# 19

PERSPECTIVES IN THE USE OF ANTIVIRAL AGENTS FOR PREVENTION AND TREATMENT OF RESPIRATORY VIRUS INFECTIONS

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## THE NEED FOR ANTIVIRAL CHEMOPROPHYLAXIS AND THERAPY

### Impact of Respiratory Viral Infections

Respiratory viral infections are the most common infectious diseases of man and a major cause of morbidity and mortality globally. In developing countries respiratory viruses remain an important cause of childhood mortality (1). Acute respiratory diseases, which are frequently caused by viral infections, account for 20% of childhood mortality and in some parts of the world are the commonest cause of death in children (1). It is estimated that 2.2 million deaths occur throughout the world annually as a result of acute respiratory diseases. These diseases account for about 13% of all deaths in Africa, Central America and the developing countries of Asia, compared to 3% in North America (1). In developed countries certain viral infections continue to cause significant mortality in high-risk groups, particularly infants and the elderly. In the United States, influenza virus epidemics have been estimated to have been associated with over 10,000 excess deaths during 18 of the past 28 years (2).

Viral respiratory tract infections of adults and children are important targets for antiviral chemoprophylaxis and therapy because of their frequency and cummulative morbidity. According to 1981 estimates, the average individual in the United States suffers 1.05 nontrivial upper respiratory illnesses or bouts of influenza per year (3). These episodes account for 36% of days lost from work and 54% of days lost from school due to acute conditions (3). The common cold has been estimated to cause about 250 million days of restricted activity and about 30 million days lost each from work and school (4). Other studies have found that the average adult experiences 2.3 (5) to 5.6 (6) common cold episodes each year. Rhinoviruses alone cause nearly one infection per adult per year (7). Annual expenditures in 1985 for over-the-counter cold symptom treatments were estimated to be $556 million (8), and others have estimated that sales of proprietary cold remedies exceed one billion

DeClercq, E. (ed.), *CLINICAL USE OF ANTIVIRAL DRUGS*. 

dollars per year (4). In the United States the economic costs of an influenza pandemic has been placed at 5-6 billion dollars (9), and in epidemic years the costs related to excess hospitalizations are measured in hundreds of million of dollars (10).

Respiratory viruses, including those like rhinovirus that typically produce upper respiratory symptoms, have been implicated in exacerbations of asthma and chronic obstructive airways disease (11-13). Respiratory syncytial virus infections commonly cause lower respiratory tract involvement in the form of bronchiolitis and pneumonia and have been associated with attacks of childhood asthma and prolonged pulmonary function abnormalities (13). Influenza virus infections are well-documented causes of acute deteriorations in asthma and chronic bronchitis patients and have been associated with prolonged small airways functional abnormalitities and airway hyperreactivity in previously healthy adults. The available evidence suggests that the successful use of antiviral agents for prevention and possibly therapy of respiratory viral infections could offer substantial clinical benefit to those with preexisting airways disorders. The role of respiratory viral infections in the pathogenesis of chronic pulmonary disease in adults (14) is unsettled but effective interventions could potentially modify the development of lower respiratory tract problems in certain groups.

Certain respiratory viruses, especially influenza (2) and RSV (15), are nosocomially transmitted agents which infect both hospital personnel and patients. Infected patients often have serious complications and prolonged hospitalizations following these infections. Such groups comprise additional target populations for the use of antiviral agents to prevent the consequences of respiratory viral infections.

Etiologic Agents and Related Clinical Syndromes

The respiratory viral pathogens accounting for the majority of illnesses are listed in Table 1. These viruses share the respiratory tract mucosa as the initial and principal sites of replication and disease expression. A number of other viruses can cause infections with respiratory tract manifestations, often as part of systemic infection, such as Epstein-Barr virus mononucleosis or cytomegalovirus pneumonitis, but these infections are considered in other chapters. The respiratory viruses differ from each other in fundamental biochemical characteristics and the nature of their host cell interactions, as well as in antigenic diversity, epidemiologic patterns, and clinical manifestations of infection. Differences in virus-host cell interactions will necessitate the development of virus-specific agents for most

infections. The differing epidemiologic patterns of infection need to be considered in identifying effective strategies for the use of antiviral agents and designing field studies of drug efficacy.

Table 1. Characteristics of the Respiratory Viruses

| Virus (Family) | Nucleic Acid | Lipid envelope | Virion diameter (nm) | #antigenic serotypes |
|---|---|---|---|---|
| Rhino (picorna) | ssRNA | - | 20-30 | 100 |
| Influenza (orthomyxo) | ssRNA (segmented) | + | 80-120 | 3 types-A,B,C, (3 A subtypes, many A & B strains) |
| Parainfluenza (paramyxo) | ssRNA | + | 150-300 | types 1-4 |
| Respiratory syncytial (paramyxo) | ssRNA | + | 150-300 | 1 (2 or 3 subtypes recognized) |
| Adeno (adeno) | dsDNA | - | 60-90 | 41 |
| Corona (corona) | ssRNA | + | 75-160 | $\geq$4 |

Abbreviations: ss= single-stranded, ds = double-stranded

This large group of viruses causes a relatively small number of overlapping clinical syndromes (Table 2), the manifestations of which depend on the site and agent of infection. The common cold, pharyngitis, laryngitis, tracheobronchitis, and pneumonia are recognized as typical clinical expressions of infection at different levels of the respiratory tract. In children, croup (laryngotracheobronchitis) and bronchiolitis are additional age-related syndromes. Many unrelated viral pathogens, and some nonviral respiratory pathogens such as mycoplasma, are capable of causing similar clinical syndromes. Not only are there potentially multiple etiologies for each syndrome, but each type of virus is capable of causing different clinical syndromes. These observations suggest that antiviral chemoprophylaxis against all or most respiratory viral illnesses would necessitate continuous use of a safe broad spectrum agent or combination of agents. This possibility appears remote at the present time.

