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COMMENTARY

NUCLEOSIDE ANALOGS WITH ANTIVIRAL ACTIVITY*

WILLIAM H. PRUSOFF and DAVID C. WARD

Departments of Pharmacology (W. H. P.) and Molecular Biophysics and Biochemistry (D. C. W.), Yale University School of Medicine, New Haven, Conn. 06510, U.S.A.

The fact that numerous nucleoside analogs have been synthesized as potential antiviral drugs demonstrates that there is no magic road to the development of an ideal antiviral agent. Many of the compounds tested evolved from programs centered on the preparation of antineoplastic agents, and several nucleoside derivatives have been obtained that are clinically useful as both antiviral and anticancer drugs. Is it, however, possible to prepare nucleosides which exhibit highly specific antiviral activity? What are the desirable features of such an ideal antiviral drug? How close do the nucleosides currently in clinical use or trial come to achieving these objectives? In this Commentary, we will attempt to answer some of these questions.

The first nucleoside approved for clinical use in the early 1960's was 5-iodo-2′-deoxyuridine (IdUrd). It is of some historical interest that Johnson and Johns [1] reported the synthesis of 5-iodopyrimidines in 1905 from Yale University. It was not until 1945, however, that Hitchings *et al.* [2] initiated a systematic study of the biological activities of various analogs of purine and pyrimidine bases. Soon after, this group [3] documented the modest inhibition by 5-bromouracil, 5-hydroxyuracil and 2,4-dithiothymine of the replication of vaccinia virus in cell culture. Visser *et al.* [4] in 1952 reported that various 5-substituted analogs of uridine (5-chloro, 5-diazo, 5-formamido, 5-hydroxy and 5-amino) inhibited the replication of Theiler's mouse encephalitis virus in mouse brain cultures. Because pyrimidine bases and ribonucleosides with substituents in the 5-position are poorly utilized in the biosynthesis of DNA precursor nucleotides, it perhaps was predictable, retrospectively, that when the 5-chloro [5], 5-bromo [6] and 5-iodo [7] derivatives of deoxyuridine were synthesized, they would exhibit, relative to the free bases, significant antiviral activity *in vitro* [8]. The subsequent demonstration that IdUrd was effective in an established virus infection in man [9, 10] was, therefore, of utmost importance. Other antiviral nucleosides were soon found; for example, 6-azauridine [11] and 1-β-D-arabinofuranosyl cytosine [12, 13].

A representative example of the type of modifications which yield nucleosides with antiviral activity is depicted in Fig. 1 for the pyrimidine series. The rationale for the synthesis of many of the known antiviral nucleosides evolved logically from our knowledge of the structure of the nucleic acids, with the hope that the synthetic nucleosides thus prepared might interfere either with the biosynthesis or function of the viral nucleic acids. There appears to be no limit to the creativity of the synthetic organic chemist in producing potential agents. However, limitations of time and funds dictate the imposition of priorities relative to the directions that one could pursue. Obviously one should not attempt to synthesize every conceivable nucleoside derivative even though the probability is that such an approach would produce some useful agents. Money and manpower not being a factor would make this a heavenly project for those chemists so inclined. One alternative to the 'shotgun' approach is to prepare derivatives of those nucleosides which have established antiviral activity in the hope that agents may evolve which have a greater selectivity for a virus-specific function and a lower, if not a total lack of, host toxicity.

As our knowledge of the molecular basis of viral reproduction becomes more sophisticated, because of applications of modern techniques of biochemistry and molecular biology, the goals of the chemotherapist in the design of potential antiviral substances have been given emphasis. Several biochemical events that are unique to the virus-infected cell or to the reproduction of the virus are now known, and these should provide novel targets in the development of new antiviral agents. Although critical biochemical differences do exist in the replication of viruses and mammalian cells, the question is whether we are astute enough to find and take advantage of such differences.

What are some of the potential sites for selective inhibition? It is well recognized that there are marked differences among the various viruses—for example, the composition of the viral nucleic acid as well as the capsid proteins, the presence within some viruses of specific enzymes, and the ability to induce the synthesis of virus-genome specified enzymes. Thus, it is not surprising that the various viruses may be affected differently by a particular physical or chemical agent. Dales [14] has recently reviewed some aspects of virus–cell interactions which are of importance to our understanding of the basis for such variation in drug effects.

