Review

Experimental human influenza: observations from studies of influenza antivirals

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Randomized, placebo–controlled trials have been conducted for nearly five decades in experimentally induced human influenza infections to assess the effectiveness, tolerability and pharmacological properties of influenza antivirals. The results of such studies have not only provided key proof–of–concept data to facilitate drug development but also contributed to our understanding of influenza pathogenesis and transmission. The lack of availability of contemporary, safety–tested virus inoculation pools in recent years needs to be resolved in order to avoid hindering the development of new drugs and vaccines.

Introduction

This article provides personal perspectives and reviews representative publications on the use of experimentally induced human influenza infections for antiviral drug studies. In addition to assessing the utility and predictive value of such challenge model findings in antiviral development, the implications for understanding influenza pathogenesis and transmission are also highlighted. These comments are based on presentations made by the author at a United States Centers for Disease Control and Prevention-sponsored meeting '*Approaches to Better Understand Human Influenza Transmission*', Atlanta, GA, USA, 4–5 November 2010 and at the National Institute of Allergy and Infectious Diseases 2011 *Influenza Antiviral Research Pipeline Workshop*, Bethesda, MD, USA, 23–24 March 2011.

Early studies

The first successful human challenge infection with influenza virus was undertaken in 1936 by Russian scientists, several years after human influenza virus was first isolated, when 72 subjects were given an inhalation of an atomized droplet suspension of two influenza A/H1N1 strains [1]. In subsequent studies of more than 200 volunteer exposures to influenza A (PR8, F-12, F-99) or influenza B (Lee) viruses, American investigators showed that the route of inoculation influenced the likelihood of fever, in that 89% of 65 volunteers infected by inhalation developed fever compared to only 12%

of 16 exposed by nasal drops [2]. The authors concluded, 'This difference between the results obtained by inhalation and intranasal instillation implies that the portal of entry for the virus of influenza may not be the nasal mucosa, but rather the lower regions of the respiratory tract.' Subsequent studies confirmed that the route of exposure, virus strain and dose, and pre-challenge serum levels of strain-specific antibodies are important factors that influence the virological and clinical responses to experimental inoculation in adults. Depending on the virus strain and study, the estimated 50% human infectious dose (HID50) of influenza virus via the aerosol challenge route was only 0.6–3.0 50% tissue culture infectious dose (TCID50), whereas it ranged from 40–<80,000 TCID50 after intranasal challenge [3–7]. These differences suggest that experimental exposure to virus in particles capable of deeper lung deposition are more efficient in causing infection, in that the HID50 appears to be at least 5–10 fold lower than for intranasal inoculation, and also more likely to induce febrile illness.

An extensive review of human challenge study data [8] from 56 studies involving different influenza A and B strains in a total of 1,091 sero-susceptible, untreated or placebo-treated participants concluded that infectious viral detection increased sharply from 12 h post-inoculation, nasal viral titres peaked on day 2 and shedding lasted an average of 4.8 days. Overall, 67% developed clinical illness and 40% develop fever

(>100°F or 37.8°C) with symptom scores peaking on day 3, such that symptoms corresponded to virus shedding with a lag period of about 1 day. Febrile responses were more common in H1 and H3-infected subjects compared to those infected with influenza B viruses. In general, illness following experimental intranasal virus inoculation is much less severe than that in naturally infected young adults who seek care and is manifested predominately by rhinitis and pharyngitis, and less often cough [9].

Safety and logistical considerations

Early investigators of influenza antivirals in the experimental challenge model recognized the potential safety issues and wrote, 'First, it goes without saying that risk from infection or drug toxicity must be minimal' [10]. The potential risks of influenza complications after experimental influenza virus inoculation, including bronchospasm, viral pneumonia and serious secondary bacterial infections, have been long-standing concerns. While such events have not been reported to date, minor complications like otitis media and suspected sinusitis leading to antibiotic therapy have occurred. In contrast to naturally infected patients with A(H3N2) illness, one comparative study found no changes in conventional pulmonary function tests, airway reactivity to cholinergic or histamine stimuli, or small airway resistance in experimentally infected subjects given one of three A(H3N2) viruses by intranasal drops (approximately 10^4 TCID50) [9]. In addition, the frequencies of fever and cough (50% versus 100%) and their duration were less in those with experimental infections compared to natural ones. Another study in subjects with allergic rhinitis infected intranasally with an A(H1N1) virus also reported no significant changes in spirometry or methacholine-induced airway reactivity [11]. Thus, experimental influenza following intranasal virus inoculation is characterized by significantly fewer lower respiratory manifestations and systemic symptoms compared to naturally acquired illness.

