Antiviral Research, 4 (1984) 303–315
Elsevier

AVR 00147

Physical and biological consequences of incorporation of antiviral agents into virus DNA*

William H. Prusoff**, William R. Mancini, Tai-Shun Lin, J.J. Lee, Scott A. Siegel and Michael J. Otto***

Yale University School of Medicine, Department of Pharmacology, 333 Cedar Street, New Haven, CT 06510, U.S.A.

(Received 23 November 1983; accepted 2 May 1984)

Summary

The molecular basis for the antiviral activity is discussed for a variety of nucleoside compounds approved for clinical use in the U.S.A. (5-iodo-2'-deoxyuridine, 5-trifluoromethyl-2'-deoxyuridine, 9-β-D-arabinofuranosyladenine, 9-(2-hydroxyethoxymethyl)guanine), or in clinical trial (*E*-5-(2-bromovinyl)-2'-deoxyuridine, 1-(2-deoxy-2-fluoro-β-D-arabinosyl)-5-iodocytosine, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), or of specific interest to our laboratory (5-iodo-5'-amino-2',5'-dideoxyuridine, 5'-amino-5'-deoxythymidine). The consequence of incorporation of idoxuridine, the 5'-amino analog of thymidine or the 5'-amino analog of idoxuridine into the DNA of herpes simplex virus type 1 on transcription and translation is emphasized.

idoxuridine, aminonucleosides, herpesvirus DNA, herpesvirus RNA, herpesvirus protein, nucleoside analogs

Introduction

The selective antiviral activity of many nucleoside analogs is related to either a unique or preferential activation of the agent by a virus encoded enzyme in the infected cell, or to a preferential trapping of the agent in the infected cell, as a consequence of augmented enzyme activity. The former is exemplified by compounds

* Presented at the International Symposium on Antiviral Compounds, Universitäts-Augenklinik Eppendorf, Hamburg, F.R.G., June 13–16, 1983.

** To whom all correspondence should be sent.

*** *Present address*: Sterling Winthrop Research Institute, Rensselaer, New York, U.S.A.

such as the 5'-amino analog thymidine (AdThd), the 5'-amino analog of 5-iodo-2'-deoxyuridine (AIdUrd), *E*-5-(2-bromovinyl)-2'-deoxyuridine (BVdU), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC), and 9-(2-hydroxyethoxymethyl)guanine (acyclovir). These compounds are uniquely or preferentially activated by phosphorylation by kinases encoded by HSV-1, HSV-2 and varicella zoster virus which are induced during the infective process.

The second group of compounds include 5-iodo-2'-deoxyuridine (idoxuridine, IdUrd) and 5-trifluoromethyl-2'-deoxyuridine (trifluridine, F_3dThd). These compounds are not selectively activated by a virus encoded enzyme, but are substrates for both cellular and viral kinases. The basis for their clinical utility is related to a marked increase in thymidine kinase activity in the infected cell, resulting in the entrapment of more of the nucleoside analog as the monophosphate than in the adjacent uninfected cell. Their use is restricted to topical administration of superficial infections, because uninfected cells in the S-phase of the cell cycle have augmented thymidine kinase activity, and will accumulate the phosphorylated drug with subsequent cytotoxicity such as bone marrow depression.

The antiviral activity of the nucleoside agents is dependent upon the initial phosphorylation, but this metabolic event does not account for antiviral activity per se. We have found a direct correlation between the uptake of idoxuridine, AIdUrd, and BVdU into the DNA of HSV-1, and the inhibition of the formation of infectious virions.

Our laboratory has initiated a program to determine the consequences of such incorporation into DNA, in an attempt to understand the molecular basis for the antiviral effect.

5-Iodo-2'-deoxyuridine

Incorporation of IdUrd into DNA produces a variety of physical and biological effects, and some of the macromolecular effects have been discussed by Szybalski [38], Brockman and Anderson [5], Prusoff et al. [30], and Prusoff and Goz [31].