INHIBITION OF VIRUS ADSORBTION

Most viruses interact with specific receptors on the cell surface. This primary event of viral infection determines host-range or cell-type specificity and precedes all aspects of viral replication. It is somewhat

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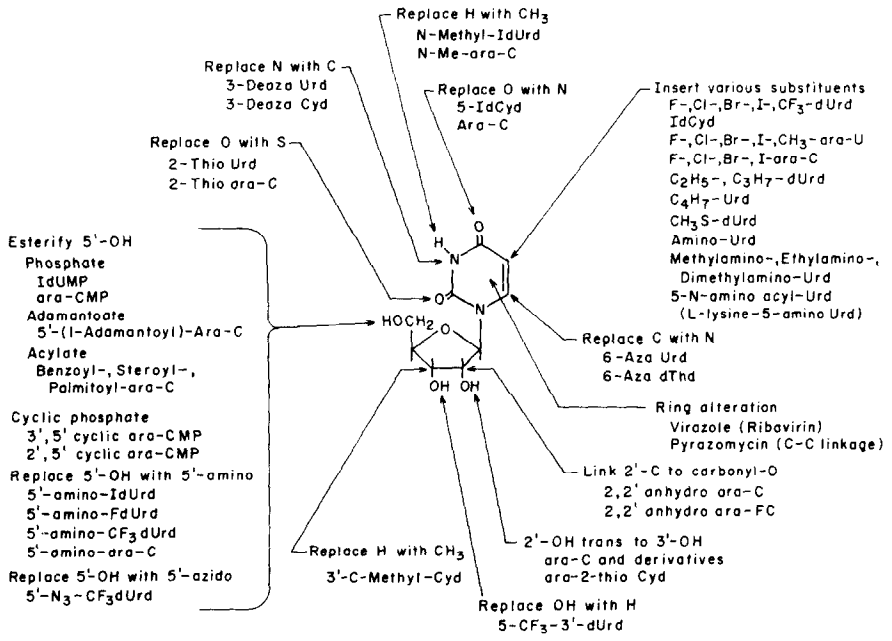

Fig. 1. Modified pyrimidine nucleosides with antiviral activity.

surprising, therefore, that the biochemical nature of virus receptors has not received more extensive investigation. While one must assume that the molecular architecture of the cell surface has a more important function than that of primarily aiding and abetting virus infection, the adsorbtion process represents a prime, yet little studied, target for specific viral inhibition. Cellular glycoproteins or glycolipids have been implicated in the attachment of Myxo, Paramyxo, Papova and Togaviruses, whereas the viral adsorbtion sites on Picorna, Reo and Rhabdoviruses contain functional sulfhydryl groups. Although a variety of polyanions, 1,2-diketones and natural glycoproteins inhibit viral adsorption to some degree, the only nucleic acid analog so far shown to exhibit such interference is 2-thiouracil [15].

INHIBITION OF VIRUS PENETRATION AND UNCOATING

The mechanisms involved in the penetration of viruses into the host cell and their subsequent uncoating have also been reviewed by Dales [14]. Four methods of penetration are now recognized. Of these, phagocytosis (viropexis) and fusion of the viral and plasma membrane are probably the most common. The other two methods propose entry through breaks in the plasma membrane or by direct passage through the membrane. After penetration into the cell, the viral genome becomes exposed within or separated from its capsid structure. The mechanisms of uncoating are often complex, and the details of the processes involved differ from virus to virus. While no nucleoside analog is known to inhibit these reactions, adamantanamine hydrochloride (amantadine HCl) and related structures prevent the initiation of progeny virus growth either by blocking the penetration or uncoating of the incoming virus particles [16, 17]. Although the exact mechanism of inhibition is unknown, the properties of amantadine HCl and related compounds have been reviewed recently [18].

INHIBITION OF AN INTRACELLULAR EVENT

Such events include inhibition of virus genome replication or transcription, viral mRNA translation, virus enzyme catalysis, or virus maturation. The various antiviral nucleosides, the active agent of interferon action, isatin β-thiosemicarbazone (marboran), guanidine, 2-(α-hydroxybenzyl)benzimidazole (HBB), ansamycins (rifamycin SV derivatives and streptovaricins), radiations sensitized by dyes (e.g. proflavin, neutral red), and phosphonoacetic acid all exert their effects in this area. Because this review is charged with discussion of antiviral nucleosides, the non-nucleoside agents will be mentioned only where appropriate for purposes of comparison.