Because aerosol inoculation produces more marked symptoms with fever and cough similar to typical influenza-like illness (ILI), aerosol inoculation would seem to be the preferred route of virus exposure in order to mimic naturally occurring influenza. However, over-riding safety concerns at present restrict aerosol challenge studies, whereas intranasal challenge appears relatively safe. However, aerosol exposure to live attenuated influenza vaccine viruses that are temperature-restricted and generally are not thought to replicate in the lower airways has recently been used in experiments studying routes of influenza transmission [12].

Serious adverse events possibly related to experimentally induced influenza infection have been described. In a 2000 study, 1 of 75 adult volunteers infected with an influenza B/Yamagata/88 virus developed new ECG abnormalities and echocardiographic evidence of asymptomatic but severe myocardial dysfunction from presumed myocarditis temporally following experimental infection [13]. The volunteer recovered and no alternative aetiologic diagnosis was found, although, in retrospect, he was noted to have non-specific ECG abnormalities prior to study enrolment. A subsequent study of 30 young adults with naturally occurring seasonal influenza illness found that 53% had abnormal ECG findings on day 4 after illness onset; this had dropped to 23% by day 28. All ECG changes were considered clinically insignificant, and no echocardiographic abnormalities were found [13]. In addition to emphasizing the importance of screening for preexisting cardiac conditions, this case experience has heavily affected the medico-legal and ethical landscapes for conducting challenge studies and increased the requirements for more detailed screening before and after experimental influenza virus challenge. In this regard, the carefully controlled environment of the human challenge model and its ready accessibility to participants for sequential sampling enables careful monitoring of the tolerability of investigational agents to be undertaken.

Even though the strains used for challenge are derived from those currently or previously circulating in the community, theoretical concerns exist about re-introduction of such viruses outside of the study facility, especially outside of the influenza season. Consequently, strict attention to infection control measures, including staff immunization and use of personal protective equipment, are required [14,15]. Related issues are the screening of subjects to prevent introduction of non-influenza respiratory viruses into the challenge unit, and initial quarantine of subjects, as well as isolation after influenza inoculation, to avoid onward transmission of influenza or other respiratory viruses that might confound study results. However, as discussed below, purposeful exposure of susceptible individuals to infected subjects may offer a means to examine possible routes of transmission and their interruption [15].

Of course, the size of the susceptible pool for a particular challenge virus diminishes over time as its parent or antigenically related strains circulate in the community. This necessitates screening of increasingly large numbers of volunteers to identify susceptible individuals and regular updating of safety-tested virus challenge pools. However, screening for susceptibility by serum hemagglutination-inhibiton (HAI) antibody may not be as predictive for infection as the more labour-intensive assays for neutralizing antibody [10,14], and the frequency of illness from a particular challenge pool appears to diminish over time, despite susceptibility based on HAI

testing. Furthermore, a major hurdle in the United States has been the lack of availability of such updated virus challenge pools in recent years. This relates largely to the high cost of producing them, in part related to the extensive testing required for adventitious agents, and the challenges in obtaining regulatory approval for their use. At present in the United States influenza virus challenge pools require an Investigational New Drug approval package that has the same basic elements as that for a live viral vaccine.

