ANTIVIRAL AGENTS

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With the recent unraveling of many of the biophysical, biological, and biochemical properties of the viruses that infect man, a groundswell of enthusiasm has gathered in anticipation of an era of viral chemotherapy. Because an impressive amount of time and money has already been devoted to the development of antiviral agents, it is appropriate, at this time, to evaluate the progress that has occurred. To do so, it is helpful and perhaps essential to consider, first, the problems that must be overcome by a successful antiviral agent and a few fundamental concepts about viruses. Therefore, this report begins with an enumeration of serious problems peculiar to the field of viral chemotherapy and continues with a short description of the general structure and classification of viruses, the sites of action of various antiviral agents in the viral replicative cycle, and finally a consideration of the structure, action, pharmacology, toxicology, and clinical efficacy of each of the candidate antiviral agents.

PROBLEMS

The first and most challenging problem is the concomitant toxicity to mammalian cells usually displayed by chemotherapeutic agents effective against viruses. Underlying the problem is the fact that a virus is an obligate intracellular parasite that must use the metabolic pathways of host cells. In contrast, a bacterium has enzymes and subcellular particles that have evolved separately from the mammalian counterparts and therefore are sufficiently different in a variety of ways to be susceptible to specific attack. Thus, while the ratio of the minimal toxic dose to the minimal therapeutic dose, or chemotherapeutic index, is high for approved antibacterial agents, it is generally quite low for the antiviral agents. There are at least two situations in which the use of a drug with a low chemotherapeutic index is morally justified: one, if it is to be used for a life-threatening illness having no other specific treatment, and, two, if it is to be used for an isolated problem accessible to therapy by local administration. It is therefore not surprising that, to date, most clinical

evaluations of antiviral agents have been confined to these two clinical situations (1–4).

The second problem is the frequent occurrence of what in bacteriology is termed the *inoculum* effect and in virology might better be termed the *multiplicity* (number of infectious particles per cell) effect. As applied to viruses, it describes the situation in which an antiviral agent is effective against virus in low concentration (low inoculum or multiplicity if being tested in vitro) but much less effective against virus in high concentration (high inoculum or multiplicity). Most antiviral agents display this multiplicity effect with the result that they are not very effective against symptomatic disease because of the high concentrations of virus present at that time. However, such drugs may still be useful as chemoprophylactic agents if used during the incubation period when virus is in low concentration. In fact, the two antiviral agents with the highest chemotherapeutic indices, amantadine and methisazone, suffer from the multiplicity effect in vitro (5, 6) and are therefore of questionable usefulness as therapeutic agents (7, 8) but nevertheless are of significant utility for chemoprophylaxis (9, 10).

The third problem is the difficulty in evaluating the efficacy of a drug to be used for a mild disease of short duration. For a disease that is so benign a question may appropriately be asked as to the necessity for any chemotherapeutic agent. The answer, of course, is that a specific therapeutic agent would be useful either for the rare occasion when the disease is quite severe or, more important, when an ordinary form of the disease occurs in a debilitated individual. To evaluate the utility of a chemotherapeutic agent in the usual presentation of mild disease is very difficult. By the time an individual is sick enough from his symptoms to seek medical attention, this type of viral illness may no longer be associated with viral replication, or the replication may be greatly reduced. Even if replication is still occurring, it may be that the viral illness will be over within 24 or 48 hr. Under such circumstances, if a drug were available that could reduce by 50% the symptomatology and duration of illness, it would be very difficult to prove, in fact, that the drug could do it. The magnitude of the challenge becomes apparent when it is realized that to solve the problem one must be able to quantify a number of subjective symptoms. We are only just beginning to understand which parameters are useful in making such an evaluation.

The fourth problem for viral chemotherapy is the emergence of drug resistant viruses. The history of drug resistant bacteria, following the use of antibiotics, has been well documented (11). In the case of antiviral agents, it has already been demonstrated that drug resistance tends to develop both in vitro and in vivo (12). Fortunately, drug resistance has not yet become a significant problem with the candidate antiviral agents, although this is most likely due to the discretion exercised in the use of agents with low chemotherapeutic indices.

To recapitulate, the four obstacles in the pathway of a candidate antiviral agent are as follows: 1. toxicity to mammalian cells resulting in a low chemotherapeutic index; 2. a multiplicity effect resulting in poor utility against virus in high concentration; 3. the difficulty in demonstrating clinical efficacy against mild disease; and 4. the emergence of drug resistant virus.

GENERAL STRUCTURE AND CLASSIFICATION OF VIRUSES

It can be seen schematically in Figure 1 that the virus particle has an inner nucleoprotein core that is either DNA (*b*) or RNA (*a*) in type. With respect to the viruses known to infect animals, the DNA viruses all have double stranded DNA, while the RNA viruses usually contain single stranded RNA. (Exceptions include the reoviruses and colorado tick fever virus.) It is also schematically shown in Figure 1 that around the inner core of a virus particle there is usually a protein coat (*c*) which may or may not have an external lipid membrane (*d*). It is thus convenient to classify the major groups of viruses according to their nucleic acid type and the presence or absence of a lipid membrane or envelope (Table 1).

SITES OF ACTION OF ANTIVIRAL AGENTS

Before indicating the sites of action of the antiviral agents, it will be necessary to consider first the normal viral replicative cycle. The replicative cycle for an RNA virus is shown schematically in Figure 2. Initially, the particle attaches to the cell membrane by physical forces in the process known as attachment, *1*. In some cases attachment is a highly specific reaction occurring only with cells having very specific receptors in the cell membrane (13, 14). In such circumstances, those cells lacking the specific receptors cannot be infected by that particular virus. The second step is the penetration of the cell membrane by the virus in a process sometimes called viropexis, *2*. Often this step consists of invagination by the cell membrane with pinocytosis of the virus particle. Step *3* is known as uncoating and consists of an opening up of the particle's protein coat with subsequent release of its nucleic acid into the cytoplasm of the cell. In the simplest viral replicative cycles, the viral RNA (input RNA) acts as RNA.