5-Iodo-2'-deoxyuridine (IdUrd, IUdR, IDU, Idoxuridine)

The first clinically effective antiviral nucleoside, 5-iodo-2'-deoxyuridine, was synthesized originally as part of an anticancer program [7]. The chemistry, biochemistry and clinical applications of IdUrd have been reviewed recently [19–21]. The importance of IdUrd lies in the demonstration that a nucleoside drug can be developed which is effective clinically in an established virus infection. The efficacy of Idoxuridine in the therapy of herpes simplex infection of the corneal epithelium in man, a disease which is the major cause of blindness due to a corneal infection in the United States, has been unequivocally established and has been approved by the FDA for such use. Juel-Jensen [22, 23] has recently reviewed the clinical utility of Idoxuridine and several other antiviral agents in man. In addition to therapy of herpes keratitis, beneficial results with Idoxuridine in man have been obtained in the therapy of herpetic whitlows, genital herpes, recurrent genital herpes, cold sores, herpes zoster, vaccinia lesions and vaccinia whitlow [22, 23].

The mechanism of the antiviral action of IdUrd has been reviewed by Prusoff and Goz [19, 21] and, in brief, it is related to the adverse biological consequences of incorporating this thymidine analog into viral DNA. The incorporation of IdUrd into the DNA of normal uninfected cells is most likely primarily responsible for the toxicity that has been found during either topical or systemic therapy. Systemic toxicity is dose related and when infused into man at a concentration of about 100 mg/kg daily for 5 or 6 days, one observes stomatitis, leukopenia and alopecia [24]. Topical therapy does not result in these toxicities since, even if the total amount used were absorbed in the systemic circulation, the concentration achieved would be several orders of magnitude below the level required to produce these symptoms in man. Nevertheless, there are hazards involved in the topical therapy of herpes keratitis with Idoxuridine. These have been reviewed recently by McGill *et al.* [25] and include contact dermatitis, punctate epithelial keratopathy, follicular conjuctivitis, narrowing and occulation of the puncta, lid changes, and lacrimation.

In addition to these undesirable features, there are other concerns whose significance to man is difficult to evaluate, and these include the ability of IdUrd to: (1) induce the formation of virus particles in cell culture, (2) increase the mutation rate in bacteria, (3) produce chromosomal damage, and (4) affect embryonic development and differentiation (references cited in 19).

Thus, it would appear that IdUrd should perhaps rest its laurels on the knowledge that it is the first compound to clearly demonstrate a successful treatment of an established virus infection in man. The corollary is that there is a need for antiviral agents with considerably less toxicity. The desirable features of such an ideal antiviral drug should include: (1) ease of preparation (low cost), (2) good solubility at or close to the physiological pH, (3) chemical stability in solution and to heating (120°), (4) metabolic stability in the circulatory system, (5) sufficient nonpolarity to avoid problems of cell transport, (6) no incorporation into the DNA of the uninfected cell, (7) no immunosuppression, (8) no activation of virus, (9) no teratogenic effects, and (10) no mutagenesis or carcinogenesis.

Let us now examine the status of other nucleosides that are either in clinical trial or appear to have good merit (Table 1).

Table 1. Some nucleosides with antiviral activity

1. 5-Iodo-2′-deoxyuridine (IdUrd, IUdR, IDU, Idoxuridine)
2. 5-Iodo-2′-deoxycytidine (IdCyd, ICdR)
3. 5-Trifluoromethyl-2′-deoxyuridine (F_3TdR, F_3dThd)
4. 5-Ethyl-2′-deoxyuridine
5. 1-β-D-arabinofuranosylcytosine (Cytarabine, ara-C)
6. 9-β-D-arabinofuranosyladenine (Vidaribine, ara-A)
7. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole, Ribavirin)
8. 6-Azauridine
9. Miscellaneous nucleosides
   a. 3-Deazauridine
   b. 3-Deazacytidine
   c. 1,3-Dideazauridine
   d. 5-Methoxylmethyl-2′-deoxyuridine
   e. Isoprinosine

5-Iodo-2′-deoxycytidine (IdCyd, ICdR)