Antiviral studies in experimental human influenza

Randomized, placebo-controlled trials in the human challenge model have been conducted for nearly five decades to assess the effectiveness of influenza antivirals and have provided key proof-of-concept data to facilitate drug development (reviewed in [14]). The first such studies in experimentally infected subjects were undertaken to test the inhibitory effects of amantadine and rimantadine [10,16]. A 1963 publication [16] on a trial of amantadine 100 mg every 12 h for 6 days starting 18 h before intranasal A(H2N2) virus inoculation reported a 46% reduction in serological evidence of infection among sero-susceptible subjects compared to placebo. A separate protective effect of preexisting serum HAI antibody was confirmed, and as subsequently found in field studies, amantadine prophylaxis in the presence of specific antibody was associated with additional protection. A 1968 report [10] showed that rimantadine 200 mg twice daily for 11 days starting 1 day before challenge with an A/Rockville/1/65(H2N2) virus reduced overall illness by 70% (86% versus 26%), diminished virus recovery, and lowered convalescent antibody titres, although not frequency of seroconversions, compared to placebo. This study had a high frequency of febrile illness in placebo recipients, perhaps related to the stringent antibody screening used to assess susceptibility (serum neutralizing antibody ≤1:2). However, one small study from the MRC Common Cold Unit found no evidence of amantadine protection against experimental influenza A/Scotland/49/57(H2N2) infection or illness, perhaps because of the use of high intranasal doses of virus [17]. Early studies employing A/Hong Kong/68(H3N2) virus at the former All-Union Research Institute of Influenza, Leningrad (former USSR), concluded that amantadine was less effective than rimantadine for prophylaxis (active at doses as low as 50 mg daily) or treatment and that rimantadine possessed therapeutic activity at higher doses in experimentally infected volunteers [18,19]. Consistent with preclinical observations, amantadine was ineffective against influenza B challenge [18]. Later studies confirmed the prophylactic

and/or early therapeutic effectiveness of these agents [14], including oral administration of other adamantane derivatives [20,21] and aerosol administration of rimantadine [22] in experimentally infected subjects.

An early interferon (IFN) prophylaxis study utilizing low intranasal doses of leukocyte-derived IFN did not reduce infection or illness rates among volunteers challenged with influenza B/Hanover/1/70 [23]. Subsequent studies with much higher doses of intranasal lymphocyte-derived or recombinant IFN-αs found evidence for reductions in symptom scores on 3–5 days post-challenge with influenza A/Eng/40/83(H3N2) [24] and in viral replication markers and illness 2–4 days post-challenge with influenza A/California/78(H1N1) [25], although intranasal IFNs did not significantly reduce overall infection or illness frequencies. However, as discussed below, these encouraging initial observations were not confirmed in subsequent field studies of intranasal recombinant IFN-αs for influenza prevention [26–28].

More recent experimental influenza studies have involved the neuraminidase inhibitors (NAIs) zanamivir [7], oseltamivir [29] and peramivir [30] for either prophylaxis or early treatment after experimental influenza A and B virus inoculation. The initial human prophylactic efficacy studies of zanamivir were conducted in 94 volunteers given intranasal zanamivir or placebo 2–6 times a day as spray or drops and then challenged intranasally with 10^5 TCID50 of influenza A/Texas/36/91(H1N1) [7]. Zanamivir prophylaxis significantly reduced the proportions of those shedding virus (73% versus 3%), becoming infected (73% versus 13%) and developing upper respiratory symptoms (61% to 26%) compared to placebo. Subsequently, a single intranasal dose of zanamivir given 4 h before challenge significantly reduced viral load and tended to reduce illness measures [31], suggesting that once-daily dosing of zanamivir was protective against influenza virus challenge. Subsequent field studies confirmed that once-daily dosing was effective for prophylaxis, when zanamivir is delivered by oral inhalation [32,33]. Similarly, in a study involving 33 subjects, oseltamivir 100 mg once or twice daily for 5 days starting 26 h before A/Texas/36/91(H1N1) challenge showed high protective efficacy (100% for virus detection, 61% for seroconversion) compared to placebo [29]. Subsequent field studies found that once or twice daily oseltamivir was effective for prophylaxis of influenza A/H3N2 illness in healthy, non-immunized adults [34].

Early treatment studies, in which drug administration was initiated between 24–32 h after virus inoculation also indicated that both intranasal zanamivir and oral oseltamivir were effective in reducing viral replication and symptoms in influenza A/H1N1-infected volunteers [7,29], findings that were confirmed in subsequent field studies. An initial proof-of-concept study of intravenous

zanamivir against the same influenza A virus challenge demonstrated high levels of protection against infection and illness measures when drug administration was initiated 4 h before virus exposure in a study involving 15 volunteers [35]. The intravenous zanamivir dose regimen used in this study (600 mg every 12 h) has been subsequently employed on compassionate-use basis for managing severely ill patients with suspected oseltamivir-resistant influenza infections, and controlled studies in hospitalized patients are currently in progress.