Table 2. Common Respiratory Syndromes and Associated Viral Etiologies

| Virus | Common Cold | Pharyngitis | Tracheo-bronchitis | Croup | Bronchiolitis | Pneumonia |
|---|---|---|---|---|---|---|
| Rhino | +++ | + | ± | | | |
| Influenza | + | + | +++ | ++[a] | +[a] | ++ |
| Para-influenza | + | + | ++ | +++[a] | ++[a] | ++[a] |
| RSV | + | + | ++ | ++[a] | +++[a] | +++[a] |
| Adeno | ++ | +++[b] | + | | + | ++[b] |
| Corona | ++ | ± | | | | |

Abbreviations: +++ = most commonly associated virus, ++ = commonly associated, + = associated, ± = possibly associated
a = infants and young children, b = military personnel

Rapid Viral Diagnosis

Rapid viral diagnosis is an integral part of the successful application of virus-specific antiviral agents for management of respiratory tract infections. Factors which are helpful in making a specific diagnosis include knowledge of the age and general condition of the host, time of year, and epidemiologic characteristics of the viruses under consideration. Certain clinical presentations, such as RSV bronchiolitis in young children or febrile respiratory illness in an adult during a known community outbreak of influenza, are sufficiently characteristic to allow a high likelihood of accurate etiologic diagnosis.

However, clinical and epidemiologic evaluation will usually not provide sufficient information to identify a specific viral pathogen, and laboratory documentation of infection is required. Serologic studies are helpful in retrospect but too slow to be beneficial for the therapeutic decisions in an individual patient. Viral isolation may also not be rapid enough to be of value in making clinical decisions for individual patients. Between two and ten days are required to isolate most respiratory viruses (16). Specimen quality and method of collection are important related variables. For example, nasopharyngeal washes or aspirates have a higher yield of virus recovery than do throat swabs for some respiratory pathogens including RSV (17) and rhinovirus, whereas throat swabs are better for isolation of adenovirus (18). At the community level, the virology laboratory can provide surveillance data, helpful in implementing antiviral prophylaxis or treatment.

The rapid detection of viral antigens by immunofluoresence and enzyme-linked

immunoassays is gaining wider availability. Several types of sensitive diagnostic test systems are commercially available for RSV. The development of rapid accurate diagnostic methods is needed for other respiratory viruses. In the future, effective intervention will probably depend on the use of narrow spectrum agents in conjunction with rapid etiologic diagnosis.

Limitations of Immunization

Active immunization would in theory be the optimal means to prevent respiratory viral disease. One major problem with immunization is the vast antigenic diversity of the respiratory viruses (Table 1). Rhinoviruses, which account for about 40% of common colds, currently number 100 different serotypes. Even for infections like influenza where effective vaccines exist, the vaccine may lose efficacy because of antigenic changes in the epidemic strains and because of waning host immunity. These limitations necessitate reformulation of the influenza vaccine and annual administration to high risk individuals. The latter requirement has probably contributed to the relatively low utilization rates of inactivated influenza vaccines in the United States. Considerable investigation has been directed at developing attenuated, intranasally-administered vaccines which would be expected to stimulate local immune responses and perhaps provide more solid and longer-lasting protection (19,20). The development of new vaccines may enable implementation of more effective immunization programs. Use of the oral, attenuated adenovirus vaccine by military recruits has proven effective in controlling acute respiratory disese caused by adenovirus types 4 and 7 in this specific population (21). For other viral pathogens, like respiratory syncytial virus, the development of a safe and effective vaccine for infants and young children has not been successful. An inactivated vaccine led to enhanced illness when natural infection was acquired (22), and an attenuated vaccine was not protective (23). According to the author of one infectious diseases monograph "Human respiratory viruses are among the most sucessful animal viruses in the world. Many show regular antigenic variation, and ... are perhaps entering their golden age, with an almost unlimited supply of susceptible hosts..." (24).

Present Status of Antiviral Agents

Both the development and acceptance of effective antivirals for respiratory viral infections has been slow. In the United States effective chemotherapy is currently limited to two licensed drugs, oral amantadine for influenza A virus infections and aerosolized ribavirin for RSV bronchiolitis and pneumonia. This situation contrasts with the extensive and growing number of effective antimicrobic agents used for treating nonviral respiratory tract infections. Advances in the

field will depend in part on identifying other potent, selective antivirals.

