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REVIEW

The threat of avian influenza A (H5N1). Part III: antiviral therapy

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Abstract Among emerging and re-emerging infectious diseases, influenza constitutes one of the major threats to mankind. In this review series epidemiologic, virologic and pathologic concerns raised by infections of humans with avian influenza virus A/H5N1 as well as treatment options are discussed. The third part discusses therapeutic options. Neuraminidase (NA) inhibitors are the most promising agents despite uncertainty about efficacy. Dosage increase, prolonged treatment or combination therapies may increase treatment efficacy and/or inhibit resistance formation. Immune system dysregulation contributes to H5N1 disease. Although current evidence does not support the use of anti-inflammatory drugs beneficial effects cannot be excluded at later disease stages.

Introduction

The spread of H5N1 avian influenza A viruses from Asia to the Middle East, Europe, and Africa raises a serious concern that a outbreak may cause a human pandemic. Until now three subtypes of avian influenza A viruses including H5N1, H7N7 and H7N3 has proven bird-to-bird and human-to-human transmission. Of all influenza A viruses circulating in birds, H5N1 is currently of the greatest public-health concern because of an increasing number of infected humans, high mortality rates (exceeding 50%) and the emergence of multiple distinguishable clades [1, 2]. The World Health Organization (WHO) and many individual nations have developed plans to detect the emergence of pandemic influenza and to limit its hazardous consequences. The clinical WHO Rapid Advice Guidelines on pharmacological management of human H5N1 infection have recently been published [3].

Different classes of antiviral agents are used for the treatment of seasonal human influenza, which may also be effective against H5N1 viruses. Neuraminidase (NA) inhibitors are the most promising drugs superior to other agents such as M2 ion channel inhibitors (adamantanes). However, direct evidence for clinical efficacy of these drugs against H5N1 influenza is very sparse [3]. Several differences between pathogenic properties of H5N1 and human adapted influenza viruses may have impact on the efficacy of antiviral therapies. H5N1 viruses possess high virulence in infected humans resulting in increased viral loads (up to ten times greater than in individuals with seasonal influenza), ability to disseminate from respiratory tract in other organs (i.e. intestinal tract and possibly central nervous system) and prolonged viral replication in target organs [4, 5]. A high viral load correlates with dysregulated immune and inflammatory responses in H5N1 infected individuals especially those who died [6]. The detection of drug-resistant H5N1 virus strains in infected humans raised concerns that viral resistance may occur either naturally or develop after treatment with antiviral drugs [7, 8]. These features of human H5N1 infection may require development of novel antiviral agents as well as modification of current treatment strategies: use of higher doses, combined use of drugs with different modes of actions, and novel routes of drug administration.

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M2 ion channel inhibitors

Adamantanes including amantadine (1-adamantamine hydrochloride; Symmetrel) and its analogue, rimantadine (α-methyl-1-adamantanemethylamine; Flumadine), represent the first class of antivirals licensed for influenza virus infection [9–11]. Both amantadine and rimantadine are active at low doses against various substypes of influenza A viruses whereas they have no activity against influenza B viruses. Adamantanes target the transmembrane domain of the M2 protein of influenza A viruses resulting in inhibition of the proton influx into the virus and in turn the release of ribonucleoprotein (RNP) from the envelope matrix (M1) ("uncoating") required for its transport into the nucleus [12, 13]. In addition, adamantanes may also exert antiviral effects at later stages of viral replication in cells infected with H5 and H7 avian strains through interference with the ion channel activity of M2 during transport through the exocytotic pathway [14, 15]. However, whether inhibition of virus release may also contribute to amantadine-induced replication inhibition of amantadine-sensitive H5N1 viruses is not clear.