In aggregate, these observations show the influenza challenge models can provide valuable proof-of-concept data with relatively small subject numbers. In general, the prophylaxis designs, in which drug administration begins before viral exposure, provides clear signals of antiviral effectiveness with smaller numbers of subjects than needed to examine therapeutic activity.

Predictive value of human challenge model

In general, the results in the experimental human challenge studies have predicted antiviral effectiveness in subsequent field studies [14], although the correspondence in the magnitude of observed beneficial effects has varied by antiviral agent, indication and virus strain. In addition, there are clear discrepancies in part related to differences in mode of administration for topically applied antivirals. For example, as discussed below, intranasal IFN-α was partially effective in the challenge model but lacked efficacy in the field for influenza. A similar series of observations emerged in studies of intranasal zanamivir. Some experimental challenge studies have provided key data for making decisions on development of particular agents. At least one putative antiviral agent with activity in animal models of influenza, the thiobendazole LY217896, was abandoned after it showed lack of prophylactic activity against an experimental influenza A(H1N1) challenge [36]. Oral administration of the NAI peramivir was found to be associated with low oral bioavailability and insufficient antiviral effectiveness when given as prophylaxis or early treatment in volunteers challenged with influenza A or B viruses [30]. Oral peramivir was not tested for prophylaxis in the field, and further study of this formulation was stopped when a treatment study in natural influenza found a nonsignificant effect on illness resolution.

The overall concordance in qualitative outcomes (effective or not) in experimental and natural influenza infections has been good for most antiviral agents and has included both prophylactic and therapeutic activities. For example, once-daily dosing with topical zanamivir and oral oseltamivir were effective for prophylaxis both in the challenge model and in subsequent field studies. Both the prophylactic and therapeutic

effectiveness of oral adamantanes were confirmed in subsequent field studies of uncomplicated illnesses due to adamantane-susceptible influenza A strains. Limited data also suggest that aerosol delivery of adamantanes to the respiratory tract has some efficacy in both experimentally induced [22] and naturally occurring influenza [37]. Oseltamivir results have also shown a good correspondence overall between experimental and natural infections, although differences in response across virus types have been observed. For example, oseltamivir prophylaxis appeared to be somewhat less effective against a high-inoculum influenza B/Yamagata/16/88 infection challenge (10^7 TCID50) compared to the earlier studies employing A/Texas/36/91(H1N1) virus at a lower inoculum (10^5 TCID50) [38]. While the findings may relate to inoculum size differences in part, it is notable that oseltamivir is less inhibitory for influenza B neuraminidases *in vitro* and that several oseltamivir treatment studies have reported slower clinical and virological responses in oseltamivir-treated influenza B-infected children compared to influenza A-infected ones [39,40].

An important issue is how well findings in the challenge model can predict dose regimen selection for subsequent field studies. One limitation of the challenge model is the relatively small numbers of subjects that can be studied at any one time, especially if a range of dose regimens are under consideration. In 69 experimentally infected volunteers randomized to one of 4 oseltamivir treatment regimens or placebo starting 28 h after virus inoculation, a broad range of doses (20–100 mg) given twice daily appeared to exert similar antiviral effects without obvious dose–response, whereas, once daily dosing at 200 mg appeared to be less effective than twice daily in inhibiting virus replication [29]. Based on such observations and the pharmacology of the drug, twice daily administration was chosen for the subsequent field studies of treatment (at doses of 75 or 150 mg twice daily). In this instance the challenge model data was more useful in determining the frequency of dosing rather than the optimal doses for field studies.

Other experiences indicate that the challenge model has provided dose-dependent signals of effectiveness that were not confirmed in later field studies. For example, high doses of oral peramivir showed significant activity in the influenza A challenge model that was not confirmed in a natural influenza treatment study [30]. Oral ribavirin also showed dose-related effects in experimental infection, in that 600 mg daily in three divided doses were largely ineffective as prophylaxis against influenza A(H3N2) or B [41,42] but 1 g daily in four divided doses starting 6 h after challenge mitigated symptom severity after influenza A(H3N2) challenge [43]. However, doses of 1 g/day were therapeutically ineffective in field studies

of uncomplicated influenza [44], whereas much higher doses (8.4 g total, given as initial loading doses and then in divided doses over 48 h) appeared to provide symptom benefit [45]. In summary, limited observations suggest that the challenge model may be more useful in predicting dose frequency, especially when linked to concurrent pharmacokinetic sampling, than target dose level. While pharmacokinetic–pharmacodynamic studies are possible in experimentally infected volunteers, they have not yet been validated for dose selection in natural infections.