Paradoxically, confusion also exists concerning the appropriate use of the few available antiviral drugs. For example, in 1968 amantadine became the first systemic antiviral agent licensed for use in the United States. Despite its documented efficacy in the treatment and especially prevention of influenza A virus-induced illness, amantadine has only recently began to gain acceptance by the medical community.

## APPROACHES TO THE CONTROL OF RESPIRATORY VIRAL ILLNESS

### Prophylaxis of Infection

Methods for controlling respiratory viral illness can be divided into those which prevent transmission of infection and those used for treatment of an established infection. Strategies aimed at protecting uninfected contacts require knowledge of the epidemiology and modes of transmission of the particular virus. In general, transmission requires exposure of a susceptible respiratory tract mucosa to infectious virus. The infective doses, initial sites of infection (nasal passages, conjunctiva, pharynx, or lower respiratory tract) and the associated mechanisms of transmission (direct contact with secretions, exposure to large droplets or inhalation of small particle aerosols) have not been defined for naturally occurring infections but appear to differ for the different respiratory viruses. These differences may have especially important implications for the use of topical antiviral agents. For example, intranasal application of an antiviral may not provide protection against viruses like influenza which can initiate infection in the lower respiratory tract.

Environmental or barrier control measures. For rhinovirus, exposure of the hands to a virus-ladden fomite or hand with subsequent self-inoculation of the conjunctiva or nasal mucosa is an efficient mechanism for spreading infection under experimental conditions (7). Thus, measures aimed at preventing rhinovirus infections might involve mechanical barriers, such as paper handkerchiefs, to prevent contamination of hands and the environment; barriers to interrupt the process of self-inoculation; and practices to remove infectious virus from contaminated hands, such as efficient hand washing. In an experimental setting, the use of paper handkerchiefs reduced rhinovirus contamination of the hands of ill subjects and decreased the frequency of transmitting infection to volunteers exposed by hand-contact with ill subjects (25).

Barrier measures have been investigated to reduce the risk of nosocomial RSV transmission to other infants and hospital staff. The routine use of masks and

gowns has been shown to be ineffective in reducing transmission of RSV to hospital personnel caring for infants with the disease (26) or to other infants (27). However, the use of disposable eye-nose goggles by hospital staff has been found to reduce the risk of nosocomial RSV infection in both infants and hospital personnel (28).

Considerable investigation is currently directed at the use of compounds that cause direct or contact inactivation of infectious virus. Such virucidal agents could supplement the effects of mechanical barriers and have been formulated in various ways to determine the feasibility of interrupting transmission. Paper handkerchiefs impregnated with virucidal materials such as citric acid and malic acid appear to be effective in blocking transmission of rhinoviruses under experimental conditions (29,30). Also, under experimental conditions, disinfection of contaminated fomites (31) or the application of virucidal substances (iodine) on the fingertips have been shown to reduce the risk of rhinovirus transmission (32). Virucidal hand lotions may have a similar effect in preventing hand contamination with subsequent self-innoculation or transmission to others. Although of considerable potential value, these approaches have not yet been proven to be effective in preventing natural rhinovirus infections.

Attention to the circulation of air from hospital rooms of persons infected with viruses known to be transmitted by aerosol such as influenza may serve to decrease nosocomial transmission. Attempts to interrupt spread of respiratory infections by air purification with ultraviolet light was found to reduce the risk of measles but not common cold transmission in schools (33).

Immunization. Passive immunization is a theoretical possibility where the agent of infection and the timing of exposure are known. Systemic administration of specific antiviral antibodies has been shown to be effective for certain viral infections like measles and varicella, which have a respiratory tract portal of entry followed by viremic dissemination. Whether systemic or topical administration of antibody would be useful in prophylaxis of infections limited to the respiratory tract remains to be determined. Such an approach would not be feasible for the common cold but might have potential value in high-risk patients after known exposure to viruses of known antigenic type, such as RSV or parainfluenza virus. Passive immunization with intravenous globulins rich in antibody to RSV for prophylaxis and treatment has proven promising in the cotton rat model and is currently under investigation for treatment in a primate model of this disease (34).

Antiviral chemoprophylaxis. Effective chemoprophylaxis generally requires administration of the agent for the duration of the exposure. This period depends on

the prophylaxis strategy (seasonal, postexposure, institution-based), the viral pathogen, and in the case of postexposure use, the duration of illness and viral shedding in the index patient.

In formulating prophylactic antiviral strategies for syndromes such as the common cold, it would be desirable to employ an agent active against a broad range of potential pathogens. Interferons have in vitro activity against most respiratory viruses but intranasal interferon alpha-2 has been ineffective in field studies for preventing infections due to viruses other than rhinovirus (35,36). Reduction in the severity of symptoms among those infected with parainfluenza was observed during one prophylaxis study with interferon alpha-2 (36), but this study and others (35,37) have not found reductions in parainfluenza infections. The success of intranasal alpha interferons for prophylaxis of influenza A and B has also been limited (38-40).

One concern regarding antiviral chemoprophylaxis is the successful prevention of infection, such that the protected patient remains suseptible to infection upon subsequent exposure. Optimally a chemoprophylactic agent would allow subclinical infection and associated natural immunization to occur without the burden of illness. In rhinovirus infections, high dose intranasal interferons can prevent both infection and illness after experimental challenge, whereas lower doses allow infection but protect against illness (41). Oral amantadine and rimantadine are more protective against influenza A virus-induced illness than against laboratory-documented infection (42) but still leave a substantial proportion of recipients susceptible to later infection.