This precursor analog of IdUrd was first synthesized by Chang and Welch [26] and found to be effective against herpes simplex virus in culture by Herrmann [8] and against experimental herpetic keratitis in rabbits by Perkins *et al.* [27]. Schildkraut *et al.* [28] reported that IdCyd and the corresponding bromo analog both inhibit herpes simplex virus in culture, but have the advantage over the deoxyuridine analogs of being significantly less toxic to uninfected host cells. This is attributed to the presence of a virus-induced deoxycytidine kinase which allows IdCyd to be phosphorylated only in the infected cell. The mononucleotide of IdCyd then is deaminated by the cellular enzyme, dCMP deaminase, to IdUMP. Although increases in deoxycytidine kinase activity after infection with herpes simplex virus have been found by a number of investigators [29, 30], failure to find such an increase has also been reported by Cheng *et al.* [31]. Thus, the selective effect of the deoxycytidine analogs against herpes simplex virus may vary with the cell infected.

Mendez and Martenet [32] found IdCyd to be more effective than IdUrd in the therapy of experimental deep herpetic keratitis. Presumably IdCyd, in contrast to IdUrd, is protected from phosphorylase action, and hence has the opportunity to reach the infected area before being converted to the active deaminated derivative.

5-Trifluoromethyl-2′-deoxyuridine (F_3TDR, F_3dThd)

This antiviral and antineoplastic agent was first synthesized by Heidelberger and has been reviewed recently [33]. The reported advantages of this agent over IdUrd are the 10-fold greater potency against herpetic keratitis in rabbits and a 10-fold greater solubility in aqueous solution. Although F_3TDR is more potent than IdUrd, clinical trials in man took advantage of the greater solubility of F_3TDR, and hence comparison was made between a 1% solution of F_3TDR and a 0·1% solution of Idoxuridine [34]. Under these conditions, F_3TDR produced a greater beneficial effect in the therapy of herpetic keratitis in man relative to IdUrd. The basis for the antiviral effect is the incorporation of this analog into the virus DNA and a subsequent effect on the transcription of late mRNA.

As with IdUrd, F_3TDR is incorporated into the DNA of both the target virus and the uninfected cells. F_3dThd exhibits teratogenic activity in a number of experimental systems [35] and is toxic to bone marrow [33]. McGill *et al.* [25] have reported that F_3TDR therapy of herpetic keratitis in man will produce punctate epithelial erosions and epithelial microcysts if given more frequently than five times a day for more than a few days. If these toxicities are ignored and treatment continued, then frank epithelial edema with stromal swelling is observed. However, in contrast to IdUrd, F_3TDR was reported by Itoi *et al.* [36] not to be teratogenic when administered topically in the eyes of rabbits. In addition, while F_3Thd is mutagenic to bacteriophage T4, it has not been found to be mutagenic to Chinese hamster cells in culture. The toxicities of F_3dThd presumably

are related to its incorporation into the DNA of the uninfected cells as well as its potent and irreversible inhibition of thymidylate synthetase, a key enzyme in the biosynthesis of dTMP, an essential precursor of DNA.

5-Ethyl-2'-deoxyuridine

The biological activity of this antiviral agent has been reviewed recently by Shugar [37, 38] and by Prusoff and Goz [20]. This thymidine analog is effective against several DNA viruses and, although incorporated into DNA, does not produce a mutagenic effect in phage or in drosophila. In addition, studies by Singh *et al.* [39] with human lymphocytes and fibroblasts in culture have shown this analog to have no effect on chromosome morphology.

Clinical studies with 5-ethyl-2'-deoxyuridine indicated a positive therapeutic effect in the treatment of herpetic keratitis (references cited in 20). Recently, Martenet [40] reported this analog to be effective in the treatment of experimental deep herpetic keratitis in rabbits.

Although this antiviral agent is not quite as effective as IdUrd or F_3TDR, it does possess the important characteristic of being non-mutagenic. The lack of mutagenicity may be related to the fact that the p*K*a values of 5-ethyl-2'-deoxyuridine and thymidine are essentially identical. The lower p*K*a of IdUrd permits a greater probability of base-pair errors after incorporation into DNA because IdUrd is in the anionic form to a significantly greater extent.