Some of these discrepancies in the predictive value of the experimental human infection model for assessing antivirals relate to its design and variability in outcomes. Virological and clinical outcome measures can vary across experiments despite use of serologically pre-screened subjects and the same challenge virus inoculum [30]. Also, in part because of high costs per subject, more recent challenge model studies have used relatively small numbers of healthy adult subjects, who are inevitably partially immune and also not representative of the broad range of persons in the general population at increased risk of severe illness or complications from influenza. As discussed above, the pathogenesis of experimental infection following nasal virus inoculation differs from typical influenza illness in those seeking care and generally causes lower levels of virus replication, a quickly resolving upper respiratory illness of usually mild–moderate severity, and paucity of lower respiratory tract manifestations. Consequently, antiviral interventions initiated during the incubation period or just as symptoms are developing favour detection of therapeutic effects. While this is an advantage with respect to establishing initial proof of efficacy, there is the potential to overestimate drug effectiveness and underestimate drug dose requirements.

In addition to quantitative virological measures (for example, viral shedding patterns in upper respiratory tract, seroconversion) and symptom profiles, virological assessments following experimental infection can also be useful in assessing the frequency, rapidity and mechanisms of emergence of drug-resistant variants. For example, the initial detection of oseltamivir-resistant A/H1N1 virus due to the H275Y mutation in viral neuraminidase was found in the nasal specimens of two volunteers who developed rebounds in viral titres during oseltamivir administration [46]. Subsequently, this mutation was shown to emerge during *in vitro* passage, and has been the predominant mutation recognized in seasonal and pandemic 2009 H1N1 viruses.

Pathogenesis studies during experimental human infections

Some challenge studies have incorporated a number of other outcome measures to assess aspects of viral and host illness pathogenesis. Various challenge studies have examined effects on peripheral blood leukocytes, innate and adaptive immune responses, mucociliary clearance and nasal patency, pulmonary and otologic function, and effects on bacterial flora [14]. With respect to study of antiviral agents, measures like nasal mucus weights, nasal lavage levels of cytokines and other mediators, and middle ear pressures (MEPs) measured by tympanometry can provide non-invasive, objective end points and enhance the value of these studies.

Early studies of innate immune responses found that experimentally induced infections were accompanied by rises in IFN concentrations in nasal secretions and/or blood [4,47,48]. Subsequent studies of placebo recipients infected with influenza A/Texas/36/91(H1N1) virus confirmed rises in nasal lavage IFN-α and other cytokines and chemokines, including interleukin (IL)-6, tumour necrosis factor (TNF)-α, IFN-γ, IL-8, IL-10, monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein (MIP)-1α and -1β [29,49,50]. Although variations in timing and magnitude of responses were noted across studies, increases in pro-inflammatory mediators generally peaked 2–3 days after infection and correlated with nasal viral titres, occurrence of fever, nasal mucus weights and symptom scores [49,50]. In particular, nasal IL-6 responses appear to be associated closely with symptom production [49,51]. Unsurprisingly, prevention of experimental infection by intravenous zanamivir prophylaxis abrogated rises in nasal cytokines and chemokines [35,49]. In addition, early therapeutic administration of oral oseltamivir markedly reduced nasal cytokine and chemokine responses [29]. Such observations indicate that a linkage exists between influenza replication in the respiratory mucosa, elaboration of pro-inflammatory mediators and symptom production in acute influenza. Furthermore, the finding that prompt inhibition of viral replication mitigates such responses shows that replication is driving these events, at least early in infection [29], although further studies of these relationships and the effect of antiviral interventions are needed in naturally infected persons.

Recent studies have also examined the host responses to experimental influenza by assaying gene transcription dynamics in peripheral blood mononuclear cells over time [52,53]. These studies have found expression patterns unique to asymptomatic and symptomatic infections, in which symptomatic subjects show multiple pattern recognition receptors-mediated antiviral and inflammatory responses, possibly related to virus-induced oxidative stress [53]. These observations raise the possibility of having novel molecular targets for both prognostic assessment and therapeutic intervention in influenza, and suggest that challenge studies would be useful for studies of potential immunomodulatory interventions.