Since most respiratory viral infections are self-limited illnesses, potential antiviral agents must have very high therapeutic indices. In particular, with drugs used for prophylaxis in healthy children or adults, only a fraction of whom will be expected to contract the infection, the antiviral must be free from significant toxicity. Because of the frequency of respiratory viral infections, antiviral agents can be anticipated to have repeated use and must also be free of significant cummulative side effects.

Treatment of Established Infection

Objectives in the treatment of an established respiratory viral infection are to reduce the severity of symptoms and decrease the risk of serious morbidity or secondary complications. Treatment of such infections may be directed principally at providing symptomatic relief with no effect on viral replication or at inhibiting viral replication. These approaches are not exclusive, and combinations of antiviral and symptomatic drugs may ultimately provide the greatest benefit.

Preventing transmission of infections is also an important goal of treatment.

This could be accomplished by use of antiviral drugs which limit viral replication or perhaps by reducing illness manifestations (mucus production, cough, sneezing) that are important in transmitting infection. For example, in a mouse model of influenza, rimantadine treatment was effective in reducing pulmonary virus titers and the risk of transmitting infection to untreated contact animals (43). Drugs which reduce symptoms without affecting viral growth could theoretically reduce the risk of transmission but this has not been tested directly.

Symptomatic regimens. Symptomatic treatments for respiratory viral disease have a wide acceptance and utilization throughout the world. In Western nations oral antihistamines and oral or intranasal decongestants are major symptomatic therapies for the common cold and have been shown to provide some reduction in cold symptoms (44). Careful study of the pathogenesis of illness production in different infections may identify host responses that could be specific targets for symptomatic interventions. For example, release of histamine does not appear to play a significant role during rhinovirus colds, whereas high concentrations of kinins have been found in the nasal secretions of symptomatic subjects (45). Drugs which modify the production or biologic effects of kinins might be candidates for symptom control.

Adverse effects of such commonly used treatment regimens need to be considered in the cost-benefit analysis of their value. Placebo-controlled trials have found mild central nervous system side effects with oral antihistamines used alone (46) or in combination with amantadine (47). In experimental rhinovirus colds (48), aspirin therapy was associated with increased viral shedding compared to placebo. If such an effect occurred in naturally occuring rhinovirus infection, it is possible that aspirin treatment could increase the risk of spread of infection to others. The association of Reye's syndrome and salicylate use during certain viral infections including influenza (49) demonstrates another undesirable unexpected adverse consequence of symptomatic therapy.

Certain traditional folk remedies which are culturally accepted as providing symptoms benefit may have biologically relevant activities that could explain their usefulness. Ingestion of hot chicken soup has been advocated by many generations as a remedy for the common cold. This common remedy may provide symptomatic relief because of its ability to increase nasal mucus velocity (50). In China, traditional herbal remedies have been used for many generations to treat upper respiratory tract disease or mild disease of the lower respiratory tract. For severe illness the herbal therapy is combined with Western therapies (1). A flavone isolated from the Chinese medicinal herb Agastache Folium has been shown to have potent *in vitro* anti-picornavirus activity, perhaps by inhibition of viral replication (51).

Additionally, it has been suggested that radix astragalus, used in traditional Chinese medicine, stimulates IgA secretion and induces interferon production when given orally or by aerosol (1). As such this remedy may have or be capable of inducing specific antiviral activity.

Antiviral chemotherapy. Antiviral therapy would be expected to provide benefit, if ongoing viral replication was central to the pathogenesis of symptom development. Since most respiratory infections are self-limited and have relatively short periods of viral replication, early initiation of treatment is needed to exert a beneficial effect. If the severity or duration of clinical illness is not related to the degree of viral replication, then specific antiviral chemotherapy would probably fail to substantially affect the clinical course. Host responses, such as the release of inflammatory mediators or immune-mediated injury, may play greater roles than on-going viral replication in causing disease in certain infections. Assessments of the duration and degree of viral replication, reflected in the concentrations of virus shed in respiratory secretions, and their relationship to the degree of illness and possibly long-term sequelae are important in this regard.

Another concern in the use of antivirals for treatment of respiratory virus infections, as in prophylaxis, is the possible inhibition of normal host immune responses to infection. This could theoretically leave the host susceptible to reinfection with the same virus serotype. No differences in seroconversion rates have been observed with the use of amantadine or rimantadine for treatment of uncomplicated influenza compared with controls (52, 53). A related concern is the possibility of a rebound in viral replication if treatment ceases before host responses are sufficient to control the infection. Increased prevalence of viral shedding without associated clinical deterioration has been described after low dose, short-term intranasal interferon prophylaxis of experimental rhinovirus infections (41) and after oral rimantadine therapy for natural influenza A virus infections in children (54). The clinical significance of these observations remains to be determined, but suggests the need for longer duration of treatment. Prolonged or recrudescent infections are of particular concern in children with primary or acquired immunodeficiency syndromes (55).