Table 1 indicates many of the consequences of incorporation of IdUrd in substitution of thymidine into cellular DNA, and some similar effects have been observed when IdUrd is incorporated into virus DNA, as well as (1) inhibition of viral replication, and (2) enzyme induction or repression. The basis for the activity of IdUrd is probably not a result of steric considerations, since Van der Waals' radii of the methyl and iodine are similar, that of iodine being 215 pm whereas that of the methyl group is 200 pm. However, the electron configuration of the pyrimidine moiety is altered because the halogen may produce two different effects. The first of these results from the high electronegativity of the halogen atom, so that electrons, by the inductive effect, are pulled away from the pyrimidine ring. The second effect is a consequence of the halogen atom having an unshared pair of electrons which can initiate a resonance effect in the pyrimidine ring by increasing the electron density of the ring. The inductive effect of the halogen is probably the dominant effect responsible for the labilization of the proton on the N3 position of IdUrd, which is readily measured by determination of the pKa of thymidine (9.8) relative to that of IdUrd (8.2).

TABLE 1

The effect of replacement of DNA thymidine by 5-iodo-2'-deoxyuridine

I. Physical	
(1)	Increased density
(2)	Increased lability to stress
(3)	Increase in stacking energy
(4)	Increase in temperature of helix-coil transition
(5)	Increased binding to proteins
(6)	Slight decrease in pH required for strand separation
(7)	Increased sensitivity to X-ray and UV-radiation
II. Biological	
(1)	Increased rate of mutation
(2)	Increased errors in protein formation
(3)	Inhibition of cellular reproduction
(4)	Inhibition of expression of differentiated cell functions
(5)	Virus induction

The antiviral activity of IdUrd has been extensively reviewed [31,33] and is a consequence of its incorporation into viral DNA.

The effect of incorporation of IdUrd into the DNA of bacteriophage T2 and T4 on the replicative and biochemical properties was studied in our laboratory by Goz and Prusoff [19]. The yield of phage particles was the same whether the DNA is substituted with IdUrd or not, and even the phage, with as much as 60% IdUrd substitution for dThd, could adsorb to bacteria and inject their DNA. However, they were not able to induce normal amounts of the phage-specific enzymes dihydrofolate reductase, lysozyme and 2'-deoxycytidylate hydroxymethylase. Aamodt and Goz [1] found that the reduced enzyme activity was probably related to a decreased synthesis, rather than the formation of an altered enzyme with less activity. Goz and Prusoff [20] studied the effect of IdUrd substitution in phage DNA by the genetic technique of marker rescue, and found from 20 to $> 90\%$ individual gene activations among 30 genes studied. The extent of inactivation of a particular gene function was related to the amount of IdUrd incorporated into the phage DNA.

A relationship has been found between the incorporation of IdUrd into the DNA of SV40 virus [6], adenovirus [39,22] and HSV-1 [13] and loss of infectivity. Smith and Dukes [36] found the ratio of the total number of HSV virions to infectious virions was increased when grown in the presence of IdUrd. Similarly Prusoff et al. [29] with IdUrd, and Easterbrook and Davern [11] with BrdUrd have shown that the incorporation of these analogs into the DNA of vaccinia virus decreased infectivity. Kaplan et al. [23] observed incorporation of IdUrd into the DNA of pseudorabies virus resulted in a failure in the assembly of the viral components into an infectious particle, as did Easterbrook and Davern [11] with BrdUrd and vaccinia virus.

The studies with adenovirus by Kan-Mitchell and Prusoff [22] revealed that the reduction of infectious virus by IdUrd was related to the formation of uninfectious virus rather than a reduction in total virions produced. Analysis by neutral and

alkaline sucrose density centrifugation of the IdUrd-substituted adenovirus DNA revealed no double- or single-stranded breaks.

The effect of incorporation of IdUrd into the DNA of adenovirus was a marked decrease in the rate of synthesis of late (9–24 h) viral proteins, but not of early (2–6 h) viral proteins. 5-Bromo-2'-deoxyuridine was found by Pennington [28] to exert a similar preferential effect on protein synthesis of vaccinia virus; however, the synthesis of only some late proteins was affected.

The effect of IdUrd incorporation into the DNA of HSV-1 has been under intensive investigation in our laboratory by Fischer et al. [13] and Otto et al. [26]. Fischer et al. [13] quantified the degree of incorporation of IdUrd into HSV-1 DNA by determination of the shift in density during isopycnic centrifugation in CsCl. There is a linear relationship between the amount of IdUrd incorporated into HSV-1 DNA and the concentration of the analog to which the virally infected cells were exposed. A 20% substitution of thymidine residues in HSV-1 DNA was associated with an 85% reduction in the formation of infectious virions.