In the process known as translation, *4*, polyribosomes form when ribosomes attach to the virus RNA and begin to translate the nucleic acid sequence into proteins. One protein will be a "turn-down" protein which will be capable of turning down the host cell's own RNA and protein synthesis. Other proteins will usually include a RNA-dependent RNA-polymerase as well as the structural proteins required for the mature virus particle. With many RNA viruses, the RNA-dependent RNA-polymerase is coded for on the viral message as described. With some viruses (15, 16) this particular enzyme is part of the mature virus particle. In other words, one of the structural proteins in the mature virus can function as an RNA-dependent RNA-polymerase. In such a case, once the virus particle enters a cell, the functional protein can separate from the particle and carry out its function without requiring prior synthesis of viral specific proteins.

In the next step of the viral replicative cycle, *5*, the RNA-dependent RNA-polymerase, regardless of whether it was synthesized in the cell or brought in preformed, will find a strand of input viral RNA and begin to synthesize a complementary strand (mirror image) to the input RNA. With completion of the complementary strand, the double stranded structure realized is known as the replicative form, *6*. The replicative form serves as template for viral RNA production. For

Table 1 Human virus families

Membrane	RNA	DNA
+	myxovirus	herpesvirus
+	paramyxovirus	poxvirus
+	rhabdovirus	
+	togavirus	
-	picornavirus	adenovirus
-	reovirus	papovavirus

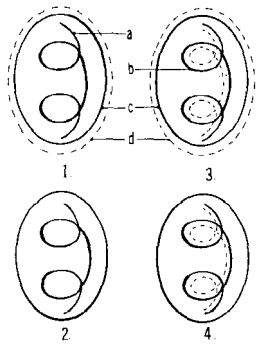

Figure 1 General structure of the animal viruses. Both RNA viruses, *1* and *2*, and DNA viruses, *3* and *4*, are depicted in this scheme. Each virus particle has a protein coat, *c*, and an inner nucleoprotein core containing either RNA, *a*, or DNA, *b*. Certain viruses have an external lipid membrane, *d*.

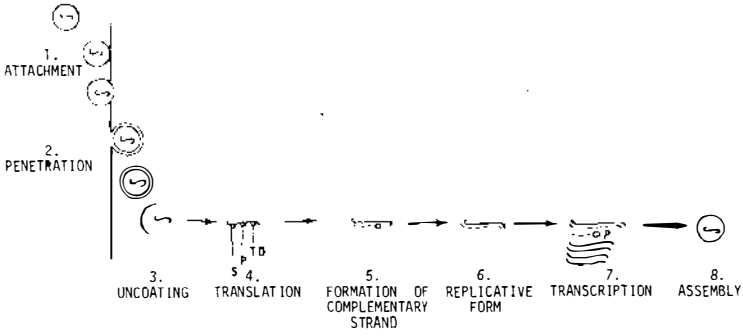

Figure 2 Replicative cycle of an RNA virus, described in text.

transcription, *7,* the same RNA-dependent RNA-polymerase or perhaps a second polymerase (which should rightfully be called a replicase) reads the replicative form with the production of new single stranded RNA that is identical with the input RNA. This new RNA can go back and serve as message for more protein synthesis, can be used to produce templates for production of more RNA, or can be incorporated into mature virus. The next step, *8,* is assembly of the new RNA, *7,* and the new structural protein, *4,* into mature virus. The mature virus is subsequently released from the cell.

One of the candidate chemotherapeutic agents against RNA viruses, amantadine, acts in the replicative cycle at step *2,* penetration. Thus amantadine prevents the penetration of some RNA membrane viruses into cells (17). Neutralizing antibody has also been shown to prevent the penetration of cells by virus (13). The substance known as interferon is believed to have at least two sites of action. Evidence has been presented that step *4,* translation, is effected such that ribosomes from interferon-treated cells become attenuated and no longer read viral RNA message, although they continue to read the host cell RNA message (18). Another site of action by interferon can be demonstrated against those particular viruses that bring into the cell a preformed RNA-dependent RNA-polymerase. In the latter situation interferon has been reported to inhibit the function of this enzyme and thereby block replication of new RNA (19, 20). Thus interferon can, at the present time, be shown to interfere with transcription for certain viruses and with translation for others. A substance known as isoprinosine has not been completely evaluated, but it is believed to work on ribosomes like interferon, i.e. attenuate ribosomes so they no longer function with viral messenger RNA. Guanidine (a chemical with a particularly low chemotherapeutic index and associated with too rapid a development of drug resistant virus to be of use in vivo) affects transcription, *7,* by inhibiting the initiation of new strands of RNA (21). Thus, antiviral agents effective against the RNA viruses in vitro include the following: amantadine and antibody, which prevent the penetration of virus particles into the cell; interferon, which can work both on translation by affecting ribosomes and on transcription by inhibiting the RNA-dependent RNA-polymerase; isoprinosine, believed to work on ribosomes like interferon; and guanidine, which will inhibit the initiation of RNA strands during transcription.