A high frequency of minor otologic abnormalities, especially abnormal MEPs determined by tympanometry, accompanies experimental influenza [54,55]. Differences in antiviral effects on these otologic changes in experimentally infected volunteers appear to have corresponded to differences reported in natural influenza. In particular, one controlled study was undertaken to determine if oral rimantadine treatment initiated 48 h after intranasal A/Kawasaki/9/86(H1N1) challenge would affect otologic end points. Rimantadine decreased viral shedding and symptoms compared to placebo, but had no effect on otologic findings, specifically the frequencies of earache, tympanic membrane abnormalities or abnormal MEPs over the week after inoculation [55]. In an earlier study of naturally infected children, rimantadine treatment reduced influenza symptoms and virus titres early but not complaints of earache on day 5 of illness compared to acetaminophen [56]. In contrast to rimantadine, intranasal zanamivir given as early treatment at 26–32 h after A/Texas/36/91(H1N1) virus inoculation significantly reduced the frequencies of abnormal MEPs (32% versus 73%) and of earache or pressure (16% versus 50%) in infected subjects compared with placebo [57]. Similar data has been seen with oseltamivir in the challenge model [29]. Insufficient data are available to know whether intranasal and/or inhaled zanamivir reduces otitis media in patients with acute influenza, but significant reductions in new otitis media diagnoses have been found in influenza-infected children given oseltamivir treatment compared to placebo [58,59]. Furthermore, a recent survey of 180 hospitalized infants less than 1 year of age also reported significantly fewer abnormalities related to head/eyes/ears/nose/throat system (for example, otitis media, conjunctivitis, rhinorrhea) during therapy with oseltamivir (1.4%) compared with recipients of amantadine or rimantadine (15.4%) [60].

Antiviral lessons regarding influenza virus transmission

Antiviral studies in experimental human influenza have also contributed insights into possible sites of influenza virus replication and mechanisms of transmission and a detailed review on this topic was recently published [15]. This paper also reported the results of an initial proof-of-concept study in which experimentally infected subjects transmitted infection to about one-quarter of susceptible close contacts within a quarantine facility. However, no formal person–person experimental transmission interruption studies utilizing antivirals have been reported to date. This is particularly pertinent for agents like IFNs and the NAI zanamivir that are delivered topically to the respiratory tract and when comparisons have been possible between outcomes in intervention studies in experimentally induced and naturally occurring influenza. As discussed below, the discrepancies in intranasal antiviral delivery effectiveness observed between studies in experimental and natural infections need to be carefully considered in extrapolation of results from the challenge model.

As noted above, early work showed that intranasal administration of high doses of lymphocyte-derived or recombinant IFN-α2 appeared to moderate illness severity and viral shedding in volunteers inoculated intranasally with an influenza A/H3N2 [24] or A/H1N1 virus [25], respectively. In household-based post-exposure prophylaxis studies, once daily intranasal administration of intranasal rIFN-α2b to household contacts was highly protective against rhinovirus-specific illness, but no protection was observed against natural influenza infections [26,27]. Similarly, no reductions in ILI or proven influenza infections were observed during 4 weeks of intranasal prophylaxis with rIFN-α2a [28] or against parainfluenza virus infections in another household-based PEP study of rIFN-α2b [61]. A 2005 trial of 1,449 military recruits that tested combined nasal and throat sprays of rIFN-α2b or placebo twice daily for 5 days found no reductions in respiratory symptoms and more epistaxis and dry throat in IFN recipients [62]. However, measurements of immunoglobulin (Ig)M antibodies by ELISA on days 0 and 15 found 76–77% reductions in the risk of influenza A, influenza B and parainfluenza virus-3 infections. The available data indicate that intranasal IFN is partially protective against experimental influenza at high doses but does not prevent natural influenza virus infection or illness. A possible protective effect of topical application of IFN to the pharynx alone or combined intranasal and pharyngeal dosing requires further study.