With widespread use, the development of resistance to antiviral agents is an increasing concern. Drug resistant influenza A viruses are readily recovered under laboratory conditions and have been reportedly isolated from non-drug exposed patients (56). During the course of experimental avian influenza (57) amantadine and rimantadine-resistant viruses were readily isolated from drug treated birds. These viruses were also shown to infect and cause illness in contact birds receiving

amantadine prophylaxis. Because large amounts of replicating virus are exposed to drug during therapeutic use, it may increase the likelihood of selecting drug-resistant virus relative to prophylaxis. Monitoring of the drug susceptibilities of respiratory viruses will be an important part of future studies.

Topical Administration of Respiratory Antivirals

Although differing in a number of characteristics, the respiratory viruses share a common affinity for the human respiratory tract. For the use of antivirals, this means that effective drug concentrations must be achieved at the site of infection within cells of the respiratory epithelium. Certain antivirals like interferons may exert activity through interaction with cellular receptors and induction of intracellular mediators, but adequate delivery of agents to susceptible areas of respiratory mucosa remains a major practical problem. The use of orally or parenterally administered antivirals is often limited by systemic toxicity. Alternatively, the topical application of antivirals to the respiratory mucosa may achieve high regional antiviral activity and reduce the risk of systemic side effects. This approach has been used successfully with aerosolized ribavirin, in contrast to oral administration (58), and with intranasal interferon.

The nature of the drug and its delivery system are important variables in topical application of antivirals to the respiratory tract. Optimal characteristics of solubility and vehicle composition have not been well defined, but water solubility appears to be advantageous. The convoluted and extensive surface area of the nasal mucous membranes and mucociliary clearance mechanisms have been obstacles to the delivery of intranasal medications. Limited studies of intranasally administered interferons have suggested that they are cleared rapidly like particulate materials. Additionally, beta interferon may be directly inactivated by nasal secretions (59). Interventions to maintain higher local concentrations, such as the use of oral antihistamines to decrease clearance or of saturated cotton pledgets, reduce the amount of interferon needed to achieve an antiviral effect but are probably not of practical value (60). Studies using radiolabelled albumin solutions have found that the intranasal distribution of coarse nasal sprays administered to volunteers is much poorer than that of nasal drops (61), especially if the subjects are upright rather than supine (62). Depending on the volume, velocity, and particle size of the spray, materials are frequently deposited in the vestibule or anterior nasal passages and do not distribute well to the nasopharynx. In volunteer studies of experimental rhinovirus colds, administration of interferon alpha-2 by nasal drops appeared to be associated with greater antiviral effects and clinical benefit than when given by nasal spray (63). Another concern with topical

administration is the development of local toxic reactions. Intranasal interferon use avoids the dose-related toxicities of systemic administration but may be associated with the frequent occurance of local irritation and mucosal histopathologic changes (64).

The type of delivery system is also critical when administering drugs by aerosol to the lower respiratory tract. The development of efficient and reliable small particle aerosol generator units has been central to the successful application of aerosolized ribavirin. Currently available delivery systems are limited to use in institutionalized patients because of the requirement for prolonged and often continuous exposure periods and because of the need to monitor the apparatus. In situations where oral administration may be unreliable and where no parenteral form exists, aerosol may provide an alternative means of drug delivery. Aerosolized rimantadine was as effective as oral rimantadine in experimental influenza illness (65) and one study suggested a beneficial effect of intermittent aersolized amantadine in uncomplicated influenza (66). On the other hand, aerosol therapy may cause local irritation or exacerbate preexisting lung disease. In this regard aerosolized amantadine was associated with reversible abnormalities (67), whereas aerosolized ribavirin has been remarkably well tolerated in infants with RSV bronchiolitis and pneumonia.

Strategies For Use

Because of the frequency of respiratory viral infections, clinical use of antiviral agents depends on developing strategies that are effective and safe during long-term or repeated drug administration. In addition, these strategies must be economically feasible and accepted by both physicians and the public. Medication taken on a seasonal basis to prevent common colds would not be successful in the market place if its cost were excessive. Acceptability will be influenced by the complexity of the dose regimen, route of administration, particular target population, and demonstration of cost-effectiveness. Acceptability will also depend on accessibility. If the availability of antivirals for respiratory tract infections is limited to physician-based prescribing, then use will be restricted principally to therapeutic administration and perhaps to prophylaxis in high-risk groups.

These points raise several important questions. If antiviral agents become available to prevent or treat common colds, should they be available without prescription, as are most symptomatic cold remedies? What is the potential for misuse by consumers and what are the risks of selecting drug-resistant viruses? What is the potential effect that effective respiratory antivirals would have on the current widespread use of antibacterials for upper respiratory illnesses? And could these

potential changes in antibiotic prescribing have an impact on bacterial resistance patterns?

Seasonal prophylaxis. Viruses which occur predictably in a given locale are potential targets for seasonal prophylaxis. This approach can be most effectively used against viral infections that occur in epidemics of short duration. This pattern is classically shown by the influenza viruses which cause annual outbreaks, lasting 6-8 weeks in a particular region. Daily oral administration of amantadine or rimantadine during community outbreaks of influenza has been proven to be protective (70-90% efficacy) against influenza A virus-induced illness in placebo-controlled studies (42). However, premature discontinuation of prophylaxis can result in an increased risk of infection (68). Prophylaxis might serve not only to protect the subject receiving medication, but also to confer protection on untreated contacts by reducing the number of infectious sources in the family unit or other closed population. For example, recent studies have found that rimantadine prophylaxis of school children during influenza A outbreaks reduces the risk of illness in untreated family contacts (69).