The replicative cycle of a DNA virus is shown schematically in Figure 3. The first three steps, attachment, penetration, and uncoating, are analogous to the similar steps in the RNA virus replicative cycle just considered. However, in subsequent steps, there is a marked difference between the two replicative cycles. Even among the DNA viruses there are differences in replicative cycles. The cycle here depicted is for vaccinia virus, which at the present time is the one most completely understood. Vaccinia has been shown to bring into the cell a preformed polymerase which, in this case, is a DNA-dependent RNA-polymerase or transcriptase, P_1. Thus as the vaccinia DNA becomes uncoated, the enzyme begins to transcribe a viral RNA message from the double-stranded DNA, *4.* This "early message" is relatively small with a sedimentation coefficient of 12S. When ribosomes begin to read the message, there is formation of an early polyribosome structure. In step *5,* there is translation of the message to proteins that include the "early enzymes" such as a DNA-dependent DNA-polymerase or replicase, thymidine kinase, and DNase. It is evident that the enzymes translated from early message are those necessary to produce

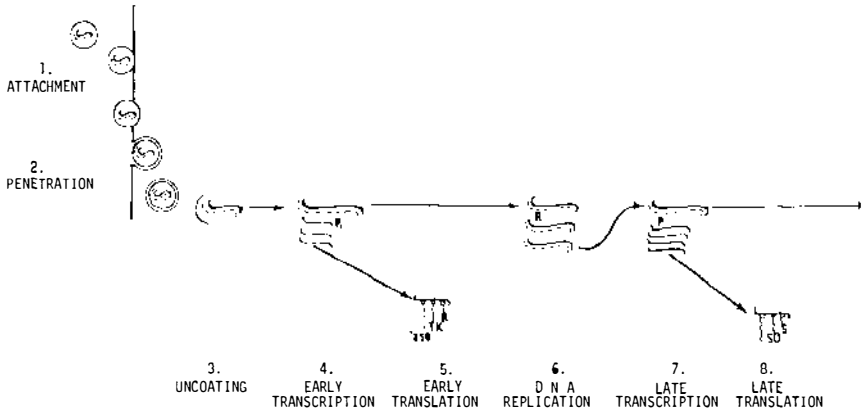

Figure 3 Replicative cycle of a DNA virus, described in text.

DNA. Hence the replication of DNA can occur as depicted in step *6.* The new DNA formed is called daughter DNA and is also capable of being transcribed. Thus in step *7,* late transcription occurs in which daughter DNA is transcribed to late mRNA (sedimentation coefficient of approximately 20S). In step *8,* late translation takes place on polyribosomes formed from the late message. Among the proteins formed are the structural protein that will go into the mature virus particles and another protein called "switch-off" protein. The latter protein appears to switch off the early message and is associated with a disintegration of the early polyribosomes. Finally, assembly occurs in step *9,* with the new DNA and structural protein brought together on a newly formed membrane (22). Ultimately, the mature virus particle is released from the cell.

The antiviral agents considered to have an effect on both RNA and DNA viruses, interferon and isoprinosine, are believed to have an action on early translation as already indicated. In addition, interferon can inhibit transcription of viruses which bring in a preformed polymerase. Thus interferon's inhibition of early transcription (step *4*) has been demonstrated with vaccinia virus which brings in a DNA-dependent RNA-polymerase, or transcriptase (23). The inhibitors of DNA production, 5-iodo-2'-deoxyuridine (IDU), cytosine arabinoside (AraC), and adenine arabinoside (AraA), all act on DNA replication in step *6.* The substances known as thiosemicarbazones act on late translation in step *8.* They may act on the switch-off protein so that it becomes less specific and switches off not only early message but also late message. In any case, there is disintegration of both early and late polyribosomes. Rifampin appears to work on assembly or, more accurately, immediately prior to assembly in step *9.* Rifampin apparently inhibits the cleavage of a protein precursor that otherwise is cleaved at the time of assembly into smaller polypeptides required for the assembly of the virus core (24). It is apparent that in considering antiviral activity against DNA viruses there are currently substances that in vitro will block each step in the replicative cycle. Thus, early transcription is blocked by interferon; early translation, by interferon and isoprinosine; DNA replication, by

IDU, AraC, and AraA; late translation, by methisazone; and assembly, by rifampin. The extent to which these in vitro activities can be translated to efficacy in man will become apparent shortly.

CANDIDATE ANTIVIRAL AGENTS

Before taking up the specific antiviral agents, it will be helpful to consider the three phases of drug evaluation as they apply to viral chemotherapy. Phase one is the preclinical phase. In this phase a prospective antiviral agent is evaluated both in cell culture and in animal models for antiviral activity and toxicity. Human experimentation begins with phase two, which is concerned with the pharmacology of the drug in man. Information concerning tolerable dosage and general toxicology is expected from this phase. With an agent having a very low chemotherapeutic index, the phase two studies can only be morally justified in patients having severe or potentially fatal disease. However, unless such studies have been appropriately set up to also determine clinical efficacy, documentation of clinical utility is neither derived nor expected from these investigations. It is the rightful objective of a phase three study, i.e. the formal therapeutic trial, to determine clinical efficacy. Because of the variable nature of the clinical course in the vast majority of virus diseases, virologists are convinced that clinical efficacy can only be demonstrated by double-blind controlled studies: netiher the patient nor the investigator knows whether the medication given is the drug being evaluated or its placebo. A rare exception to this requirement might occur with a drug being evaluated, for example, against symptomatic rabies, which is considered to be 100% fatal with the presently available modes of treatment.

There are many chemical and biological substances that have some antiviral effects in cell culture and even in laboratory animals but do not show sufficient potency and/or freedom from toxicity to be considered likely candidates to leave phase one for human studies. A much smaller number of antiviral agents have received phase two evaluation for toxicology and dosage in man, although most of these studies were not controlled in a manner that would document efficacy. There are a handful of antiviral agents that have received phase three evaluation with double-blind controlled studies. The candidate antiviral agents to be considered below are those that have either already made phase three or have good reason from the phase two studies to be seriously considered for phase three.

