. The most common adverse effects observed with zanamivir inhalation were respiratory effects (bronchitis and cough). However, there was no significant difference in the incidence of adverse effects between the zanamivir inhalation group and the placebo group. The study group stated that zanamivir was well tolerated among high-risk patients.[32]



Zanamivir (GG 167)



R=H GS 4071
$R=CH_2-CH_3$ Oseltamivir

Fig. 5. Chemical structures of neuraminidase inhibitors zanamivir and oseltamivir (GS 4104) and GS 4071.

Drug-resistant viruses were isolated by passaging the virus in MDCK cells in the presence of zanamivir. Mutations in HA and NA were found in the resistant virus. The HA mutation, 198Thr to Ile, reduced the affinity of the virus for the receptors found on susceptible human cells (Siaα2-6Gal). Weak affinity of HA for the receptor may facilitate the release of the virus from cells, and NA activity may not contribute significantly to the release of the virus. Thus, zanamivir is thought to inefficiently inhibit the release of the resistant strains with mutations in HA. Two types of NA mutants were also isolated from the culture described above. One had a mutation in the catalytic site of NA (Arg292 to Lys) and the other a mutation in the framework residue of NA (Glu119 to Gly, Asp or Ala) with which the guanidino group of zanamivir interacts.[33]

The same research group also isolated zanamivir-resistant strains from an immunocompromised child infected with the influenza B virus. The isolates had a Thr198 to Ile mutation in HA and an Arg152 to Lys mutation in NA. The HA mutation reduced the affinity of the virus for human cell receptors and the NA mutation reduced the enzymatic NA inhibitory activity of zanamivir.[34]

A large scale US clinical trial of zanamivir in the treatment of influenza during 1995 to 1996 was reported by Monto et al.[35] In this trial, 1182 patients were randomised to receive double-blind treatment with placebo or zanamivir via inhalation (10mg, 2 or 4 times daily) and nasal spray (6.4mg, 2 or 4 times daily) for 5 days. Overall, 722 patients had virologically confirmed influenza [including A (H3N2), A (H1N1) and B in similar numbers]. Zanamivir reduced the duration of illness by 1 to 1.5 days when given within 30 hours of onset of disease ($p = 0.001$ when zanamivir was given 4 times daily, 10mg via inhalation or 6.4mg via nasal spray). Possible drug associated adverse events were reported equally in placebo and zanamivir groups. 35 patients were withdrawn from the study because of adverse events; numbers were similar in each group (12 in the placebo group, 13 in the zanamivir 2 times daily group, 10 in the zanamivir 4 times daily group). Thus, this large randomised trial of zanamivir for the treatment of influenza confirms the clinical efficacy of zanamivir when given early in the course of infections and also its tolerability.

6.2 Oseltamivir

More recently, a group from Gilead Sciences developed a carbocyclic transition state analogue of sialic acid, oseltamivir (GS 4104) [fig. 5]. Oseltamivir is well absorbed from the alimentary tract (30 to 70%), in contrast to zanamivir which has only a low degree of absorption (3 to 4%) from the alimentary tract. After absorption from the alimentary tract, oseltamivir changes to its nonester active form, GS 4071, and is distributed efficiently to the lungs and other tissues.[36,37]

Therapeutic efficacy of oral oseltamivir in reducing the symptoms of influenza in both experimentally infected volunteers and patients with acute febrile respiratory illness have been reported. 80 infected adults were randomised to receive oral oseltamivir (20 to 200mg twice daily for the volunteers

and 75 to 150mg twice daily for the patients) or placebo for 5 days. Treatment was initiated 28 hours after the inoculation of the volunteers and 36 hours after the onset of symptoms in the patients. In both trials, oral oseltamivir significantly reduced the time to alleviation of symptoms compared with placebo.[38,39]

Similar HA and NA mutants were obtained as for the case of zanamivir resistance (see section 6.1) following *in vitro* culture of influenza A virus in the presence of GS 4071. The mutants were all resistant to the inhibitory action of GS 4071. The HA mutants had substitutions at Ala28 to Thr and Thr Arg124 to Met. The NA had an Arg292 to Lys mutation. The substitution of NA Glu119 to Gly or Asp did not induce resistance for the activity of GS 4071 as an NA inhibitor.[40] From the observations of these investigators,[40] mutant strains resistant to zanamivir or GS 4071 are difficult to generate both *in vitro* and *in vivo*.