Like amantadine, rimantadine is also effective for the prevention of infection and illness caused by seasonal influenza A viruses [16]. When used as prophylactic treatment, either drug can prevent about 50% of infections or 70–90% of morbidity. Notably, prophylactic treatment does not increase the emergence of resistances [16, 17]. Amantadine proved to be safe in a pandemic situation with Russian (H1N1) influenza [18]. Moreover, the therapeutic use of amantadine or rimantadine reduced the duration of symptoms by approximately 1 day when administered within 48 h of onset of symptoms [16]. However, these drugs have not been shown to reduce the rate of influenza A virus-associated complications [19]. Adverse events detected under amantadine and rimantadine therapy include effects on the central nervous system (CNS) such as anxiety, depression, and insomnia in about 10 and 2% of subjects, respectively [16]. These side effects may limit the use of these drugs in influenza pandemic. In addition, both amantadine and rimantadine can exaggerate gastrointestinal symptoms, including nausea and vomiting in influenza A patients [16].

In contrast to prophylactic treatment, adamantane resistance emerges rapidly following therapeutic use in human influenza A infection. Adamantane-resistant viruses are fully transmissible and pathogenic [20, 21]. Up to 30% of influenza A patients treated with amantadine may shed resistant viruses, sometimes as early as day 2–3 after onset of treatment [22]. The actual numbers might even be higher if more rigorous detection techniques were used [23]. Molecular characterization of amantadine-resistant influenza A variants has revealed that a single substitution at one of five codons in the transmembrane region of the M2 protein may confer drug resistance [24, 25]. H3N2-infected individuals may shed more amantadine-resistant variants than their H1N1-infected counterparts [25]. A particular resistance mutation (Ser31Asn) has been detected in over 70% of community A/H3N2 isolates in China and Hong Kong in 2004–2005 [26] and in over 90% of such isolates in North America in late 2005 [27]. Most recently, an amantadine-resistant H7N7 virus strain (influenza A/Netherlands/219/03) was identified that did not harbour any known mutation in its M2 protein that has been associated with amantadine resistance [28]. Studies using reverse genetics revealed that the HA of this H7N7 strain caused amantadine resistance. This shows that influenza viruses can have non-M2 protein-determined resistance to amantadine [28].

Published case study data report on amantadine treatment of 10 H5N1 patients. No final conclusion can be drawn from this uncontrolled clinical use [4]. However, all (4 out of 4) patients that were treated with amantadine within 5 days after onset of symptoms survived. In contrast, four out of six patients who received treatment after day 5 died.

The rapid emergence of adamantane-resistant virus strains observed in patients with seasonal influenza raises concerns that rapid resistance development may also occur in adamantane-treated H5N1 infected patients. In addition, H5N1 variants with native (pre-treatment) resistance to amantadine have been found. More than 95% of the H5N1 isolates from the Indochina clade 1 isolated in Cambodia-Thailand-Vietnam naturally contained dual mutation motif Leu26Ile and Ser31Asn in the M2 protein, which is invariably associated with resistance to amantadine and rimantadine. Therefore, adamantanes are ineffective in the treatment and prevention of infections from this clade [7, 29, 30]. However, it is important to note that only 6.3–8.9% of H5N1 isolates from the China-Indonesia clade 2 were resistant to adamantanes [7]. Interestingly, no M2 mutations were found in H5N1 viruses isolated in Myanmar (Burma) [30]. Thus, based on extrapolation from trials in seasonal influenza, amantadine and rimantadine might offer clinical benefit as a first-line agent for chemoprophylaxis of H5N1 infection when NA inhibitors are not available and the virus is known or likely to be susceptible [3]. In addition, the long shelf life of amantadine of >25 years and its low cost make it an attractive choice for stockpiling.

Neuraminidase inhibitors

The break-through in anti-influenza virus chemotherapy was reached by development of blockers of viral NA activity. NA inhibitors arose from over nearly six decades of scientific achievements and their development is one of the

first examples of so-called rationale drug design [11]. NA inhibitors were entered in clinical practice in 1999, and oral oseltamivir has quickly become the principal drug of choice for treating influenza and pandemic stockpiling [31].

The NA of influenza virus hydrolyzes terminal sialic acids of sialoglycans, and it is generally accepted that NA promotes the release of progeny virus from infected host cells by destroying receptors on the host cell and the virus itself, following the intracellular viral replication cycle [32, 33]. The NA inhibitors interfere with this process and inhibit the release of progeny influenza virus from infected host cells [33]. This prevents infection of new host cells and thereby halts the spread of infection in the respiratory tract [31]. NA activity is also important for the initiation of influenza virus infection in human airway epithelium by removing of decoy receptors (α-2,6-linked sialic acid) on mucins, cilia, and cellular glycocalix, which would impede virus access to functional receptors on target cells [34] and/or promoting virus entry into sensitive cells [35]. These initial steps of influenza virus replication were efficiently inhibited by treatment with NA inhibitors and may explain at least in part why early administration of NA inhibitors to influenza patients is essential for the therapeutic effects.

