A Perspective from Controlled Investigations on Chemotherapy for Viral Respiratory Infections

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Failure to translate discoveries about the etiology of viral respiratory infections into effective vaccines has stimulated research into antiviral chemotherapy. Volunteers whose antibody status before challenge is known and who have been exposed to a known dose of virus at a defined time provide an objective system for controlled observation of antiviral chemotherapy. A perspective on experience with drugs of five different categories of mechanisms of action is presented. Prophylactic and possibly therapeutic control of influenza and rhinovirus infections is possible. Field trials have confirmed that some regimens have beneficial effects and have shown the clinical restrictions of others. Chemotherapy is enhanced by immunoprophylaxis, and vice versa. If the potential of antiviral chemotherapy for clinical use is to be realized, complex virologic problems (some related to drugs and others unrelated) must be overcome. The groundwork is laid for new directions and new successes in the prevention and treatment of viral respiratory diseases with antiviral drugs.

A few more than two score years ago, the first isolation, laboratory propagation, and controlled transmission of influenza A virus changed viral respiratory diseases from descriptive syndromes to etiologically specific and epidemiologically characterized diseases. There was much confidence during these years that etiologic identification would cause viral respiratory infections to yield to medical control in the form of vaccines.

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Instead, the shifting antigenic coat of influenza viruses, the many serospecific strains of rhinovirus, the recurrence of infections with the same types of paramyxovirus, the prevalence of diverse types of adenovirus, and the periodic emergence of different strains of coronavirus, reovirus, and respiratory enterovirus have permitted a continued uncontrolled prevalence of these diseases.

Failure to use our new knowledge about the etiology of viral respiratory diseases for effective control by immunoprophylaxis provides a continued impetus for the development of antiviral chemotherapy. However, only the use of amantadine for the chemoprophylaxis of influenza A, a few investigational drugs, and preformed interferon against influenza or rhinovirus infections have been effective in controlled studies in man [1-8]. Other controlled trials have added negative data that are important in a realistic evaluation of the potential of antiviral chemotherapy.

Controlled investigation of chemotherapy for viral respiratory infections has depended largely upon measurements in volunteers. Influenza virus and rhinovirus have been the respiratory viruses most commonly used for challenge. In subjects whose antibody status before challenge is known

and who have been exposed to a known dose of virus at a specified time, symptoms have been a sensitive indicator of the biologic events associated with infection. When complemented by virologic and serologic tests, such investigations provide an objective system for the observation of antiviral chemotherapy and its clinical effects. Confirmatory field trials have been limited to a few drugs with clinical potential. Tests of effectiveness in an open population are expensive and laborious because of the large number of different kinds of infection, different host susceptibilities, and variability in the disease produced.

Drugs that have been studied in man for potential value in the treatment of viral respiratory infections operate by various mechanisms. Some categories of mechanism are: (1) inactivation of virus by contact; (2) metallo-chelation; (3) alteration of virus-cell interaction; (4) inhibition or misdirection of macromolecular synthesis (protein, nucleic acid, enzyme); and (5) induction of increased host resistance.

Compounds that have a virucidal action by contact with the virus are the most numerous. Many are also cytotoxic, but some have minimal toxicity for mammalian cells. Drugs capable of inhibiting or misdirecting the synthesis of infectious virus or viral products are most analogous to drugs used in antibacterial chemotherapy and have received more attention in the form of laboratory study than in the form of trials in humans. Drugs capable of specific blockade of virus-dependent or virus-directed processes provide a theoretical ideal for chemotherapy. One reservation is that strain-to-strain variation among the viruses might preclude antiviral activity, even among different strains of the same type of virus.