Amantadine

The structure for 1-adamantanamine hydrochloride (amantadine) is given in *1* of Figure 4. This uniquely symmetrical primary amine has a pK_a of 9.0 and therefore exists as an ammonium ion at physiological pH. In cell culture, amantadine and certain other ammonium salts will prevent the penetration into cells of several RNA membrane viruses: myxoviruses (types A and C influenza), a paramyoxovirus (Sendai virus), and a togavirus (rubella) (17, 25–27). Unfortunately, the multiplicity effect is usually significant. In addition there is considerable variation in susceptibility among strains believed to be similar, except for minor differences in their outer membranes. Strains of influenza A_2 virus are clearly the most susceptible, and in mice the drug has been shown to have both prophylactic and therapeutic activity against such strains (25, 26, 28, 29).

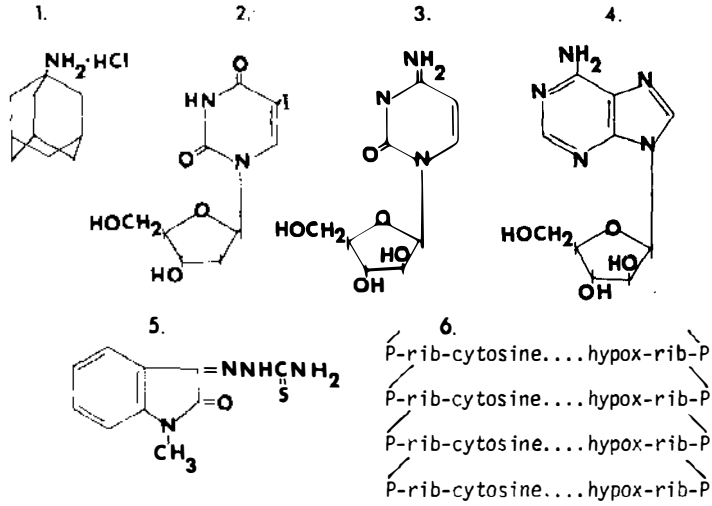

Figure 4 Chemical structure of candidate antiviral agents. Represented are the structures of amantadine *1*, idoxuridine *2*, cytosine arabinoside *3*, adenine arabinoside *4*, methisazone *5*, and poly I · poly C *6*.

Human studies have revealed that amantadine administered by the oral route is absorbed slowly but probably completely, has a long half-life, and is almost entirely recoverable in the urine (30, Table 2). With a dose of 100 mg, twice daily, gastrointestinal upset and transitory central nervous system phenomena are noted in 1–2% of patients (31, 32). In one study, when the drug was given over a one year period for treatment of Parkinson's disease, the only additional adverse effects were livedo reticularis and mild ankle edema (33).

Early double-blind efficacy studies demonstrated that amantadine prophylaxis would significantly reduce the clinical and/or serological attack rate of either naturally acquired (34–37) or intranasally administered (38) Asian strain influenza A_2 virus. Further double-blind studies were carried out in student volunteers when the Hong Kong strain of influenza A_2 virus became prevalent. At least 50% protection was again found when amantadine was used in prophylaxis against natural infection (9) or against challenge with intranasally administered virus (39) (Table 3). Thus, it is evident that amantadine is effective as a chemoprophylactic agent against sensitive strains of influenza A_2 virus. However, there is no evidence that it is more effective than a vaccine containing the appropriate antigen. Therefore, in years when an appropriate vaccine is available, it would appear reasonable to immunize, rather than chemoprophylax, those debilitated individuals whom the Public Health Service recommends protecting against influenza (40). A different practice must be recommended for years when the prevalent virus is an antigenic variant of influenza A_2, for which a vaccine has not yet been prepared. In this case, it would appear prudent, during the epidemic period, to chemoprophylax with amantadine those individuals

Table 2 Clinical pharmacology of antiviral agents

	mol wt	pK	Route	Single Dose (mg)	Peak Time (hr)	Serum Concen (μg/ml)	$T_{½}$ (hr)	Renal Clearance[a] (xGFR)	Elimination Urine (%)	Elimination Metab (%)	(CSF) (Serum)	References
Amantadine	151	9.0	po	100.	1-4	0.2	15.	5.	86	--	–	30
Idoxuridine	354	8.25	iv	80/Kg 2 hr[b]	during	80.0	0.5	0.5	20	80	0.02[d]	56-58
				24 hr	during	≤2.5[c]						49
AraC	246	4.5	iv	10/Kg 24 hr	during	0.5	0.2	0.6	8	76	0.4	67
AraA	271		iv	15/Kg 12 hr	during	–	1.5	1.0	49	–	0.5	75
Methisazone	238		po	200/Kg	6.	20.	2.7	–			–	85
Interferon	23,000		iv	8×10^5 u[e]	during	--	ca 12.[a]	<0.1	–	>95[a]		100, 103, 104
Poly I · Poly C			iv	x $1/m^2$	during	–	<0.5[a]		–	>95[a]		100, 112

[a] author's estimate.

[b] infusion interval.

[c] μg (0.4 m).

[d] in the dog.

[e] units.

who require protection, provided the strain has been shown to be in fact sensitive to amantadine (41).