7. Conclusion and Future Direction

The clinical importance of anti-influenza chemotherapy is evident as antigenic shifts of virus and pandemics of new subtype strains of influenza are predicted in the near future. The prophylactic use of amantadine and rimantadine for influenza A virus infection is recommended, and ribavirin, in the form of an aerosol for patients with severe influenza A and B infections, has been reported to be efficacious.

Recent progress in anti-influenza chemotherapy has been made with the development of the NA inhibitors, zanamivir and oseltamivir. Clinical trials have shown both drugs to be efficacious in alleviating the main symptoms of influenza. A polyoxometalate, PM-523, was inhibitory for the growth of influenza virus *in vitro* and *in vivo*. PM-523 inhibited fusion of virus envelope and cell membrane and showed broad spectrum antiviral activity against ortho- and paramyxoviruses.

What remains to be done in the future is to examine clinically the combination of 2 or more drugs with different antiviral activity against influenza virus growth. Also, the development of drugs which have broad spectrum antiviral activity against ortho- and paramyxoviruses is needed.

References

1. Togashi T. Influenza in children [in Japanese]. J Jpn Med Assoc 1998; 12: 1038-43
2. Health and Welfare Statistics Association. Jpn J Health Welfare Stat 1998; 45: 414-5
3. Subbara BK, Klimov A, Katz J, et al. Characterization of an avian A(H5N1) virus isolated from a child with a fatal respiratory illness. Science 1998; 279: 393-6
4. Claas EC, Osterhaus AD, van Beek R, et al. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 1998; 351: 472-7
5. Influenza A (H9N2) in Hong Kong special administrative region of China (SAR). Communicable Disease Surveillance and Response (CRS) [online]. 1999 April 7. Available from: URL: http://www.who.org/emc/outbreak_news [Accessed 1999 Sep 13]
6. Stewart-Harris CH, Shield GC. Influenza: the virus and disease. London: Edward Arnold, 1976
7. Kaji M. What is 'cold syndrome' [in Japanese]. Rinshou Kenkyu 1996; 73: 2677-81
8. Jackson GG, Muldoon RL, Akers LW. Serological evidence for prevention of influenzal infection in volunteers by anti-influenzal drug adamantanamine hydrochloride. Antimicrob Agents Chemother 1963; 3: 703-7
9. Isunoda A, Massah HF, Cochran KW, et al. Antiviral activity of alpha-1-adamantanemethylamine hydrochloride. Antimicrob Agents Chemother 1965; 5: 553-60
10. Monto AS, Ohmit SE, Hornbuckle K, et al. Safety and efficacy of long-term use of rimantadine for prophylaxis of type A influenza in nursing homes. Antimicrob Agents Chemother 1995; 39: 2224-8
11. Dolin R, Richman RC, Madore HP, et al. A controlled trial of amantadine and rimantadine In the prophylaxis of Influenza A Infection. N Engl J Med 1982; 307: 580-3
12. Belshe RB, Burk B, Newman F, et al. Resistance of influenza A virus to amantadine and rimantadine: results of one decade of surveillance. J Infect Dis 1989; 159: 430-5
13. Gilbert BE, Knight V. Biochemistry and clinical applications of ribavirin. Antimicrob Agents Chemother 1986; 30: 201-5
14. Smith DW, Frankel LR, Mathers LH, et al. A controlled trial of aerosolized ribavirin in infants receiving mechanical ventilation for severe respiratory syncytial virus infection. N Engl J Med 1991; 325: 24-9
15. Stein DS, Creticos CM, Jackson GG, et al. Oral ribavirin treatment of influenza A and B. Antimicrob Agents Chemother 1987; 31: 1285-7
16. Hayden FG, Sable CA, Connor JD, et al. Intravenous ribavirin by constant infusion for serious influenza and parinfluenza-virus infection. Antiviral Ther 1996; 1: 51-6
17. Luo G, Torri A, Harte WE, et al. Molecular mechanism underlying action of a novel fusion inhibitor of influenza A virus. J Virol 1997; 71: 4062-70
18. Hosoya M, Matsuyama S, Baba M, et al. Effects of protease inhibitors on replication of various myxoviruses. Antimicrob Agents Chemother 1992; 36: 1432-6
19. Hosoya M, Shigeta S, Ishii T, et al. Comparative inhibitory effects of various nucleoside analogues on replication of influenza virus types A and B *in vitro* and *in vivo*. J Infect Dis 1993; 168: 641-6