The chelation of trace metals, especially Mg^{++} , Mn^{++} , and Ca^{++} , can result in the inhibition of viral growth in tissue culture; however, expectations have not been great for the use or effectiveness of drugs with this mechanism of action in man. With amantadine, a novel mode of antiviral chemotherapy was introduced, in which the drug exerts no direct effect on the virus or on viral replication and none on cell metabolism, but early virus-cell membrane interactions are disturbed. Perhaps oldest in concept but most unique of the methods of antiviral chemotherapy investigated is the use of drugs for the induction of local cellular

resistance to viral infection or direct use of the mediators of natural resistance, such as interferon.

Controlled Trials in Man

(1) *Contact inactivation.* Caffeic acid was found to have a direct antiviral effect on influenza A virus in vitro. Caffeic acid was also used for treatment of infections with influenza A virus in mice (R. Pollikoff, personal communication) and in ferrets (K. W. Cochran, personal communication). Of 20 mice given 75 mg of drug/kg per day (perorally), beginning at the time of infection or shortly thereafter, 64% had lung consolidation (vs. 89% of 30 controls), and 50% survived infection (vs. 13% of controls); the mean number of days of survival for drug-treated mice was 11.3, compared with 9.1 for controls. Thus this regimen was protective. In 18 ferrets given the same dose of drug by the same route, the opposite effect was observed; fever was noted for a mean of 7.1 days (vs. 5.0 days in nine control animals), and the mean titer of antibody to influenza A virus was 1:1,990 (vs. 1:1,760 in controls). These findings suggested that treated ferrets had more viral product than untreated animals. The effect of treatment with caffeic acid in a divided dose of 6 g per day was tested in a controlled trial in antibody-free volunteers. The rate of infection with influenza A2 virus was not changed, but the symptomatic expression of illness among those infected was exaggerated (figure 1). The score for respiratory disease symptoms, beginning after an incubation period of approximately 48 hr and ending six days after challenge, was notably greater in subjects given drug than in controls. Although the number of subjects was small and the severity of the infection among control subjects was minimal, other experience (including virus excretion) confirms the augmenting role of treatment with caffeic acid on influenza in man.

Famotone and memotone (two substituted isoquinolines) and calcium elenolate (a glycoside) have a direct virus-inactivating action on influenza viruses. These compounds have been investigated in man, and the results showed insignificant benefit against influenzal disease [9-12].

The accumulated experience suggests that drugs for which contact or extracellular inactivation of

Figure 1. Clinical illness in caffeic acid-treated and control (lactose-treated) volunteers after intranasal inoculation of influenza A2/Bethesda/10/63 virus. Treatment was begun one day before viral challenge and continued for six days. All observations were made on a double-blind basis.

virus is the principal mechanism of action are poorly effective for the prevention of viral respiratory infections. This judgment appears to be valid for drugs administered systemically or topically. Another noteworthy principle is that some drugs can paradoxically increase illness and viral replication.

(2) *Chelation.* Oxolinic acid is an antibacterial drug that interrupts bacterial synthesis of DNA. In an *in vitro* screening test, this drug was found to inhibit the replication of rhinoviruses and (when the drug was added to a previously infected cell culture) to repair monolayers of infected cells. Because rhinoviruses have no known DNA transcription or DNA-dependent phase, studies were made to elucidate a mechanism for the antiviral action. It was found that oxolinic acid chelates Mg^{++} , Mn^{++} , and Ca^{++} . The antiviral effect was reversed by a 10-fold increase in the $MgCl_2$ concentration in the tissue culture medium. Under these conditions, an inhibitory concentration of oxolinic acid had no effect, and the excess $MgCl_2$ enhanced the capability of the cells to replicate virus.

A pharmacologic observation showed that oxolinic acid is secreted in tears and respiratory

secretions. As a result, the continuous delivery of drug to the surface of infected respiratory epithelium appeared to be feasible. On this basis and with the safety of the drug established, a clinical trial of oxolinic acid was performed in volunteers. The results with two sizes of inoculum of rhinovirus type 30 are shown in figure 2. In subjects infected with a very large inoculum of virus, 10^5 TCID₅₀ (an infectious dose for one-half of inoculated cultures), drug treatment had little or no influence on the proportion of persons infected. With the smaller (but still very large) challenge inoculum of 10^3 TCID₅₀, drug treatment reduced the incidence of infection by one-third. In the group of subjects receiving the latter inoculum, a significant drug effect was also demonstrated by a decrease in the percentage of nasal specimens from which infectious virus was recovered (figure 3).