HSV-1 DNA isolated from control cultures and labeled with [^{14}C]thymidine and from cells exposed to IdUrd and labeled with [^{3}H]thymidine were cosedimented in both neutral and alkaline sucrose density gradients. Under neutral conditions both the control and IdUrd-substituted DNA sedimented as single peaks, however, the substituted peak was slightly more dense. Thus there was no evidence of double-stranded breaks in the DNA.

McCrea and Lipman [25] reported extensive fragmentation of vaccinia virus DNA substituted with IdUrd, which may be related to the method of preparation used for electron microscope visualization, or to differences in the synthesis or packaging of the vaccinia DNA.

Buettner and Werchau [6] did find IdUrd produced single-stranded breaks in the DNA of SV40 virus which they reported was dependent on exposure of the SV40 DNA to light. Incorporation of F_3dThd into the DNA of vaccinia virus was found by Fujiwara and Heidelberger [17] to produce a decrease in the size of the DNA relative to control as evidenced by sucrose density gradient centrifugation.

Although extensive incorporation of IdUrd into HSV-1 DNA had no apparent effect on its physical integrity, as analyzed in a neutral or alkaline sucrose density gradient for possible single- or double-stranded breaks, the substituted DNA produced abnormal patterns of transcription and subsequent translation.

The effect of IdUrd on herpesvirus gene expression has been under investigation in our laboratory. Since early transcription does not depend on newly synthesized viral DNA, it is not affected during exposure to IdUrd. However, marked effects are seen in the formation of late RNA, since this process is dependent upon newly synthesized DNA.

Incorporation of [^{3}H]uridine into total RNA of HSV-1 infected Vero cells was not appreciably affected, but within 8 h after infection the amount of viral poly A^+ RNA formed in the presence of IdUrd was about 50% of that produced in the untreated infected cells (Fig. 1). This differential increased almost 3-fold at 18–24 h post infection (p.i.). Concomitantly the amount of viral poly A^- RNA formed in the presence of IdUrd within 8 h p.i. was 50% greater, and at 18–24 h p.i. the virus-speci-

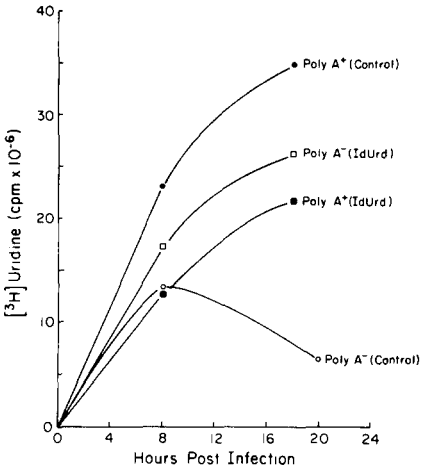


Fig. 1. The effect of 5-iodo-2′-deoxyuridine (IdUrd) on the synthesis of HSV-1-specific RNA.

fic poly A^- RNA was about 400% greater. These effects which are related to the substitution of IdUrd for thymidine in the newly synthesized viral DNA may be produced by an inhibition of polyadenylation, or a stabilization of late viral transcripts.

Studies on the effect of IdUrd on the translation of viral RNA, as expected, showed no inhibition of early alpha proteins, but there was significant reduction in late beta and gamma proteins. There was great variation in the amount of the reduction of specific proteins, with levels ranging from 0 to 65%.

As indicated earlier, IdUrd produced no significant decrease in the total number of virions produced, but the progeny virions had altered protein patterns as revealed by SDS–polyacrylamide gel electrophoresis relative to control HSV-1 [26].

The IdUrd-substituted virions were defective in their ability to induce a normal protein pattern upon subsequent infection of Vero cells in the absence of added IdUrd, in that there was a decreased ability to synthesize all viral-induced proteins and glycoproteins.

Thus the IdUrd-substituted virions have: (1) a reduced ability to produce plaques; (2) an altered ability to code for new proteins; (3) an increased particle to pfu ratio; and (4) an ability to interfere with the replication of unsubstituted (control) HSV-1 virus.

5′-Amino-nucleosides

5-Iodo-5′-amino-2′,5′-dideoxyuridine (AIdUrd, AIU) and 5′-amino-5′-deoxythymidine (5′-AT, AdThd) are both highly selective antiviral agents with essentially no cytotoxicity. Both inhibit HSV-1 and HSV-2, and in addition AIU also inhibits varicella zoster virus and the Epstein–Barr virus in cell culture. Both amino analogs are efficacious in the topical therapy of experimental herpetic keratitis in rabbits, but

they are not as potent as idoxuridine. AIU is also effective against oral HSV-2 infection in mice.