The efficacy of amantadine in the treatment of influenza has also been investigated with double-blind studies. In these investigations, a major problem has been the already discussed difficulty of demonstrating the influence of chemotherapy on a relatively benign viral disease of short duration. Although the studies appear to show a tendency for symptoms to be less severe and recovery to be earlier with amantadine, a significant difference (when compared with placebo) has been difficult to document. One objective parameter which has been easily quantitated is the interval of temperature elevation. In studies of natural outbreaks with either the Asian (42–44) or Hong Kong (7, 45) strain of influenza A_2 virus, the average interval of fever following initiation of treatment has been found to be significantly shortened by intervention with amantadine (Table 4). Whether aspirin will reduce the febrile period and other symptomatology as much or more is not known. An appropriately designed study comparing aspirin with amantadine is indicated at the present time. An important question is whether amantadine should be used for cases of influenza associated with pneumonia, particularly those pneumonias that occur in debilitated individuals. Although superinfecting bacterial pneumonia is a well-recognized complication of influenza, the importance of an underlying viral pneumonia should not be overlooked. In fact, 20–25% of the lungs from patients dying with influenza and pneumonia grow out influenza virus but no bacteria (46, 47). Therefore, it is important to find out if amantadine can 1. prevent the development of viral pneumonia in patients with influenza and/or 2. decrease the mortality of patients who contract influenza pneumonia.

Idoxuridine

5-Indo-2'-deoxyuridine (idoxuridine, IDU) has been the most thoroughly evaluated of the halogenated pyrimidine nucleosides. The contribution of the iodo group at the 5-position (*2* in Figure 4) makes this molecule a halogenated analog of thymidine. Although IDU appears to inhibit many of the enzymes used in the synthesis of DNA, its important action is considered to be its incorporation into a bogus DNA (48). IDU has been shown in cell culture to be consistently effective against the following human pathogens: herpesvirus hominis types 1 and 2 (49), cytomegalovirus (also a herpesvirus, 50), and vaccinia, a poxvirus (51). Its effect against varicella-zoster (a herpesvirus) has been equivocal (52, 53). Although IDU is found in animal studies to be consistently effective against corneal infections with either herpesvirus hominis (1) or vaccinia (54), its efficacy against systemic viral infection in animals is much less convincing (55).

In man, idoxuridine is rapidly metabolized to iodouracil and iodide giving a serum half-life of about 30 min (56) and resulting in only 20% of the intravenously administered dose appearing in the urine as active compound (Table 2) (57). To maintain the presence of active IDU, continuous intravenous infusions over as long as 24 hr have been used in the past. However, recent work indicates that such infusions result in serum levels no greater than 2.5 μg/0.4 ml (49). It has therefore been recommended that the daily dose be divided into 1 hr infusions given every

Table 3 Chemoprophylaxis with amantadine against infection with influenza A_2/Hong Kong

Transmission (Ref.)	No.	Serological Infection (%)		Clinical Infection (%)	
Induced (39)					
Amantadine	17	24		35	
Placebo	16	88		82	
Protection			73%[a]		57%[a]
Natural (9)					
Amantadine	192	14.1		11	
Placebo	199	29.6		26	
Protection			52%[a]		58%[a]

[a] [(Percentage of Placebo − percentage of Amantadine) ÷ percentage of Placebo] x 100.

Table 4 Treatment of naturally acquired influenza A_2 infection: effect of amantadine on febrile interval

Population (Ref.)	AMANTADINE No.	AMANTADINE Mean Interval (Hr $T > 99^{O}$)	PLACEBO No.	PLACEBO Mean Interval (Hr $T > 99^{O}$)	p VALUE
ASIAN STRAIN					
Prison (44)[a]					
Texas #1	23	40.9	20	66.4	0.01
Texas #2	17	26.3	17	59.9	0.01
Maryland	12	46.7	13	69.3	ns
Prison (42)	20	23[b]	39	45[b]	0.01
Home for aged (43)	13	37	15	75	0.05
HONG KONG STRAIN					
Family practice (45)	72	46.6	81	75.1	0.01
Prison (7)	13	44.5	16	71.3	0.05

[a] Double-blind, placebo controlled studies in which patients with serologically proven influenza were treated within 48 hr of the onset of symptoms; the mean febrile interval following initiation of treatment was statistically analyzed.

[b] Interval with $T > 100^{O}$.

12 hr (49). Studies in the dog indicate that even with intravenous doses adequate to give good serum levels, no significant quantities of IDU reach the cerebral spinal fluid in the absence of meningeal inflammation (58).

Toxicity from IDU is a direct result of its action on cells having rapid turnover. Thus the effect on muscosal cells of the gastrointestinal tract may be manifest by anorexia and stomatitis; the effect on bone marrow, by leukopenia, anemia, and thrombocytopenia; and the effect on epidermal tissues, by alopecia and loss of nails (57). The severity of the toxic effects varies with the total 5 day dose, the daily dose, and the rate of administration of individual infusions (59). With continuous infusion of the recommended dose, the nadir of marrow depression can be found 5 to 18 days after the onset of therapy (3). Thrombocytopenia and hemorrhage should be expected in those patients who have received a total dose greater than 20 g (3).

Numerous anecdotal studies have purported to demonstrate the efficacy of IDU in the treatment of either local or systemic infection with viruses of the herpesvirus and poxvirus families. However, efficacy has been definitely demonstrated only for epithelial keratitis due to herpesvirus hominis. Double-blind studies have shown that 0.1% IDU or placebo administered locally (at 1 hr intervals during the day and 2 hr intervals at night) will, on the average, result in healing of the corneas in 72% or 24% of the cases, respectively (60). It is generally recognized that IDU is ineffective for corneal stromal lesions associated with herpesvirus hominis infection, and its efficacy for herpetic skin lesions remains controversial (61, 62).