The results provide some grounds for encouragement, when the excessive size of the challenge dose relative to natural infections is considered. Perhaps more important is the probable pharmacologic advantage of the secretion of drug into the fluids bathing the respiratory tract cells. This event may have permitted a local chelation effect and amplified a modest level of antiviral activity.

Figure 2. Results of a double-blind study in antibody-free volunteers given prophylactic treatment with oxolinic acid (250 mg four times daily) or a placebo for two days before and four days after challenge with 10^5 or 10^3 TCID₅₀ of rhinovirus.

Figure 3. Effect of treatment with oxolinic acid on the nasal excretion of virus after a challenge dose of 10^3 TCID₅₀ of rhinovirus type 30 in antibody-free volunteers. The difference between the level of excretion in the treated subjects and that in untreated controls was statistically significant.

Because of the rapidity with which topical instillations are removed from the nose, the secretory properties of a drug may be among the most important considerations in the chemotherapy of viral respiratory diseases.

(3) *Inhibition of synthesis.* Of the drugs capable of inhibiting viral replication by interference with macromolecular synthesis, most are too toxic to be considered for antiviral chemotherapy of viral respiratory infections. Nevertheless, some such drugs have been sufficiently safe for use in trials with volunteers. The activity against rhinovirus of a substituted indole-triazine (SKF 30097) was investigated in primary and secondary cultures of human diploid lung fibroblasts (WI-38 cells). In primary tissue cultures, the drug inhibited viral replication at a concentration of 20 μ g/ml; at this concentration cytotoxicity was inapparent. At lower concentrations there was a proportional decrease in the inhibition production

of virus. The transfer of harvests to secondary cell cultures in drug-free medium indicated that the action of the drug had prevented or restricted viral replication. The titer of virus in the supernatant and in the washed cells of infected cultures was from several hundred to several thousand times lower in treated than in control cultures.

When the indole-triazine was given to volunteers in oral doses of 250 mg three times daily beginning 48 hr before challenge with rhinovirus, a statistically significant reduction in the rate of infection and illness was demonstrated (table 1). If treatment was delayed for several hours after challenge, however, no benefit was obtained; thus the benefit of the drug was restricted to prophylaxis. A further difficulty was encountered as a result of side effects of the drug. Approximately one-quarter of the treated volunteers had severe headache that required cessation of the treatment in 10%. This symptom was noted only among subjects given drug, and the frequency was statistically significant compared with that in controls. Unfortunately, although the effective prophylaxis of rhinovirus infections in man confirms the antiviral activity observed in vitro, the drug has no clinical potential because of the absence of therapeutic activity and the production of drug-related symptoms.

(4) *Amantadine.* The prophylactic effectiveness of amantadine (1-adamantanamine hydrochloride) against influenza A has been validated in various test systems. The initial observations in mice, in which a threefold or 75% reduction in the number of deaths from a highly lethal infection was produced [13], have been reproduced as reductions in frequency of illness and seroconversion in volunteers and in persons observed in field trials [1-4, 14-23]. The effect is consistently one of a 50%-70% reduction in the signs of infection among persons treated prior to exposure, and

Table 1. Clinical trials of an indole-triazine (SKF 30097) against infection with rhinovirus type 44 in antibody-free volunteers.

Condition	Prophylaxis			Therapy		
	Placebo	Drug	P	Placebo	Drug	P
Infected	14/18*	3/16	<0.001	10/16	12/18	NS†
Ill	8/18	0/16	<0.01	8/16	9/18	NS
Side effects	0	3/16	<0.05	0	6/18	<0.01

* Number of volunteers with indicated condition/total number tested.