1-β-D-Arabinofuranosylcytosine (ara-C, Cytarabine)

The antiviral potential of ara-C has been well reviewed [21, 41–44] and the activity against experimental herpetic and vaccinia keratitis established. Although initial studies of the therapy of varicella-zoster infections in man looked encouraging, a subsequent double-blind study showed no enhanced efficacy relative to a placebo. Similarly, reservations exist for its use in therapy of herpetic encephalitis or cytomegalovirus infections. Its use in herpetic ketatitis in man is restricted because of its toxicity and similar considerations apply to its potential systemic use. The marked antiviral activity *in vitro* is diminished *in vivo* because of the high rate of deamination to ara-U, a compound with no antiviral activity.

Associated toxicities include teratogenesis, potent immunosuppression, chromosomal aberrations, corneal speckling, leukopenia, thrombocytopenia, megaloblastosis, and mild hepatic and gastrointestinal toxicity. Lauter *et al.* [45], following a study of the cytotoxicity, minimal antiviral concentrations, and the pharmacokinetics of ara-C, concluded that ara-C would not be a useful antiviral agent in man because its therapeutic to toxic ratio approaches unity. Relative to IdUrd, ara-C is 10 times more toxic to cell cultures.

Because if its marked toxicity the use of this compound should be restricted to those situations where a controlled double-blind study has established its efficacy, and then only if other less toxic agents are not available.

9-β-D-Arabinofuranosyladenine (ara-A, Vidarabine)

Ara-A, like IdUrd, was first synthesized as an anticancer agent by Lee *et al.* [46]. A number of reports have appeared recently that review the antiviral activity of ara-A and its derivatives [41–44, 47]. A broad spectrum of DNA viruses as well as oncogenic RNA viruses, such as Rous sarcoma virus and Gross murine leukemia virus [48] are sensitive to ara-A. Most non-oncogenic RNA viruses are not, however, inhibited by ara-A.

Cutaneous herpetic lesions in hairless mice were treated with either ara-A, IdUrd or 6-azauridine by intraperitoneal administration, and beneficial effects were found with both ara-A and IdUrd, but not with 6-azauridine [49]. Neither ara-A nor ara-C had any significant effect on the final mortality of newborn mice infected with herpes virus hominus type 2 [50].

Ara-A appears to be equivalent to IdUrd in the therapy of herpetic keratitis in man, but not better. Herpes keratouveitis in man is not affected by IdUrd but was benefically treated by intravenous ara-A [51]. A pilot study by Ch'ien *et al.* [52] indicates a possible beneficial effect of ara-A in neonatal herpes if administered early after onset of neurological symptoms.

The unique feature of ara-A, which has encouraged clinical trial, is its relative lack of toxicity. It has been reported to be beneficial when given systemically in deep stromal infections of herpes keratitis, in herpes genitalis and is, at present, in clinical trial for therapy of herpes encephalitis. Ara-A is not immunosuppressive, a fortunate characteristic because ara-A requires an effective uncompromised immunological host defense mechanism for its antiviral activity [53].

Ara-A, after phosphorylation to the triphosphate derivative, has been reported to either inhibit DNA polymerase or to be incorporated into DNA. In contrast to ara-C, no incorporation into RNA has been found. Whereas ara-C is rapidly deaminated to an inactive substance, ara-hypoxanthine (ara-H), the deaminated product of ara-A, retains activity. Considerable effort [54] is in progress to find agents, such as coformycin [55] or erythro-9-(2-hydroxy-3-nonyl) adenine [56], which inhibit adenosine deaminase, the enzyme responsible for metabolic alteration of ara-A.

Although no acute toxicity has been reported in man, nausea and vomiting is a problem in about 30 per cent of the patients who receive ara-A. A reduction in hemoglobin, white blood cells and platelets has also been observed but this is not considered to be serious. Chromosomal aberrations have been found in human leukocytes exposed to ara-A in culture, and teratogenic effects in experimental animals.

A major disadvantage of both ara-A and IdUrd is their very low solubility, a property that necessitates the infusion of very large volumes when given systemically. Increased solubility of ara-A is afforded by the 5'-phosphate derivative or by the recently described arabinofuranosyladenine-5'-formate [57]. Since the efficacy of ara-A *in vivo* may be due to ara-H, the development of ara-H derivatives with increased solubility is in progress [47].

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole, Ribavirin)

This compound, synthesized by Witkowski *et al.* [58], has a broad spectrum of antiviral activity against RNA and DNA viruses both *in vitro* and *in*

vivo. Protection against a lethal infection by influenza virus, types A and B, in mice was best achieved when the drug was administered immediately after inoculation with the virus [59]. The compound is also effective in experimental herpes and vaccinia keratitis in rabbits, and recent studies by Sidwell *et al.* [60] showed Ribavirin to have a modest effect on Friend leukemia virus infections in mice. Again, the efficacy of the drug is related to the time of treatment.