Many other viruses, including respiratory syncytial virus, parainfluenza virus types 1 and 2, rhinovirus, and coronavirus have unique seasonal patterns, but the longer duration of their periods of activity, the variable age-associated illness rates, and the occurrence of overlapping periods of activity are important variables in considering seasonal prophylaxis with antiviral agents which are virus-specific. The broad *in vitro* antiviral spectrum of interferons and the encouraging results in volunteer models of experimental infection engendered hopes that long-term or seasonal use would protect against a wide range of respiratory viruses. As discussed above, intranasal interferon alpha-2 has been proven to prevent only natural rhinovirus infections in studies to date, and its prolonged use (weeks) has been associated with frequent nasal side effects (64). These shortcomings appear to preclude its long-term use in healthy adults. Studies of intranasal interferon alpha-2 in asthmatic children have found some evidence of protection against respiratory illness during intermittent use over a period of three months (70), but further studies are needed in high-risk groups. A recent tolerance trial with interferon beta-serine$_{17}$ indicates that it may have lower potential for side effects but its efficacy remains to be established (71).

Postexposure prophylaxis. Prophylaxis after exposure to a person with respiratory illness, such as commonly occurs in the family setting, is an effective approach where there are high rates of secondary transmission. The prinicipal reservoir of many viruses appears to be the upper respiratory tract of school

children (72). The common pattern of transmission involves spread within the school system and subsequently within the household. Epidemiologic studies have shown this to be the case for rhinovirus colds, as well as for influenza and respiratory syncytial virus infections, where school-aged children are frequently implicated as introducing the virus into the household. Short-term (7 days) prophylatic use of intranasal interferon has been recently shown to be an effective means of preventing transmission of rhinovirus colds in families (73,74). One study of oral amantadine (10 days) found marked reductions in influenza illness occurence in amantadine-treated household contacts compared to placebo (75), but a subsequent study by the same investigators failed to confirm their initial observations (76). Further studies are needed to determine whether this approach is an effective and practical one.

Institution-based prophylaxis. Work sites as a place of viral transmission have received increasing study. In contrast to the household setting, rhinovirus infections do not appear to spread very efficiently among persons at work. Other viruses, particularly influenza and RSV, are well documented nosocomial pathogens (2,15). Use of antivirals to prevent transmission of these agents from patient to staff and from staff to patient would be one strategy for controlling this problem. One placebo-controlled study found oral amantadine administration to hospitalized patients was efficacious in preventing nosocomial influenza during a documented community outbreak (77). An uncontrolled study suggested that amantadine administration to patients and staff may prevent the further spread of an established nosocomial outbreak of influenza (78).

Prophylaxis of other instutitional populations, such as those in nursing homes, boarding schools, or day care centers is appropriate where there is a documented risk of outbreak occurence. Amantadine and rimantadine have proven prophylactic efficacy in such settings (79).

Treatment. Therapeutic strategies for the use of an antiviral agent depends on the frequency of infection and its associated morbidity including both short and long-term sequelae. Since use is limited to those individuals who are symptomatic, some drug side effects may be acceptable, particularly in individuals who are at high risk for serious complications. Important targets for therapy include influenza virus infections in children and adults, and respiratory syncytial virus, parainfluenza virus, and adenovirus infections in children. Oral amantadine and rimantadine have significant therapeutic effects in uncomplicated influenza A virus infections of adults. Amantadine has been shown to speed the resolution of small airways functional abnormalities (80). However, it remains undetermined whether either drug can prevent the complications of influenza in high-risk patients or

accelerate the resolution of established viral pneumonia (81). Similarly, aerosolized ribavirin has significant antiviral and clinical effects in RSV bronchiolitis and pneumonia of hospitalized children (82), but it is unclear whether its use can decrease the need for ventilatory support during short-term management or reduce the risk of long-term complications.

Other viruses, such as rhinovirus and coronavirus, are such common causes of infection that they have a high cumulative burden of morbidity and economic losses. They may be associated with important complications, including exacerbations of airways disease in those with chronic lung disease and bacterial infections of the ear and sinuses in previously normal patients. For these reasons, these infections are also appropriate targets for treatment. However, recent studies using intranasal interferon for treatment of natural colds have found no evidence of symptomatic benefit compared to placebo (83). An earlier study of intranasal enviroxime also found that it was ineffective in natural colds (84).

## DEVELOPMENTAL TESTING OF RESPIRATORY ANTIVIRAL AGENTS

### Types of Antiviral Agents

The development of selective antiviral agents depends on identifying drugs which inhibit virus-specific events with no or minimal effects on host cell function. Neutralizing antibodies can interact with virus particles to prevent attachment. Agents which competitively bind to specific host receptor sites could also prevent virus attachment. For example, human rhinoviruses can be divided into a major group representing nearly 90% of serotypes that share a single type of host cell receptor (85) and a minor group which utilizes a different receptor. Monoclonal antibodies directed against the major human cell receptor sites are potent inhibitors of rhinovirus replication *in vitro* (86). Intranasal administration of rhinovirus receptor monoclonal antibody has recently been shown to delay the onset of symptoms and virus shedding following experimental rhinovirus infection in man (87).