The efficacy of IDU for systemic viral infection has not been established, in part because the natural history of the disease under study has frequently been unknown; in part because the dose and schedule of administration of IDU has varied among investigators with the optimal schedule still unclear; and in part because suitable controls have always been lacking. The disease that has received most attention is herpesvirus hominis encephalitis. The largest individual study shows no difference in mortality (33%) when patients given IDU are compared with untreated patients cared for in neighboring hospitals (3). Evidence given for the occurrence of significantly fewer neurologic residua in the treated patients would have been convincing if control patients had been used in a double-blind fashion. Clearly, a double-blind controlled evaluation of IDU for herpes encephalitis is vitally needed; fortunately one is now in progress. If it should later become apparent that IDU is not effective for systemic viral disease of man, this information would be understandable in view of the past difficulty in showing efficacy for IDU against systemic infection in animals. It is of interest that for herpetic keratitis, the effective topical dose (given on 20 occasions/24 hr) has a concentration of 1000 μg/ml, which is at least 12 times the maximal level of IDU reached in the serum, when the recommended total daily systemic dose for herpes encephalitis is rapidly infused over 2 hr (56).

Cytosine Arabinoside

The structure of cytosine arabinoside, 1-β-D-arabinofuranosyl cytosine (araC), is given in *3* of Figure 4. The molecule differs chemically from cytidine by having arabinose instead of ribose for its sugar moiety which, in fact, merely makes the 2'-hydroxyl *trans* instead of *cis* to the 3'-hydroxyl. Because the sugar then lacks a

2'-*cis*-hydroxyl, it is handled by enzymes as deoxyribose. Thus, araC is really an analog for cytosine deoxyribose or deoxycytidine. As the di- and triphosphorylated derivatives, araC competes with the similar forms of deoxycytidine for enzymes such as DNA polymerase used in the production of DNA. In cell culture, araC has activity against human viruses of the herpesvirus family as well as the poxvirus, vaccinia, and the rhabdovirus, rabies (63). With experimental infections in animals, topical activity has been found for herpesvirus hominis and vaccinia keratitis (64, 65), but efficacy against systemic viral infection has not yet been documented (66).

In man, intravenously infused araC is rapidly deaminated to 1-β-D-arabinofuranosyl uracil (araU), which lacks antiviral activity. Therefore, araC has a serum half-life of only 12 min and only 8% of the active compound is recovered in urine (Table 2, 67). For this reason araC is usually given as a continuous infusion. Deamination is much slower in the spinal fluid where araC has been found at a concentration equal to 40% of the steady state serum level (67). AraC resembles IDU with dose- and schedule-related toxicities resulting from its action against rapidly dividing cells in the bone marrow and gastrointestinal mucosa (68). In addition araC, even in low dosage, has a profound immunosuppressive effect resulting in a reduced response to the induction of cellular immunity (established cellular immunity persists) and a depression of both primary and anamnestic antibody response (69).

There have been a number of enthusiastic but anecdotal reports concerning the use of araC in the treatment of infection with varicella-zoster, herpesvirus hominis, or cytomegalovirus in immunosuppressed or nonimmunosuppressed individuals. However, the clinical efficacy of araC for any viral infection of man remained unclear until recently, when the results of a double-blind placebo c
finally became available (70). Not only did this study fail to show any efficacy for araC in the treatment of varicella-zoster infections, but it also clearly defined a group of lymphoma patients who did poorer with araC than placebo, presumably because of the immunosuppressive activity of araC. Such results again focus on the strict necessity for further double-blind studies in order to establish the clinical efficacy of araC for any viral infection of man.

Adenine Arabinoside

The most promising of the arabinosyl nucleosides at the present time is adenine arabinoside, 1-β-D-arabinofuranosyl adenine (araA), which differs from araC by having a purine base, adenine, rather than a pyrimidine base, cytosine (*4* in Figure 4). Because the 2'-carbon lacks a *cis*-hydroxyl group, araA is an analog of deoxyadenosine. As the triphosphate it presumably competes with deoxyadenosine triphosphate for enzymes such as DNA polymerase used in the synthesis of DNA. With human viruses in cell culture, araA is quite active against herpesvirus hominis, varicella-zoster, cytomegalovirus, and vaccinia (71, 72). In experimental animals, araA has been found effective both topically for herpesvirus hominis keratitis (72) and systemically for encephalitis due to vaccinia or herpesvirus hominis type 1 or 2 (73).

In man, araA is first quickly deaminated to 1-β-D-arabinofuranosyl hypoxanthine (araHx), which has comparable activity against viruses in vivo (74). The further metabolism of araHx to inactive products is comparatively slow. As a result, the potency of araA has a serum half-life of 90 min and appears in the urine in 49% of the administered dose (Table 2, 75). The concentration of araA in the cerebral spinal fluid has been reported to be 50% of the simultaneous serum level. Based on the results of phase two studies, it is currently recommended that araA, for systemic use, be given as a continuous infusion over 12 hr. Although the relevant studies in man are not published in detail, it appears that the expected marrow depression is mild with the dosages being evaluated (76). There is a transient elevation of serum glutamic oxaloacetic transaminase, and a preliminary report showed a significant increase in leukocyte chromosome breakage (77). Thus far, there has been no evidence of immunosuppression by araA.