Oseltamivir (Tamiflu) and zanamivir (Relenza) represent two clinically used NA inhibitors. Oseltamivir is an oral formulation, while zanamivir is a powder that is inhaled from a breath-activated plastic device. Both drugs are effective against all NA subtypes and, therefore, against all strains of influenza [31]. Zanamivir exhibits better in vitro antiviral activity against influenza B viruses, whereas oseltamivir is more effective against influenza A isolates [36, 37]. Clinical trials demonstrated that NA inhibitors are highly effective in the treatment of seasonal influenza. Since replication of influenza virus in the respiratory tract reaches its peak between 24 and 72 h after the onset of the illness, drugs such as the NA inhibitors that act at the stage of viral replication must be administered as early as possible [31]. Studies in widely diverse geographic locations showed that when otherwise healthy adults with influenza received oseltamivir or zanamivir within 36–48 h after the onset of illness, the duration of symptoms was by 1–2 days shorter [38–42]. In 5–12-year-old children, zanamivir reduced the duration of symptoms by average of 1.25 days [43]. Moreover, NA inhibitor treatment reduced complications associated with influenza infection both in adults and children [44–46] thus providing an important advantage over M2 ion channel inhibitors [19]. Controlled studies have demonstrated that zanamavir and oseltamivir are effective in preventing clinical influenza in healthy adults when the drugs are used prophylactically after exposure to influenza of close contacts, such as household members [47–50] or as seasonal prophylaxis in the community [51, 52]. Oseltamivir reduced the incidence of laboratory confirmed influenza by 76% during seasonal prophylaxis in the community [51]. Zanamivir provided comparable (67%) protection [52]. When used as post-exposure prophylaxis in household, oseltamivir and zanamivir reduced incidence of laboratory confirmed influenza by 89 and 79%, respectively [47, 49].

Controlled clinical trials on the efficacy of NA inhibitors for treatment and prophylaxis of human avian influenza H5N1 infection have not been performed. The use of NA inhibitors for avian influenza is therefore based on in vitro data and animal experiments. In a mouse model, both oseltamivir and zanamivir protected animals infected with H5N1 (A/Hong Kong/156/97), H9N2 (A/quail/Hong Kong/G1/97) or H6N1 (A/Teal/HK/W312/97) avian viruses against death. Moreover, the treatment reduced viral titres in the lungs and blocked the spread of virus to the brain [53, 54]. The time of commencement of antiviral therapy was directly related to the survival of animals. Highest levels of protection were seen when NA inhibitors were administered within 48 h of infection. This is consistent with clinical efficacy of the drugs for human influenza [54, 55]. Ferrets, which represent the most suitable animal model for human H5N1 pneumonia, were also used to observe activity of NA inhibitors against avian influenza viruses. Oseltamivir was tested for early post-exposure prophylaxis and for treatment in ferrets exposed to representatives of two clades of H5N1 virus [56]. Ferrets were protected from lethal infection with the A/Vietnam/1203/04 virus by oseltamivir 5 mg/kg/ day given 4 h after virus inoculation, but 25 mg/kg day were required when treatment was initiated 24 h after virus inoculation. For the treatment of ferrets inoculated with the less pathogenic A/Turkey/15/06 virus, 10 mg/kg day of oseltamivir starting 24 h after virus inoculation was sufficient to reduce the lethargy of the animals, to significantly inhibit inflammation in the upper respiratory tract, and to block virus spread to the internal organs. These findings suggested early onset of oseltamivir therapy to be crucial for treatment of highly pathogenic H5N1 viruses and that higher doses may be needed for the treatment of more virulent viruses.