† NS = not significant.

the significance of the difference in rates of infection is no longer doubted.

Acceptance of this conclusion permits examination of other problems in antiviral chemoprophylaxis. The effect of such treatment is most apparent when the incidence and severity of influenza are great (figure 4). As the amount of disease in a population declines, the absolute difference between the amount in treated groups and that in untreated groups diminishes. Although the proportional reduction accomplished by prophylaxis is the same, a relatively large number of persons must be treated before the benefit is observed. Thus the cost of the apparently unnecessary prophylactic treatment of many persons may retard recommendation of such a program. Cost, however, is a calculable factor, and the prevention of a few cases of influenza (especially those requiring hospitalization or leading to death) usually establishes a cost-benefit position in favor of prophylaxis. At a ratio of treated cases per case of influenza prevented of 25:1, the cost for amantadine prophylaxis during epidemic influenza should not be prohibitive.

A more important consideration in the use of a drug for prophylaxis is freedom from toxicity. Early concerns about the narrow range between

the effective and intoxicating doses of amantadine have been assuaged by further experience. In a dose of 100 mg twice daily, almost no toxicity is produced, and the regimen can provide protection after about 48 hr. The experience accumulated with many patients given amantadine over a long period as treatment for Parkinson's disease has provided reassurance about the safety of this drug regimen [24]. Because of the peculiar pharmacologic properties of absorption and distribution of amantadine, an even safer and more effective regimen for prophylaxis of influenza might be expected from smaller, more frequent doses given initially. Even at this early point, with more attention to the optimal drug regimen, toxicity of amantadine need not be a restricting factor in its use for prophylaxis against influenza A.

Amantadine chemoprophylaxis has not been used extensively, primarily because physicians and lay persons have failed to involve themselves in this form of influenza prophylaxis. Epidemic influenza is not well anticipated with regard to recommendations during the epidemic period. Patients who might require drug protection are not adequately identified in advance; both the profession and the community are unprepared regarding a plan for the logistics of chemoprophylaxis, and,

Figure 4. Influence of the incidence of influenzal disease on the magnitude of the prophylactic effect of amantadine. The proportional reduction in incidence in each experimental system was the same (50%–70%). All members of each group received placebo or drug, and the ratios of treated cases per case of influenza prevented were 1.7:1 (mice), 2.5:1 (ill volunteers), and 25:1 (ill persons in institutions). (Figure is taken from [18] with permission of the Pan American Health Association.)

as a prophylactic drug, amantadine must be available quickly at the time of need and throughout the epidemic period. Delayed initiation of treatment or its premature discontinuation readily nullifies the potential benefit (table 2) [25]. Rapid access to the product and advice for proper use is particularly difficult with a prescription drug like amantadine. All of these problems of implementation are behavior-related rather than drug-related factors that must be overcome with any prophylactic product. If the benefit of amantadine is to be obtained, acceptance and prior planning by physicians and education of patients will be required.

(5) *Interferon and interferon inducers.* Since a wide variety of viruses cause respiratory diseases, interferon or drugs capable of stimulating or accelerating its production offer an attractive approach to chemotherapy. The use of species-specific exogenous interferon is an alternative method. Although the protective value of interferon has been abundantly demonstrated in cell cultures and animals, only modest evidence is available for its prophylactic or therapeutic use in respiratory disease in man [5–8, 26–28].

In studies with volunteers, the dominant effect of drug-induced interferon has been to decrease the symptoms of infection; decreased viral shedding is a second and less marked effect [27]. The rate of infection after challenge may be only slightly decreased by interferon. The time relation of the presence of interferon to virus exposure is critical. Any measurable titer of interferon in the

respiratory secretions at the time of infection greatly lessens the severity of the subsequent illness. Above this minimum, the height of the titer has little appreciable importance. Whether or not a sustained level of interferon is necessary for several days after the exposure to virus is unknown.