Preventing penetration of virus into host cells and viral uncoating are also sites for selective inhibition of viral replication. Certain types of chemical compounds (chalcones, WIN 51711) appear to directly bind to the rhinovirus protein-shell and inhibit replication by essentially trapping the genome inside the virion particle (88,89). To be effective such antivirals must have pharmacological properties that allow direct interaction with the virus particle. The adamantane compounds (amantadine, rimantadine) may exert antiviral activity at the stage of uncoating of the viral genome although their exact mechanism of action is undetermined (90).

With the identification of specific viral enzymes it may be possible to develop antiviral agents which act preferentially on the enzyme or substrate (91). Synthetic peptides are being developed to competitively inhibit the function of critical viral enzymes. For example, the amino acid sequence of the fusion activity region of the influenza HA has been identified and small peptides of the region have been synthesized with the hope of competitively inhibiting its activity. Similar fusion sequences have been synthesized from the parainfluenza F proteins (92). Glycoprotein biosynthesis can be inhibited by interfering with intracellular transport, proteolytic cleavage, or glycosylation of the protein (91). Cleavage of influenza HA is required for virus infectivity and is performed by host cell proteolytic enzymes. Certain protease inhibitors have been shown in animal models to reduce influenza virus titer and NA activity (93).

Other potential sites for antiviral action include inhibition of viral nucleic acid transcription or translation. For example, ribavirin monophosphate inhibits inosine monophosphate dehydrogenase, an enzyme responsible for the synthesis of guanine necleotides (94). After conversion to the triphosphate, ribavirin inhibits steps in the capping and elongation of mRNA. The assembly and release of viruses from infected cells are additional possible targets for antiviral agents.

An approach to more effective antiviral chemotherapy might be the use of combination therapy. By using two agents with different modes of action, an enhanced or synergistic antiviral effect can occasionally be achieved. Synergistic anti-rhinovirus activity has been demonstrated *in vitro* when various interferons are combined with other anti-rhinoviral agents (95). Interferons also have a synergistic effect *in vitro* in combination with rimantadine or ribavirin against influenza viruses (96). *In vitro* and *in vivo* animal model data indicate that combinations of ribavirin and amantadine or rimantadine also offer promise (97,98), but clinical trials have not yet been conducted.

Preclinical Testing

The obvious goal of research in antiviral chemotherapy is the development of agents which are both effective and safe when used in man. As for other antivirals, efficacy and toxicity testing are performed initially in appropriate cell culture and animal model test systems. The relationships between inhibitory concentrations under *in vitro* conditions and achievable drug levels in blood or respiratory secretions have not been established for respiratory antivirals. This relates to both a lack of standardized *in vitro* test methods and an incomplete understanding of relevant pharmacokinetic parameters in man. Such correlations would be very useful in helping to select optimal dosing regimens. For influenza A viruses, a plaque

inhibition assay (99) found inhibitory concentrations of amantadine and rimantadine (0.2-0.4 μg/ml) that could be readily achieved in blood and respiratory secretions in man and appears to predict the therapeutic efficacy of these agents. For other compounds, like ribavirin which undergoes intracellular phosphorylation to its active form, correlations between concentrations of the parent drug active in vitro and achievable drug levels in vivo are even less certain.

The identification of a compound which has potent in vitro activity and little or no cell toxicity unfortunately does not guarantee similar effects in vivo. For example, enviroxime which has significant anti-rhinovirus activity in cell and organ culture had little effect on rhinovirus replication when administered intranasally (84), and oral administration was associated with unacceptable gastrointestinal side effects. Additionally, different serotypes of a certain virus may have differing susceptibilities to certain agents. Rhinovirus sensitivity to various interferons, for instance, has been shown to vary with serotype in certain cell culture systems (100).

Adequate small animal models that reflect the virologic and clinicopathologic events seen in man do not exist for some human respiratory virus infections such as rhinovirus or coronavirus. A recently described mouse model of rhinovirus infection remains to be validated. Certain nonhuman primates have been used for antiviral testing (101), but maintaining these animals and wide-scale testing are very costly. The testing of certain antivirals, like interferons, is also limited by species-specific activity and toxicity. Furthermore, considerable differences may exist in the pharmacology of drugs between different species of test animals and man. For example, amantadine metabolism and excretion differ markedly in rodents, other small animals, and man (102). Such observations highlight the difficulty in extrapolating the results of in vitro or animal model testing to practical applications of antivirals in man.

Volunteer Testing

Because of the limitations of available animal models and the uncomplicated course of most respiratory viral infections in adults, human volunteers experimentally challenged with one of the respiratory viruses are often used to determine drug efficacy under carefully controlled conditions. Subjects, whose susceptibility to infection is based on serum antibody titers, are inoculated with a quantity of virus known to cause infection and usually an associated illness. The study drug is evaluated in a double-blind, randomized comparison with an appropriate placebo given by the same route. By altering the time of initiating drug administration in relation to virus challenge, it is possible to assess prophylactic

and/or therapeutic activity. These models have also been used to address the questions of dose regimen and mode of drug administration. Human models of experimental rhinovirus, coronavirus, influenza A and B viruses, respiratory syncytial virus, and parainfluenza virus infections have been used in antiviral studies.