Direct data on effects of NA inhibitors in humans came from a case series that described 37 H5N1 patients, of whom 25 were treated with oseltamivir (19 deaths) and 12 patients did not receive oseltamivir (9 deaths) [4]. Treatment regimens differed across these patients, beginning between day 4 and day 22 of illness. In the Thai series in 2004, patient who had survived after oseltamivir treatment appeared to have received the agent earlier than those who subsequently died (4.5 vs. 9 days after disease onset) [57]. A benefit of oseltamivir therapy was also suggested by treatment of eight patients in Vietnam in 2005 [8]. A rapid decline in the pharyngeal viral load to undetectable levels was observed in four out of four survivors. In contrast,

virus was still detectable at the end of treatment in three out of four patients who died of the infection after receiving the full course of treatment (one patient who died had insufficient follow-up). High viral loads resulting in intense inflammatory responses were identified as a central mechanism to H5N1 pathogenesis in patients in Vietnam [6]. These results suggest that successful control of viral replication by antivirals is essential to reduce inflammatory responses and in turn to improve clinical outcome. Even small relative risk reductions could lead to large net benefits in mortality [3].

A major advantage of NA inhibitors over M2 ion channel inhibitors is that they are less prone to select resistant influenza viruses. Until recently, there was little evidence of naturally occurring resistance to NA inhibitors [17, 58]. Both influenza A H1N1 and H3N2 isolates are highly susceptible to NA inhibitors. The susceptibility of avian influenza viruses to NA inhibitors is variable and may be drug specific. The influence of different mutations in NA genes on virus susceptibility to oseltamivir, zanamivir and peramivir, a further NA inhibitor under clinical investigation, was studied in vitro [59]. Only two mutations conferred resistance to all three NA inhibitors investigated [60] (Fig. 1). Clinically achievable concentrations of NA inhibitors inhibit replication of influenza A/H5N1 viruses isolated in 1997 and in the recent outbreaks [31]. However, recently oseltamivir-resistant H5N1 viruses were isolated from two of eight Vietnamese patients during oseltamivir treatment [8]. Both patients died of influenza A H5N1 virus infection, despite early initiation of treatment in one patient. These observations suggest that resistance can emerge during the currently recommended regimen of oseltamivir therapy and may be responsible for clinical deterioration. The resistance of H5N1 isolates resulted from the substitution of a single amino acid in N1 neuraminidase (His274Tyr) [8]. A 2000–2001 study in Japan revealed such resistant variants in up to 16% of children with human influenza A (H1N1) who had received oseltamivir [61]. In 2004, another study in Japan found that 9 of 50 oseltamivir-treated influenza A (H3N2) virus infected children (18%) had a virus with drug-resistance mutation in the NA gene (Arg292Lys, Asn294Ser, or Glu119Val) [62]. Oseltamivir resistant H5N1 viruses were isolated from two infected humans in Egypt [63] just 2 days after the initiation of oseltamivir treatment, an unusually short period to develop resistance. The virus in both patients had a rare Asn294Ser mutation, seen only in one previous H5N1 patient in Vietnam [63] and in H3N2 isolates from children treated with oseltamivir in Japan [62]. Consequently, the Egyptian patients may have been infected by a sick bird that already harboured the mutated virus or resistance formation occured in a very short time. Notably, oseltamivir resistant viruses have not been detected before day 4 of treatment of seasonal influenza [61]. It is probable to see more oseltamivir-resistant influenza H5N1 viruses when the number of human cases increases and the use of this drug for prophylactic or therapeutic purposes becomes more common. Moreover, influenza virus mutations in the NA confering oseltamivir resistance have been generally regarded to be subtype specific [64]. Since a Asn294Ser mutation was found in H3N2 isolates [62] as well as in H5N1 isolates [63] this may not be true for H5N1 (Fig. 2; Table 1).