The height of the titer of interferon is of obvious importance in the lessening of symptoms when the interferon is induced only after infection has been established. Fifty units or more of interferon in the nasal wash (more at the cell surface) appears to be related to relief from symptoms, and this effect is greater than the apparent antiviral effect of interferon. The therapeutic use of interferon may require larger and more frequent doses than prophylactic use. The function of a high titer of interferon may be the profusion of a wider area of cells or the binding and removal of interferon inhibitors [29]. Also, interferon cannot be considered homogeneous in its quality and properties. We have found that the physical and biologic characteristics of interferon in nasal secretions late in the illness are different from those noted earlier in the same person. Thus, the symptomatic response might be related to the appearance of a new type of interferon rather than to its titer.

Two other factors that will influence the potential usefulness of topical interferon inducers in respiratory infections are the hyporeactivity of the patient after a few days of drug administration and the ability of drug to prime mucosal cells for an accelerated and increased response to viral in-

Table 2. Effect of chemoprophylaxis with amantadine and its withdrawal during epidemic influenza.

Subjects (no.), condition	Period				
	1 (18 days, prophylaxis)		2* (16 days, no treatment)	3* (10 days, prophylaxis)	
	Yes	No		Yes	No
Group 1 (53)					
Influenza	1	...	9	...	3
Infected but not ill	5	...	2	...	5
Total	6/53		11/47		8/36
Group 2 (52)					
Influenza	...	8	2	2	...
Infected but not ill	...	2	3	0	...
Total		10/52	5/42	2/37	

NOTE. During each period, the differences between treated and untreated groups was statistically significant ($P < 0.05$). Table is taken from [25] with permission of the American Review of Respiratory Disease.

* Persons infected during the earlier period(s) were removed from the susceptible populations before data were compiled.

duction of interferon. The critical nature of the time relation in the administration of an interferon-inducing drug before or during the course of infection is a major deterrent to the potential for clinical use. Similarly, the time of application and the quality of exogenous interferon can be expected to influence the dose required and the results obtained. There is no experience by which we can judge the importance of these factors to the potential of preformed preparations of interferon for clinical use.

(6) *Other measures.* Several other drugs that have been used in controlled trials have yielded negative or equivocal results. Often the results of such studies are not reported. Some of the drugs that have had considerable study are isoprinosine (used against rhinovirus and influenza virus infection [30, 31]) and isoquinoline drugs (used against rhinoviruses [30–33]). Large doses of vitamin C had a period of popular appeal for the prevention of common viral respiratory disease. The rationale, which has no basis from investigational experience, is the creation of an increase in natural host resistance. In volunteers, the effect of large doses of ascorbic acid against a rhinovirus infection was negligible [34]. In controlled field trials, oral doses of 2 g per day failed to alter the frequency of acquisition of colds. Intermittent doses of as much as 8 g of vitamin C per day at the time of exposure to infection may have reduced the duration of symptomatic illness, but continuous large doses had no beneficial effect in preventing common acute respiratory disease of diverse etiology [35, 36].

Investigational studies in our laboratory show a discernible role for specific cell-mediated immune responses in acute viral respiratory disease. Substances with a potential for augmenting the cellular response, usually on a nonspecific basis, have been developed, but they have had limited trial in viral respiratory disease. Drugs that suppress delayed-type sensitivity, such as aspirin, adversely affect some measures of rhinovirus infection [37].

The Collaborative Roles of Immunoprophylaxis and Chemotherapy

The potential for antiviral chemotherapy is enhanced by immunoprophylaxis or natural immu-

nity of the person. The presence of specific antibody in the plasma when not protective by itself increases the effectiveness of chemotherapy, and vice versa. An example of this collaborative role is presented in figure 5. The results of rhinovirus challenge with or without drug treatment are shown for subjects with and without detectable antibody in serum before challenge. Drug alone and antibody alone produced a modest reduction in the frequency of infection after challenge with a large inoculum. The difference between results among persons with either of these factors and the results among persons with neither is not statistically significant.