The clinical efficacy of topical araA has been demonstrated in a double-blind study in which araA ointment, 33 mg/ml (three to five times per day) was found to be as effective as the standard IDU ointment, 5 mg/ml, for herpesvirus hominis epithelial keratitis (2). From patients included in phase two studies, there is also evidence suggestive of a favorable response in systemic infection with herpesvirus hominis, varicella-zoster, vaccinia, or cytomegalovirus. However, all of these latter experiences are anecdotal. To determine the true clinical efficacy of araA for systemic viral infection, a multihospital double-blind placebo controlled study is presently being carried out. There is optimism that araA may prove to be more efficacious than araC for the following reasons: araA has successfully been used to treat systemic infection in animals; araHx, its deamination product, is also active; araA does not immunosuppress; and the marrow depression from araA is not severe.

Methisazone

Methisazone, N-methylisatin-3-thiosemicarbazone (*5* in Figure 4), is considered the best candidate antiviral agent of several N-substituted analogs of isatin-3-thiosemicarbazone (isatin) (78). The analogs lose antiviral activity when substitution is made on either the side chain or the aromatic ring, or if the sulfur of the side chain is replaced by oxygen. As already indicated, the action of the thiosemicarbazones occurs late in the viral replicative cycle and, at least for vaccinia virus, is at the level of translation from late mRNA, resulting in disintegration of both early and late polyribosomes (79). In cell culture, methisazone and its analogs are known to have activity against vaccinia and the other human poxviruses, varicilla-zoster virus, and adenoviruses (80, 81). Surprisingly, activity has also been reported for several human RNA viruses (82). In experimental encephalitis in mice, methisazone has been found effective against vaccinia, alastrim, and variola major viruses (83, 84).

Methisazone has too low a solubility to be given as a solution (in the concentrations needed for man or large animals) and therefore is recommended only by the oral route as a micronized preparation in sucrose syrup (78). Although its clinical efficacy has been widely studied, there is still very little information available on the absorption, distribution, and elimination of methisazone in man (Table 2). From the scant number of patients studied, it appears that the peak serum level occurs at

about 6 hr and that the serum half-life is approximately 2.7 hr (85). The only important side effect of methisazone is vomiting, which has been reported in 11–66% of subjects taking the drug, usually about 4 to 6 hr after ingestion (10, 86, 87). Vomiting may have significantly interfered with the clinical evaluation of methisazone in that patients who vomited, retained an unknown proportion of the medication, knew they had not received placebo, and may have elected to omit further doses.

A major thrust in the efforts to establish the clinical utility of methisazone has been to determine its efficacy in the chemoprophylaxis of smallpox. In countries where smallpox is endemic, close contacts of index cases have been given methisazone in doses ranging from 3–6 g per day for 1–4 days. Control contacts, picked in alternating of random fashion, have been given either no drug or placebo (10, 86–88). It is evident from the summary of these studies in Table 5 that the evidence for the efficacy of methisazone is based on the first two studies. However, despite dealing with impressive numbers of contacts, these studies were unfortunately not designed with double-blind placebo controls. Because in each of the studies the group treated with methisazone had a lower incidence of smallpox than the respective control group, it is likely that methisazone did have a chemoprophylactic effect. It is important that this speculation finally be verified by a double-blind study with sufficient numbers of cases. In countries that have discontinued routine smallpox immunization, e.g. the United States, the nonimmunized population now has an increased vulnerability to smallpox (note study 3, which used contacts lacking previous immunization). Contacts of a case introduced from an endemic area, would be expected to benefit greatly if an effective chemoprophylactic agent could be added to primary immunization given after exposure.

Although a number of reports describe the use of methisazone therapeutically, its efficacy as a chemotherapeutic agent has yet to be extablished. A controlled study of its use in the treatment of smallpox failed to show significant effect (8). Unfortunately, reports of its successful use in the therapy of serious dermal complications

Table 5 Methisazone: chemoprophylaxis for smallpox contacts

Study	Double-Blind Design	Total No. Contacts		Clinical Disease		p value
		Methisazone	Control	Methisazone	Control	
				(%)	(%)	
1. Bauer (86)	No	2610	2560	0.69	3.99	0.001
2. do Valle[a] (87)	No	384	520	2.1	8.1	0.05
3. Rao[b] (88)	Yes	17	20	11.8	40.	ns
4. Heiner (10)	a. No	156	157	1.9	5.	ns
	b. Yes	106	103	3.7	4.8	ns

[a]Epidemic of alastrim.

[b]Contacts lacked previous immunization.

of vaccinia immunization, i.e. eczema vaccinatum and vaccinia gangrenosa, have all been anecdotal in nature (78, 89, 90). In light of the rising number of individuals with depressed cellular immunity, the time is ripe for a good double-blind study to determine the efficacy of methisazone as an adjunct to vaccinia immune globulin in the treatment of the serious vaccinial infections that occur in such individuals.

Interferon

Interferon is a proteinacious substance, released from cells during viral infection and under certain other circumstances, that has the capacity to convey on other cells a resistance to challenge with virus. Since interferon has been identified in both man and the lower animals during natural infection with virus, it is considered likely to be an important natural defense mechanism (91, 92). In addition, there is widespread anticipation of eventual fulfillment of a therapeutic potential for the interferon system, i.e. the use of exogenous interferon itself or an inducer of endogenous interferon, to prevent or treat viral disease in man.

Human interferon has never been fully purified, but has been found to behave chemically like a glycoprotein (93). Viral-induced human interferon has several molecular species that may be multiples of a predominant dimer form having a molecular weight of 23,000 and an isoelectric point of 5.6 (94, 95). The antiviral activity of interferon is still under investigation but is known to require the production of a second protein (96). Evidence has been presented that the resulting antiviral action can either attenuate ribosomes so they will no longer read viral RNA (18) or inhibit the activity of those RNA dependent polymerases brought into the cell by a virus (19, 20). In cell culture, interferon produced by cells of human or other animal origin has been shown to protect cells of the same or phylogenetically related species against a great variety of RNA and DNA viruses (97). Against experimental local or systemic viral infection in animals, interferon from the same or related species has also been found to be protective (98, 99).