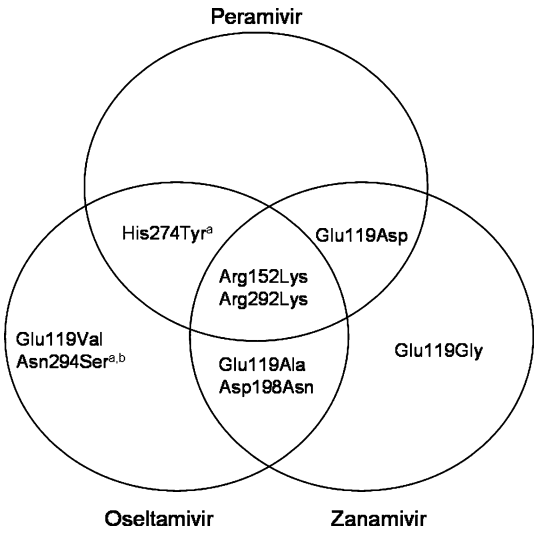

Fig. 1 Resistance to neuramindase inhibitors of influenza A and B viruses mediated by mutations in the neuraminidase gene, selected in humans, mice and/or in cell culture. [a]found in human H5N1 isolates; [b]cross-resistance to other neuraminidase inhibitors was not tested

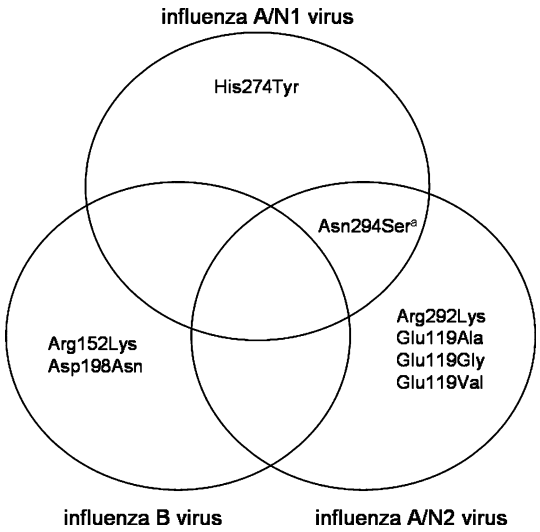

Fig. 2 Mutations in the neuraminidase genes of different influenza subtypes that mediate oseltamivir resistance. [a] mutation detected in H3N2-infected oseltamivir-treated children in Japan and in H5N1-infected patients (one patient from Vietnam in 2004, two patients from Egypt in 2006)

Table 1 Antiviral drugs for treatment of H5N1 infection in humans

Drug class	Mechanism	Virus sensitivity	Common adverse effects	Possible role in pandemic
M2 inhibitors amantadine rimantadine	Prevents uncoating by inhibition of the influenza A M2 protein	Highly sensitive; rapid resistance development, H5N1 from Indochina clade 1 naturally resistant	Dose-related central nervous and gastrointestinal adverse effects	Limited, prophylaxis for sensitive strains; combination with neuraminidase inhibitors (?)
Neuraminidase inhibitors oseltamivir zanamivir	Prevents release of progeny virus from infected cells by inhibition of neuraminidase of influenza A and B	Highly sensitive; resistance development described, but to a lower extent than for M2 inhibitors, no natural resistance reported	Mild gastrointestinal adverse effects (oseltamivir), bronchospasm (inhaled zanamivir)	Prophylaxis and treatment, oral oseltamivir for stockpiling; limited systemic effects of inhaled zanamivir
Nucleoside analogues ribavirin	Inhibition of RNA replication and translation: -inosine monophosphate dehydrogenase inhibition -inhibition of mRNA capping -lethal mutagenesis of virus RNA	Low therapeutic index	Dose-related anaemia, raised transaminases, brachycardia, risk of teratogenic effects	Combination with neuraminidase inhibitors?