Studies in these models of induced infection have correctly predicted the prophylactic efficacies of oral amantadine and rimantadine for influenza A virus and of intranasal interferons for rhinovirus and the theraputic activity of aerosolized ribavirin in RSV infections. In other circumstances the results observed in experimentally induced infections have not corresponded to observations from field studies of naturally occuring infections. Such discrepancies could relate to a number of factors, including the variable virologic and clinical course of both experimental and natural respiratory viral infections, differences in the pathogenesis of infection between the induced models and natural illness, and the relatively small sample sizes studied in volunteer trials. For example, these models usually employ intranasal administration of the virus, sometimes in high concentrations as for influenza viruses, whereas the site of acquisition, the infectious inoculum and duration of exposure may be different in natural conditions.

Studies of drug toxicities and pharamacokinetics in uninfected volunteers, particularly members of target populations, can provide important data for selecting drug regimens. For example, a placebo-controlled trial of of the structurally related drugs, amantadine and rimantadine, found significant differences in toxicity that related to differences in pharmacokinetics (103). There is a need for long-term studies to evaluate cumulative toxicity and the effect of repeated use on efficacy for those agents which may be subject to frequent and repetitive usage. For interferons, the question of immunogenicity is important, as the production of secretory neutralizing antibody might reduce efficacy.

Less controlled conditions occur when such studies are conducted in naturally occurring illness. One important new variable is determining the specific viral etiology of the subject's clinical syndrome. Other variables include the increased difficulty encountered in assessing compliance with the treatment regimen and the potential for unblinding of the study, either of which could bias study results. One study with vitamin C was flawed by the ability of study subjects to determine their treatment status (104,105). Ascorbic acid (vitamin C), which has been espoused for the prevention and treatment of the common cold, was found to have negligible effectiveness in controlled clinical trials (106-108). Similarly, a study claiming effectiveness for oral zinc lozenges in treating common cold symptoms (109) may have

been compromised by the lack of an appropriately blinded placebo (110).

Pharmacokinetics and Drug Delivery

The effective application of antiviral agents depends on the site of virus replication in the host. Knowing that rhinovirus replication occurs in the nasal mucosa, for example, enables targeting of anti-rhinoviral agents to this site. On the other hand, anti-influenza agents need to be distributed to the lower respiratory tract. Assessing the delivery of systemically administered antivirals to the respiratory tract mucosa has not been standardized. Direct measure of active drug concentrations in respiratory mucosal biopsies or scrapings may appear to offer the best assessment. However, in vitro studies with amantadine suggest that much intracellular drug, which is concentrated in lysosomes, is not biologically active and that concentrations in the extracellular medium are more predictive of antiviral activity (111). Consequently, measurements of drug that penetrates into respiratory secretions after systemic administration may be more predictive of activity in some instances. Such measurements are complicated by the difficulty of obtaining appropriate respiratory secretions for analysis. Nasal washings have been used but introduce a variable dilution effect. A method for collection of induced nasal secretions that provides small volume samples of much higher concentration, as assessed by IgA measurements has been described (112). This technique was employed to demonstrate that after oral administration of equivalent dosages, rimantadine nasal secretion levels are similar to those of amantadine and higher than those present in the plasma (113), observations which may in part explain its clinical effectiveness despite lower plasma levels than amantadine (103).

In the case of topically applied antiviral agents, such as aerosolized ribavirin or intranasal interferon, the value of measuring nasal mucus or lower respiratory tract secretion levels is also uncertain. Collection of nasal secretions after intranasal application of a drug is confounded by the fact that drug is probably recovered from sites where it is not exerting an antiviral effect. Careful studies of the respiratory tract distribution, systemic absorption, and possible systemic effects of topically applied antivirals are important in the development of such agents. A practical problem is possible interference with virus isolation, when residual drug is present in respiratory specimens. Although this has been reported not to be a problem associated with ribavirin (114), the addition of anti-interferon antibody to collection broth is necessary to increase the yield of rhinovirus in samples containing interferon (115).

SUMMARY

Respiratory viruses continue to be major causes of morbidity and mortality. Several antiviral agents with clinical usefulness in respiratory viral infections are currently available, but more effective agents for treatment and prophylaxis are needed. As newer agents become available and indications expand for available antivirals, physician and patient education will be needed to foster their optimal utilization. Increased knowledge of the epidemiology of respiratory viruses, their mechanisms of transmission and disease production, and rapid diagnosis should allow development of effective strategies for their application. Barrier-type methods of prevention, perhaps incorporating virucidal compounds, may prove useful in decreasing transmission of some respiratory viruses. Combinations of specific antiviral agents with drugs that provide symptomatic relief might provide the best means of treatment. Until effective methods for control are available for most respiratory viruses, medical considerations and economic incentives will continue to stimulate research on antiviral therapy and chemoprophylaxis.

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