Because the methods of producing large quantities of human interferon are only now being developed, and because full purification of interferon has still not been perfected, clinical studies in man have been quite limited, and the pharmacology of human interferon remains largely unknown. From the rate of disappearance of serum interferon after induction with a synthetic polymer, the serum half-life of human interferon can be estimated at less than 12 hr (100). It is also known that human interferon given intravenously to a rabbit has a serum half-life in the rabbit of about 73 min (101). Because serum, urine, saliva, spinal fluid, bile, and feces each have been shown capable of inactivating human interferon in vitro, it is likely that the fate of natural human interferon in vivo is almost entirely accounted for by metabolic degradation (102, 103). Partially purified human viral-induced leukocyte interferon has been associated with fever and chills when given intravenously to terminal cancer patients (104). However, these effects are considered secondary to leukocyte incompatability, and sufficiently pure human interferon would presumably have no toxicities because it is a native metabolite.

The same factors that have held up the pharmacological evaluation of interferon have made the determination of its clinical efficacy all but impossible. Several

volunteer studies, using highly purified interferon for prophylaxis against local infection, are currently under way. The best study so far reported a double-blind investigation using a total of 14 million units of interferon per volunteer. The interferon was given locally by nasal spray in multiple doses beginning 24 hr prior to innoculation of rhinovirus 4 (105). A significant decrease in both severe symptoms and frequency of virus-shedding was found. Although the therapy was far from being practical, at least the ability of exogenous human interferon to prophylactically attenuate a local viral infection was clearly established. For systemic viral disease, clinical trials to determine the efficacy of prophylactic or therapeutic exogenous human interferon, unfortunately, must await both a perfection in the purification of interferon and further development in the logistics of its production.

Polyriboinosinic Acid and Polyribocytidylic Acid

Because of the still unresolved problems in establishing a practical use for interferon itself, attention has turned to the various nonviral inducers of interferon as alternative means for prophylactic or therapeutic intervention. The best studied nonviral inducer of interferon is polyriboinosinic acid • polyribocytidylic acid (poly I•poly C, Figure 4–6). Poly I•poly C is the equal molar complex of the synthetic homopolymers of the nucleotides, inosinic acid, and cytidylic acid. By X-ray diffraction study, sedimentation coefficient, ultraviolet absorption, and melting point determination, poly I•poly C is a double stranded helical complex of the two homopolymers (106, 107). In low concentration poly I•poly C will protect cells in culture from challenge with virus. In higher concentrations the complex with both protect the cells and release assayable interferon into the medium (108). The mechanism by which interferon is induced is not fully worked out, although it is known to require derepression of the host cell genome with host directed protein synthesis (109) and may accomplish this without entering the cell (110). When given to a variety of animals by topical or parenteral administration, poly I•poly C is associated with a rapid rise in interferon levels and definite protection against local or systemic viral infection, respectively (111).

Studies with human plasma in vitro have shown it capable of almost completely inactivating poly I•poly C within 1 hr, presumably through circulating nucleases (112). It is likely therefore that in man metabolism accounts for the elimination of almost all of the administered dose. Biologically, it is found in man that parenteral poly I•poly C leads to a hyporeactive state lasting several days during which additional injections will not induce further interferon (100) [animal studies have indicated that protection against virus is not present during such a hyporeactive state (113)]. Unfortunately, the intravenous administration of poly I•poly C in a dose of 2.5 mg/m^2 to man is associated with increased fibrin split monomers, a prolongation of thrombin time, and fever; even with a dose of 1.0 mg/m^2, there is a detectable increase in fibrin split monomers. In dosage low enough to avoid toxicity there is no detectable interferon in the serum (114).

A double-blind study of topical poly I•poly C (1000 μg/ml) has shown it to be as effective as topical IDU (0.2%) for the treatment of superficial herpetic keratitis (115). Another double-blind study using nasal drops of poly I•poly C both pro-

phylactically and therapeutically has shown it to induce only minimal amounts of nasal interferon and to be associated with a small reduction in the severity of respiratory symptoms [following innoculation of either rhinovirus 13 or the Hong Kong strain of Influenza A_2 (116)]. As already noted, phase two studies have demonstrated that intravenous poly I•poly C is rapidly degraded and toxic and in addition has a tendency to produce a hyporeactive state. Further trials to determine its clinical efficacy via parenteral routes of administration have not been attempted.

Conclusion

It is evident that a monumental amount of hard work has been devoted to establishing practical viral chemotherapy. Nevertheless, it is quite clear that clinical efficacy has been established in a very few circumstances (Table 6). Clearly, there is a place for the topical use of antiviral agents in the treatment of herpetic keratitis, but at the present time there is no systemic viral disease for which we have a proven means of chemotherapy. It is encouraging that the use of amantadine and methisazone in the prophylaxis of influenza A_2 and smallpox, respectively, has been accepted. Hopefully, there will be a day soon when the problems peculiar to the field of viral chemotherapy will be effectively overcome and a proven treatment for systemic viral diseases will be at hand.

Table 6 Established clinical efficacy of antiviral agents

	TOPICAL		SYSTEMIC	
	Prophylaxis	Therapy	Prophylaxis	Therapy
Amantadine			+	
IDU		+		
AraC				
AraA		+		
Methisazone			+	
Interferon	+[a]			
Poly I-Poly C	+[a]	+		

[a] Protection against challenge inoculation but without adequate field trial against natural infection.

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