Murine studies indicated that as compared with influenza H5N1 strain from 1997, the strain isolated in 2004 required higher doses and more prolonged administration (8 vs. 5 days) to induce similar antiviral effects and survival rates [65]. These differences were ascribed to enhanced pathogenicity of 2004 H5N1 isolate characterised by higher replication efficiency and increased neuroinvasiveness [66]. Previous observations had demonstrated that oseltamivir treatment should be continued as long as lung virus titers remain high [66]. In accordance, improved antiviral effects were observed after oseltamivir treatment for 8 days, the time at which the lung virus titre had begun to decrease [65]. Another reason for the extension of oseltamivir treatment is that neutralising antibodies are detected much later in patients infected with H5N1 than with human-adapted influenza viruses. The delayed production of antibodies may be explained by the lack of prior exposure to H5N1 viruses and absence of immunological memory against cross reacting antigens in most humans. Therefore, high-dose therapy with NA inhibitors may improve treatment of H5N1 infection, thus enhancing treatment efficacy and preventing emergence of resistant virus strains. Such possibility was suggested by clinical trials in which oseltamivir has been administered to healthy adults and elderly volunteers in high doses without significant adverse reactions [67]. However, current clinical data do not support the use of higher drug doses or more prolonged treatment of H5N1 infected humans [3]. Moreover, oseltamivir at a dose of 150 mg twice a day (two fold of standard recommended oral dose) did not provide greater antiviral or clinical effects in adults with uncomplicated seasonal influenza [40, 41].

The evidence for pharmacological chemoprophylaxis of H5N1 infection with oseltamivir or zanamivir is very low and indirect [3]. As mentioned above chemoprophylaxis trials in seasonal influenza have shown significant reductions in the influenza incidence. Moreover, pre-exposure prophylaxis with NA inhibitors was active in animal models of H5N1 infection. On the basis of extrapolation from these trials, both oseltamivir and zanamivir might cause reductions in frequency of H5N1 infection. The chemoprophylaxis courses should begin as soon as possible after exposure status is known and be continued for 7–10 days after the last exposure [3]. However, trials with post-exposure prophylaxis with oseltamivir were performed in humans infected with avian influenza virus H7N7. In the Netherlands (between February and June, 2003), 85 of the 453 people who reported symptoms including influenza-like illness or conjunctivitis (or both) had H7N7 isolated from their lacrimal fluid or upper respiratory swabs [68]. Ninety individuals in the case registry probably had prophylactic treatment. Infection with H7N7 virus was detected in 1 of the 38 (3%) people who used oseltamivir, compared with 5 of 52 (10%) who reported that they had

not taken prophylactic medication. Nevertheless, data from this study are insufficient to reach conclusion on the effectiveness of oseltamivir because of the small numbers and the late nature of the commencement of post-exposure prophylaxis.

Antiviral combinations

Treatment of H5N1 infection may be potentially optimised by using combination therapy with two NA inhibitors, one NA inhibitor plus one adamantane (if the circulating genotype is susceptible to adamantanes), or one NA inhibitor plus ribavirin. The synergistic effects of these combinations, if any, need to be studied urgently first by in vitro and animal studies. Despite lack of treatment data for zanamivir in human H5N1 inefection, zanamivir should be considered for pandemic treatment either alone or in combination with oseltamivir. Studies with nebulized preparations suggest that zanamivir has good safety and efficacy, even in patients with respiratory distress [38, 69, 70]. Zanamivir is an attractive antiviral drug for combined treatment of H5N1 infection because of non-overlapping resistance patterns with oseltamivir. The oseltamivir-resistant H5N1 virus isolate from Vietnam described above remains fully susceptible to zanamivir [8]. Thus, combined treatment using these two NA inhibitors would be expected to reduce the opportunity for the selection of resistant mutants, in a manner akin to the use of dual nucleoside analogues in antiretroviral therapy. However, the routine use of licensed inhalation formulation of zanamivir could be problematic in very young or elderly patients, and patients with severe co-morbidities who are not able to inhale the powder properly [31]. Moreover, administration by inhalation delivers zanamivir predominantly to the upper respiratory tract and the blood levels achieved are almost five times lower than that of oseltamivir [31, 71]. This observation raises a concern that H5N1 infected patients would develop easily resistant viruses during treatment with inhaled zanamivir since H5N1 viruses have a tendency to replicate outside the respiratory tract. Therefore, other formulations of zanamivir may be more appropriate for treatment of H5N1 infection. In preclinical trial, intravenous zanamivir, was very well tolerated and achieved very high levels in blood and respiratory secretions. Protective efficacy was also demonstrated in experimental human infections [69, 71].