The molecular basis for its antiviral activity has been reviewed by Simon *et al.* [61]. Ribavirin is first converted to the 5′-phosphate derivative through the action of deoxyadenosine kinase [62]. This nucleotide is a potent inhibitor of the enzyme inosinate dehydrogenase (K_i of $2{\cdot}7 \times 10^{-7}$ M), which is responsible for the conversion of inosinate (IMP) to xanthylate (XMP), the immediate precursor of guanylate.

Unfortunately, Ribavirin is most effective as a prophylactic agent, a characteristic which severely limits its utility. In addition, teratogenesis has also been observed. The great significance of this compound is the demonstration that a single nucleoside can have an effect against a wide variety of both DNA and RNA viruses. The spectrum of antiviral activity of other nucleosides is more limited.

6-Azauridine

The biological activity of azapyrimidine nucleosides has been thoroughly reviewed by Škoda [63]. 6-Azauridine in high concentrations has a broad spectrum of antiviral activity *in vitro* which includes both DNA and RNA viruses. Limited experiments with herpetic keratitis in rabbits and in man indicate a potentially benefical effect. 6-Azauridine was reported also to reduce the mortality in patients with smallpox.

This agent is immunosuppressive and is teratogenic. Although in man large doses generally produce no serious toxicities, Dantzig *et al.* [64] found a high incidence of serious CNS disturbances. The potential of 6-azauridine as an antiviral agent is limited both by its low potency and its toxicity, which may be related to either the presence of 6-azauracil in the preparation or to the sensitivity of the patient.

Miscellaneous nucleosides

Schabel and Montgomery [65] have compiled an extensive list of purine and pyrimidine nucleosides that have been evaluated for antiviral activity, and more recently Bloch [66] has reviewed the structure–activity relationship of a large number of biologically active nucleosides. Modest antiviral activity against several RNA viruses was exerted by the pyridine nucleoside derivatives 3-deazauridine [67, 68] and 3-deazacytidine [67]. More recently, Sharma *et al.* [69] reported that 4-(β-D-ribofuranosyl)-1,3-dihydroxybenzene (1,3-dideazauridine) inhibits herpes simplex virus type 1 *in vitro*.

5-Methoxymethyl-2′-deoxyuridine, the methyl ether of 5-hydroxymethyl-2′-deoxyuridine, was found by Meldrum *et al.* [70] to markedly inhibit the herpes virus of infectious bovine rhinotracheitis.

Of interest is the ability of the 'normal' nucleosides, thymidine [71, 72], deoxyguanosine [72] and deoxycytidine [72], to inhibit the replication of herpes simplex virus type 2 but not that of herpes simplex virus type 1. Borman and Roizman [73] had previously found deoxyadenosine and uridine to inhibit the replication of herpes simplex virus at relatively high concentrations.

Isoprinosine, the *para*-acetamidobenzoic acid salt of inosine dimethylaminoisopropanol in a molar ratio of 1:3, has been reported to inhibit a number of RNA and DNA viruses [74]. Conflicting reports have appeared concerning its efficacy as an antiviral agent in animal systems [75]; however, negative effects have been found [76, 77] in man challenged with influenza virus or rhinovirus. The mechanism responsible for its antiviral effect in cell culture has not been clarified as yet.

NEW DIRECTIONS

None of the above nucleosides possess all of the characteristics of the ideal antiviral agent. Perhaps the two most troublesome properties are toxicity and poor solubility. Because incorporation of drugs into nucleic acids of normal cells may be responsible for many of the toxicities associated with certain antiviral agents, we have embarked on a program concerned with the synthesis of compounds which hopefully would not be incorporated into the DNA of normal tissues. While such compounds might have little selective activity against those viruses suspected to be totally dependent on cellular enzymes for nucleotide metabolism and viral genome replication, the majority of clinically significant viruses do not fall into this category.