20. Tomassini J, Selnick H, Davies ME, et al. Inhibition of Cap (m7GpppXm)-dependent endonuclease of influenza virus by 4-substituted 2,4-dioxobutanoic acid compounds. Antimicrob Agents Chemother 1994; 38; 2827-37
21. Cianci C, Chung TDY, Meanwell N, et al. Identification of N-hydroxamic acid and N-hydroxyimide compounds that inhibit the influenza virus polymerase. Antiviral Chem Chemother 1996; 7: 353-60
22. Abe T, Suzuki S, Hatta T, et al. Specific inhibition of influenza virus RNA polymerase and nucleoprotein gene expression by liposomally encapsulated antisense phosphorothioate oligonucleotides in MDCK cells. Antiviral Chem Chemother 1998; 9: 253-62
23. Miztu T, Fujiwara M, Hatta T, et al. Antisense oligonuleotides directed against the viral RNA polymerase gene enhance survival of mice infected with influenza A. Nature Biotechnology 1999; 17: 583-7
24. Barnard DL, Hill CL, Gage T, et al. Potent inhibition of respiratory syncytial virus by polyoxo-metalates of several structural classes. Antiviral Res 1997; 34: 27-37
25. Huffman JH, Sidwell RW, Barnard DL, et al. Influenza virus inhibitory effects of a series of germanium- and silicon-centred polyoxometalates. Antiviral Chem Chemother 1997; 8: 75-83
26. Shigeta S, Mori S, Watanabe J, et al. *In vitro* antimyxovirus activity and mechanism of anti-influenza virus activity of polyoxometalates PM-504 and PM-523. Antiviral Chem Chemother 1996; 7: 346-52
27. Shigeta S, Mori S, Watanabe J, et al. Synergistic anti-influenza virus A (H1N1) activities of PM-523 (polyoxometalate) and ribavirin *in vitro* and *in vivo*. Antimicrob Agents Chemother 1997; 41: 1423-7
28. Chou TC. Quantitation dose-effect analysis and algorithms. A theoretical study. Asia Pac J Pharmacol 1987; 2: 93-9
29. Chou J, Chou TC. Dose-effect analysis with microcomputers. Cambridge: Biosoft, 1987: 33-56
30. von Itzstein M, Wu W-Y, Kok GB, et al. Rational Design of potent sialidase-based inhibitors of influenza replication. Nature 1993; 363: 418-23
31. Hayden FG, Osterhaus A, Treanor JJ, et al. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. N Engl J Med 1997; 337: 874-80
32. Silagy CA, the MIST study group. Randomised trial of efficacy and safety of inhaled zanamivir in treatment of influenza A and B virus infections. Lancet 1998; 352: 1877-81
33. Gubareva LV, Robinson MJ, Bethell RC, et al. Catalytic and framework mutations in the neuraminidase active site of influenza viruses that are resistant to 4-guanidino-Neu5Ac2en. J Virol 1997; 71: 3385-90
34. Gubareva LV, Matrosovich MN, Brenner MK, et al. Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. J Infect Dis 1998; 178: 1257-62
35. Monto AS, Fleming DM, Henry D, et al. Efficacy and safety of neuraminidase inhibitor zanamivir in the treatment of influenza A and B infections. J Infect Dis 1999; 180: 254-61
36. Eisenberg EJ, Bidgood A, Cundy C. Penetration of GS4071, a novel influenza neuraminidase inhibitor, into rat bronchoalveolar lining fluid following oral administration of the prodrug GS4014. Antimicrob Agents Chemother 1997; 41: 1949-52
37. Sidwell RW, Huffman JH, Barnard DL, et al. Inhibition of influenza virus infections in mice by GS4104, an orally effective influenza neuraminidase inhibitor. Antiviral Res 1998; 37: 107-20
38. Hayden FG, Lobo M, Treanor JJ. Efficacy and tolerability of oral GS4104 for early treatment of experimental influenza in humans [abstract]. 37th Internscience Conference on Antimicrobial Agents and Chemotherapy; 1997 Sep 28-Oct 1; Toronto, 14
39. Aoki F, Osterhaus A, Rimmelzwaan G, et al. Oral GS4104 successfully reduced duration and severity of naturally acquired influenza [abstract]. 38th Internscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sep 24-27; San Diego (CA), 22
40. Mendel DB, Sidwell RW. Influenza virus resistance to neuraminidase inhibitors. Drug Resist Updates 1998; 1: 184-9

Correspondence and reprints: Dr *Shiro Shigeta*, Department of Microbiology, School of Medicine, Fukushima Medical University, 1 Hikarigaoka, Fukushima, 960-1295, Japan.
E-mail: sshigeta@ccfmu.Ac.jp