Because of the uncertainties regarding initial sites of influenza acquisition and spread within the respiratory tract in natural influenza illness, different routes of administration (that is, intranasal sprays, oral dry powder inhalation) were tested for topical zanamivir in early field studies. One household-based, four-way randomized PEP trial found that intranasal zanamivir for 5 days was ineffective in preventing ILI in household contacts, but that inhaled zanamivir (with or without intranasal zanamivir) reduced the risk by about 50%, although the study had few influenza-positive patients [63]. Subsequent placebo-controlled studies showed that if the ill index case in a household tested positive for influenza, inhaled zanamivir reduced symptomatic influenza in contacts with 80% efficacy [32] and that seasonal prophylaxis with inhaled zanamivir at a dose of 10 mg once daily for 4 weeks reduced laboratory confirmed clinical influenza by 67% [64]. Thus, intranasal zanamivir is highly protective against experimental influenza following

intranasal virus inoculation but not against natural influenza, whereas orally inhaled zanamivir is highly protective against natural influenza illness. Of note, zanamivir is detectable in nasal lavages after oral inhalation but the levels are more than 50-fold lower than after nasal dosing [31]. Studies have not determined whether orally inhaled zanamivir reduces nasal virus replication in experimentally infected volunteers.

When initiation of zanamivir dosing was delayed until 32 h after viral inoculation in the challenge model, therapeutic effects were also observed in terms of reduced nasal viral titres and symptoms [36]. The initial zanamivir treatment study in seasonal influenza found that orally inhaled zanamivir, with or without intranasal zanamivir, reduced fever and symptoms when administered early to patients compared to placebo [65]. The addition of intranasal zanamivir had a significant effect on reducing viral titres in nasal washes and may have decreased nasal symptoms but did not improve overall time to recovery. Of note, in a pooled analysis of clinical studies, inhaled zanamivir alone did not reduce upper respiratory complications, such as sinusitis and otitis media, but intranasal plus inhaled zanamivir appeared to have an effect [66]. While orally inhaled zanamivir quickly reduces pharyngeal viral replication [67], inconsistent effects on nasal replication have been found in studies of seasonal influenza treatment. One [68] but not another [65] found decreased nasal viral replication with orally inhaled zanamivir, so that an effect of orally inhaled zanamivir on decreasing the risk of nasal acquisition or transmission of seasonal influenza remains possible. A re-analysis of four household-based studies of inhaled zanamivir and oral oseltamivir with respect to their effect on preventing secondary cases confirmed high protective efficacy of PEP for both antiviral drugs [69]. However, the estimated effect of index case treatment on reducing the infectiousness for contacts was much lower for zanamivir compared to oseltamivir. In contrast, a retrospective analysis of household-based antiviral use in Japan during the 2009 H1N1 pandemic found that early inhaled zanamivir treatment of ill index cases appeared to reduce secondary illnesses in contacts by about 40% [70]. Thus, orally inhaled zanamivir treatment of natural influenza in adults does not appear to consistently reduce nasal viral replication or upper respiratory tract complications, but may diminish the likelihood of secondary infections in household contacts.

In summary, in contrast to findings with intranasal IFNs or intranasal zanamivir in experimental human influenza, administration of these antivirals to the nose alone does not prevent seasonal influenza infection or illness. These findings suggest that the nose is not the only or even principal site for influenza acquisition, although nasal viral replication and symptoms in the index case might be important for seasonal influenza transmission to others under close contact conditions. The fact that orally inhaled zanamivir prophylaxis is highly protective against seasonal influenza illness and also effective for its treatment suggests that the pharynx and/or tracheobronchial tree are key anatomic areas for initial virus acquisition and replication. The available data do not address the relative importance of nasal deposition following large droplet transmission but suggest that it may be less important in seasonal influenza than for other respiratory viruses.

Conclusion

Experimentally induced human influenza infections have been used for decades to assess the effectiveness, tolerability and pharmacological properties of influenza antivirals. The results of such studies have provided key proof-of-concept data to facilitate drug development and also contributed to our understanding of influenza pathogenesis and transmission. Such insights should facilitate the development of both novel therapies, including potential immunomodulatory ones, and non-pharmacological interventions, respectively. However, the current lack of approved influenza virus challenge pools in the United States and most other countries has hindered development of new drugs and vaccines and needs to be resolved, so that these important studies can go forward in the future.

Disclosure statement

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