Among subjects with antibody who also received drug, infection was reduced to a level significantly lower than the level produced by drug in antibody-free subjects. Other experience confirms this effect of antibody in improving the effectiveness of a chemotherapeutic agent. Thus, although immunoprophylaxis has been logistically impractical in the control of viral respiratory diseases, its development for specific diseases should

Figure 5. Synergistic effect of antiviral chemotherapy (oxolinic acid) and antibody in the prevention of rhinovirus infection in volunteers observed in a controlled, double-blind study.

be sought and immunoprophylaxis should be practiced as an adjunct measure for chemotherapy and vice versa. Less than optimal progress and benefit will be gained if the two methods are considered as alternatives for management. This combination approach is especially valid for influenza during the interpandemic years, when both vaccine and amantadine are available for control of the disease.

Comment

Perspective is gained from an assessment of prior experience and future needs. The remarkable advances during the past two decades in our knowledge about the viruses that cause respiratory infections have in a way been counterproductive. So great is the complexity of the problem, with the number of strains, changes in antigens, and reinfections without lasting immunity, that a sense of hopelessness is easily generated. As with other complex problems, the prevention and control of the common viral respiratory infections by chemotherapy is not likely to be found in one drug, one procedure, or one method or to be accomplished at one time. Individual parts of the problem will need to be isolated and investigated for specific information.

In the experience reported herein and in the literature, the feasibility of prophylactic and possibly therapeutic control of viral respiratory infection under investigational conditions is demonstrated. If the potential of antiviral chemotherapy for clinical use in these diseases is to be fulfilled, several problems must be overcome. Among them are the development of methods for the rapid identification of specific strains of virus or properties of virus that are drug-susceptible. Some loosening of the restrictions of drug specificity and the critical time factor in the administration of drugs for prophylaxis or therapy must be attained. Topical and systemic routes for the delivery of drugs to infected or susceptible portals must be developed, with regimens that cause little toxicity. In addition, there are non-drug-related problems in the logistics of making effective prescription drugs readily accessible with understanding of their safe and proper use; this is a complicated educational and social problem that will need to be solved.

As exemplified by interferon, amantadine, and the chelating agents, antiviral chemotherapy need

not be directly antiviral, in the sense of killing the virus or even specifically interrupting its synthesis. This fact permits potential freedom from some of the toxicity involved in interrupting basic cell functions with which viral replication is so intimately related. Other new directions and mechanisms of chemotherapy are sure to be found. In the interim, new attitudes may be required that differ from the traditional prescription of drugs for recovery of a clinically ill patient. Although treatment beneficial to the ill patient is a *sine qua non* for successful chemotherapy, prophylaxis against viral infections is clearly easier than cure. Guidelines for early recognition of proper indications and use of prophylaxis must be developed and put into practice. During the development of effective curative chemotherapy, consideration should be given to the treatment of the ill patient for the primary purpose of diminishing his or her role as a transmitter of infectious virus. This socially directed measure might decrease household and community exposure to the virus, and the lower rate of transmission might provide "herd protection" equivalent to the "herd immunity" of immunoprophylaxis. Drugs and treatments are known that will significantly reduce the amount of virus released from infected cells or the amount of infectious virus recoverable from the respiratory secretions. Other drugs and treatments increase the amount of virus shed and might be generally avoided.

Progress in the chemotherapy of viral respiratory infections has not been as rapid as would be desirable to sustain the effort and commitment required for success. Nevertheless, real accomplishments have been made, and optimism about the feasibility of practical clinical antiviral chemotherapy has increased. The groundwork is laid for new successes and new directions in the use of chemotherapy as a major method for relief of morbidity and death from these common infections.

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