Combination therapy of oseltamivir with rimantadine was found to be synergistic in preventing mortality from H9N2 infections in animal studies [54, 55]. However, animal studies on other avian influenza viruses have not been performed. Recent in vitro observations demonstrated that combination chemotherapy with adamantanes and NA inhibitors may reduce the emergence of drug-resistant influenza variants. Prolonged treatment of cultured cells infected with different influenza strains including H5N1/97 virus with a combination of oseltamivir and amantadine prevented emerging of resistant viruses which were obtained in the presence of amantadine or oseltamavir monotherapy [72]. Since as mentioned above H5N1 viruses (Indochina clade) with naturally occurring resistance to adamantanes were frequently detected [29], the introduction of such combination will depend on our knowledge of sensitivity of circulating H5N1 genotype to adamantanes.

A combination of NA inhibitors with ribavirin may provide another possibility to improve therapy of H5N1 infection. The broad spectrum antiviral ribavirin, a nucleoside analogue, is a recognised inhibitor of influenza A and B virus infections in vitro and animal models [73, 74]. Ribavirin inhibits replication of viruses by inhibiting cellular enzyme inosine monophosphate dehydrogenase, which is required for the synthesis of guanosine triphosphate. Moreover, inhibition of viral polymerase activity by the 5′-triphosphate metabolite of ribavirin, inhibition of viral mRNA capping, and lethal mutagenesis of the RNA genome may contribute to the antiviral effects of ribavirin [75, 76]. Ribavirin has been used in the treatment of human influenza A virus infections, usually administered orally or by aerosolisation, and occasionally intravenously for severe infections in immunocompromised hosts [77–79]. A consistent benefit has not been observed in clinical studies, and currently ribavirin is not considered to be a drug of choice for influenza A infection. In vitro data on the activity of ribavirin on avian influenza viruses is limited [80]. In vitro efficacy of ribavirin against two strains of influenza H5N1 was in the range of concentrations similar to those inhibiting replication of human influenza strains. Ribavirin was used for the treatment of two patients in Vietnam in 2004 and one patient in Hong Kong in 1997 infected with H5N1. No clinical benefit was observed and all patients died.

However, ribavirin was shown to be highly effective in reducing mortality in a mannan-enhanced mice model infected by influenza B even when treatment was delayed for 3 days after infection, when oseltamivir treatment was no longer effective [81]. Combination of oseltamivir with ribavirin treatment started at such delayed timing does not increase the efficacy in this mice model. Since the use of ribavirin has been limited by relatively small therapeutic index, haemolytic anemia at high doses and potential teratogenic effects, the carboxiamidine analogue of ribavirin, viramidine was developed [76]. Viramidine, currently examined in Phase III trials for the treatment of hepatitis C [76], has been shown to act primarily as a prodrug of ribavirin, being converted to ribavirin by adenosine deaminase [82]. Viramidine inhibited replication of H1N1, H3N2 and H5N1 viruses in cultured cells and showed similar effects to ribavirin in animal models against human influenza A

infection [80]. Considering the lesser toxicity than ribavirin, viramidine may warrant further evaluation as a possible therapy especially in a combination with NA inhibitors for influenza including infections with H5N1 viruses [80].

Immunomodulatory treatment

Clinical observations indicate that high viral load and the resulting immune dysregulation and inflammatory responses, are central to H5N1 pathogenesis [4, 6]. These observations suggest that timely suppression of viral replication should remain the mainstay for treatment of influenza H5N1. The seemingly limited clinical efficacy of antiviral treatment of H5N1 influenza may be due to the inability of antivirals to interfere with the sequence of events leading to dysregulated immune and inflammatory responses, when treatment is started late in the course of the illness. At this stage of H5N1 infection the treatment with immunomodulatory agents may have positive effects on outcome of the H5N1 disease due to suppression of hyperactivated immune and inflammatory responses.

Corticosteroids have been used for small numbers of patients with H5N1 pneumonia in Hong Kong, Vietnam and Thailand. The numbers of patients who received corticosteroids in the three outbreaks were three (2 deaths), seven (6 deaths), and eight (6 deaths), respectively [4, 57, 83]. In general, the use of immunomodulators is associated with a risk of interference with both innate and adaptive immune responses. Among survivors, specific humoral immune responses to H5N1 infection are detectable by microneutralisation assay 10–14 days after the onset of illness. Corticosteroid use may delay or blunt these responses. Moreover, corticosteroid-induced suppression of systemic inflammatory mediators such as interferons (IFNs) may impair host response against virus infection [84]. Therefore, it would be counter-productive if the immune defence against viral replication is impaired by immunomodulators, while antiviral therapy is not optimised.