Previous studies [78, 79] have shown that 5′-amino-5′-deoxythymidine is a potent inhibitor of mammalian thymidine kinase, an enzyme that shows enhanced activity in many neoplasms and virus-infected tissues. This compound in addition has mild antivirus activity against herpes simplex virus type 1 in cell culture [80]. Therefore, the 5′-amino analog of 5-iodo-2′-deoxyuridine (5′-amino-2′,5′-dideoxy-5-iodouridine, AIU) was synthesized with the hope that we would retain the antiviral activity of IdUrd, decrease or eliminate incorporation into DNA of normal tissues (in an attempt to obviate host toxicity), and possibly increase its solubility (by virtue of the amino group in the 5′-position affording the possibility of salt formation).

Initial studies with AIU showed that it was a potent inhibitor of the replication of herpes simplex virus type 1 at concentrations which were totally devoid of apparent cellular toxicity [80, 81]. The antiviral activity and cytotoxicity of AIU relative to IdUrd, F_3dThd, ara-C and ara-A were determined. AIU is less potent than IdUrd, F_3dThd or ara-C on a molar basis; however, it is considerably more potent than ara-A. The most striking finding, however, is the marked cytotoxicity of IdUrd, F_3dThd and ara-C relative to AIU, when comparison is made at comparable antiviral concentrations [81]. Although an important feature of ara-A is its relative lack of cellular toxicity, we found that, at a concentration of ara-A that produced less antiviral activity than AIU, significant cytotoxicity was nevertheless observed. The total lack of toxicity of AIU to cells in culture was extended to include murine, avian, simian and human cells. Studies of toxicity in newborn and 8-day-old

mice revealed no evidence of gross or histological toxicity. These findings encouraged studies of the therapeutic effect of AIU on experimental herpetic keratitis in rabbits, and AIU (0·8%) and IdUrd (0·1%) were essentially equally successful [82]. The obvious extension of these studies to determine the effect of AIU on other herpes viruses, such as herpes zoster and cytomegalovirus, is in progress.

An additional remarkable feature of this agent is the restricted spectrum of antiviral activity. Of various DNA viruses challenged with AIU, herpes simplex virus type 1 is uniquely sensitive to inhibition. This selective antiviral activity and complete lack of host-cell toxicity would imply that the site of inhibition may be that of a virus-specific function. This is supported by the finding that radioactive AIU is taken up only by the herpes virus-infected cells. Furthermore, only in the infected cell is there a large increase in the pool size of dATP, dGTP and dCTP. Normally, infection of a cell with herpes virus results in a very marked increase in the pool size of dTTP; however, in the presence of AIU, a significant decrease in the magnitude of such an increase was observed. This may be attributed to a decreased virus effect as a result of the inhibition by AIU of herpes virus propagation or possibly by a feedback inhibition of ribonucleoside diphosphate reductase. Studies on the site of inhibition by AIU, as well as its mechanism of action, are in progress.

A potentially very important finding, which is in its early stages of exploration, is the inhibition of the replication of the oncogenic RNA viruses—Maloney and Rauscher murine leukemia virus. This direction is under intensive investigation in view of the relationship between certain RNA viruses to oncogenesis in experimental animals and the theoretical extension of such a relationship to man.

A very important characteristic of 5′-amino analogs of nucleosides may be their unique spectrum of antiviral activity. Indeed, the 5′-amino analog of ara-C [1-(5-amino-5-deoxy-D-arabinofuranosyl)cytosine; (Am-ara-C)] has been found to inhibit vaccinia virus but not herpes simplex virus. Therefore, the 5′-amino analogs appear to represent a unique class of antiviral agents with a highly restricted spectrum of antiviral activity, and a decreased toxicity which ranges to total absence as with AIU.

In conclusion, we have seen a very interesting transition in our thinking about antiviral agents in general and that of nucleosides in particular. Not too many years ago the concept of finding an antiviral drug was considered bordering on ludicrous. Today we are no longer concerned with the concept of an antiviral agent, but rather with the development of effective antiviral agents with little or no host cell toxicity. AIU appears to represent that type of compound since studies in cell culture, as well as with experimental animals, show excellent antiviral activity that is accompanied with an apparently complete absence of host-tissue toxicity. Whether this relationship will extend to man remains to be seen—the presumption based on animal experiments is that such indeed will be the case.

Thus, a new era may be evolving in the search for clinically effective antiviral drugs in which the emphasis will be on the development of compounds with little or no host-cell toxicity. Whether we can eventually find a compound that has all the characteristics of our ideal antiviral agent (see above) remains to be seen—but why not!

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