Interferons are well known to protect against virus infection both through direct antiviral effects and immunomodulatory activity [85, 86]. Unfortunately highly pathogenic H5N1/97 viruses were resistant to IFNs in vitro and the resistance was associated with the presence of glutamic acid at position 92 of NS1 protein [87, 88]. These in vitro data extend to in vivo findings, since pigs infected with a recombinant human H1N1 virus possessing NS1 of the H5N1/97 virus experienced higher virus titres and body temperatures than those infected with a control H1N1 virus [87, 88]. Although sensitivity of H5N1 viruses from outbreaks after 1997 is not known, it is not probable that IFNs may be used as antiviral agents for H5N1 infected humans. Blood concentrations of both IFNγ and IFNα were increased in patients with H5N1 infection [4, 89]. Moreover, levels of chemokines such as IP-10 (interferon-gamma-inducible protein-10; CXCL10) or MIG (monokine induced by interferon-gamma; CXCL9) which are strongly induced by IFNγ in bronchial epithelial cells [90] were elevated in H5N1 infected individuals and particularly high in those who died [6]. Therefore, there is a concern that exogenous IFNs would increase production of proinflammatory cytokines/chemokines that are associated with severity of H5N1 influenza in humans.

There are a number of novel applications of non-antiviral drugs not normally employed in the treatment of viral pneumonia (e.g. statins, macrolide antibiotics) or even natural products (flavonoids, flavones and polyphenols) that may be used for control of severe respiratory inflammation that accompanies influenza infections [91]. Inflammatory modulator acetylsalicylic acid (ASA) was suggested to inhibit replication of human adapted influenza viruses almost 20 years ago [92]. In recent observations ASA was shown to inhibit replication of highly pathogenic avian viruses including H7N7 (A/Bratislava/79; FPV) and human H5N1 isolate (AThailand-1; KAN-1) in cultured cells and in mice [93]. Although ASA seems to inhibit influenza virus replication independently of effects on cellular transcription factor NF-κB [93], the suppression of inflammation by ASA in different organs including respiratory tract was associated with inhibition of NF-κB whose activity is relevant for expression of most inflammatory genes [94]. Since ASA showed no toxic side effects or the tendency to induce resistant virus strains, existing salicilate-based aerosolic drugs may be suitable treatment for influenza due to both antiviral and anti-inflammatory activities.

Conclusions

Controlled clinical trials would be required to provide evidence on efficacy of antiviral therapy for prophylaxis and treatment of H5N1 influenza. However, given the severity of the disease, such clinical trials cannot be performed during the next outbreaks due to ethical reasons. Therefore, treatment regimens should be carefully examined first in in vitro and animal experiments. Ferrets represent the most suitable animal model for human H5N1 pneumonia [95] and were already used to study the activity of antiviral agents against avian influenza viruses [56]. NA inhibitors are most promising agents for treatment and prophylaxis of H5N1 disease at present and oseltamivir is regarded as the drug of choice [3], despite uncertainty concerning efficacy. Although early antiviral treatment and supportive care remain key features in the management of H5N1 patients, treatment with oseltamivir may be beneficial even when initiated as late as 8 days after the onset of symptoms, if

there is an evidence of ongoing viral replication [4, 8]. Resistance formation to antiviral drugs may further reduce the efficacy of antiviral therapies. Therefore, careful monitoring is required. New treatment modalities such as dosage increase, prolonged treatment period and combinations of antiviral agents with different modes of action may increase the efficacy of antiviral therapies and/or inhibit the development of resistant virus strains. Vigorous dysregulation of immune and inflammatory responses contribute to severity of H5N1 disease [8]. Although current evidence does not support a beneficial role of corticosteroids or other immunomodulators in the management of severe H5N1 infections [4] beneficial effects of anti-inflammatory drugs when used at later stages of the disease cannot be excluded.

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