Metabolic studies established that these compounds are substrates for viral but not cellular thymidine kinase. The viral enzyme initially phosphorylates the amino moiety forming a phosphoramidate bond, and then adds a second phosphate in a phosphodiester linkage to the phosphoramidate (Fig. 2). A third phosphate is then added in a phosphodiester linkage by a cellular enzyme. There is a direct correlation between incorporation of these analogs into the HSV DNA and inhibition of the formation of infectious virions. AIU and AdThd are incorporated internally into the DNA structure with the formation of a phosphoramidate bond (Fig. 3).

Fischer et al. [13] using alkaline and neutral sucrose gradients found that the presence of AIU, in the DNA of HSV-1 results in double- and single-stranded breaks, which increased proportionately to the amount of incorporation of this analog into the DNA.

In contrast, the incorporation of AdThd into the viral DNA, which also forms a phosphoramidate bond, produced no single- or double-stranded breaks. Hence AdThd and IdUrd behaved similarly with respect to the physical integrity of the substituted DNA. It thus appears that the combination of the 5-iodo and the 5′-amino moieties may be responsible for a decreased stability of the DNA, since the presence of only the 5-iodo moiety, as with IdUrd substitution, or the presence of only a phosphoramidate bond, as with AdThd substitution, does not introduce any detectable instability. X-ray crystallography and NMR studies of AIU by Birnbaum et al. [4] revealed an unusual O(1′)-endo pucker in the conformation of the deoxyribose moiety, whereas the usual conformation of nucleosides is C(2′)-endo or C(3′)-endo. The relationship between this unusual structure and the instability of AIU-substituted DNA is not clear. Unfortunately we have not been able to crystallize AdThd for X-ray crystallography to determine whether it does or does not have a conformation similar to AIU.

The effect of incorporation of AIU and AdThd into the herpesvirus DNA on gene expression was investigated. Early transcription does not depend on newly synthesized viral DNA, and was unaffected during exposure to AIU, AdThd, or IdUrd. Total accumulation of [^{3}H]uridine into RNA in HSV-1-infected Vero cells is not significantly affected, but the amount of HSV-1-specific poly A$^+$ RNA begins to decline with respect to infected control cells as early as 8 h after infection. The reduced levels of HSV-1-specific poly A$^+$ RNA is more pronounced at 18–24 h p.i. since poly A$^+$ RNA in

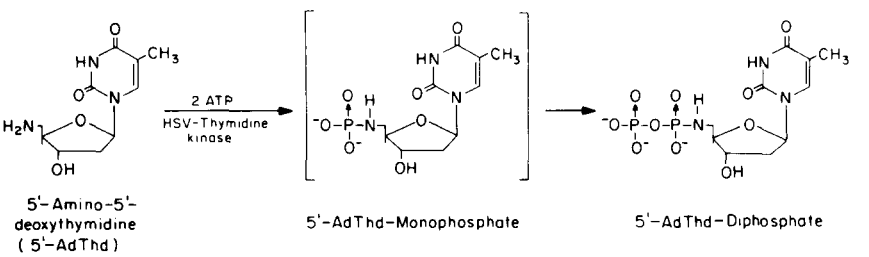


Fig. 2. Metabolism of 5′-amino-5′-deoxythymidine (AdThd) by HSV-1 thymidine kinase.

control cells continues to rise whereas that in the AIU- and AdThd-treated cells remains essentially unchanged (Figs. 4 and 5).

AIU produced a small increase in the HSV-1-specific poly A^- RNA at 8 h p.i., which appears more pronounced at 18 h p.i. because the control poly A^- RNA is markedly decreased at this latter time (Fig. 4). However, AdThd produced about a 50% increase in HSV-1- specific poly A^- RNA at 8 h p.i., and by 18 h there was a marked increase in poly A^- RNA which was about 8-fold greater than that produced by the control virions. In contrast to the RNA produced by the control virions 18 h p.i., where the ratio of poly A^+ RNA to poly A^- RNA was about 5–6 : 1, that seen with all three analogs was the reverse, the most striking being that seen with AdThd in which the ratio of poly A^+ RNA to poly A^- RNA was about 1 : 10 (Fig. 5).

These effects are, as with IdUrd, a consequence of incorporation of AIU and AdThd into the viral DNA in substitution of DNA thymidine. The increase in poly A^-

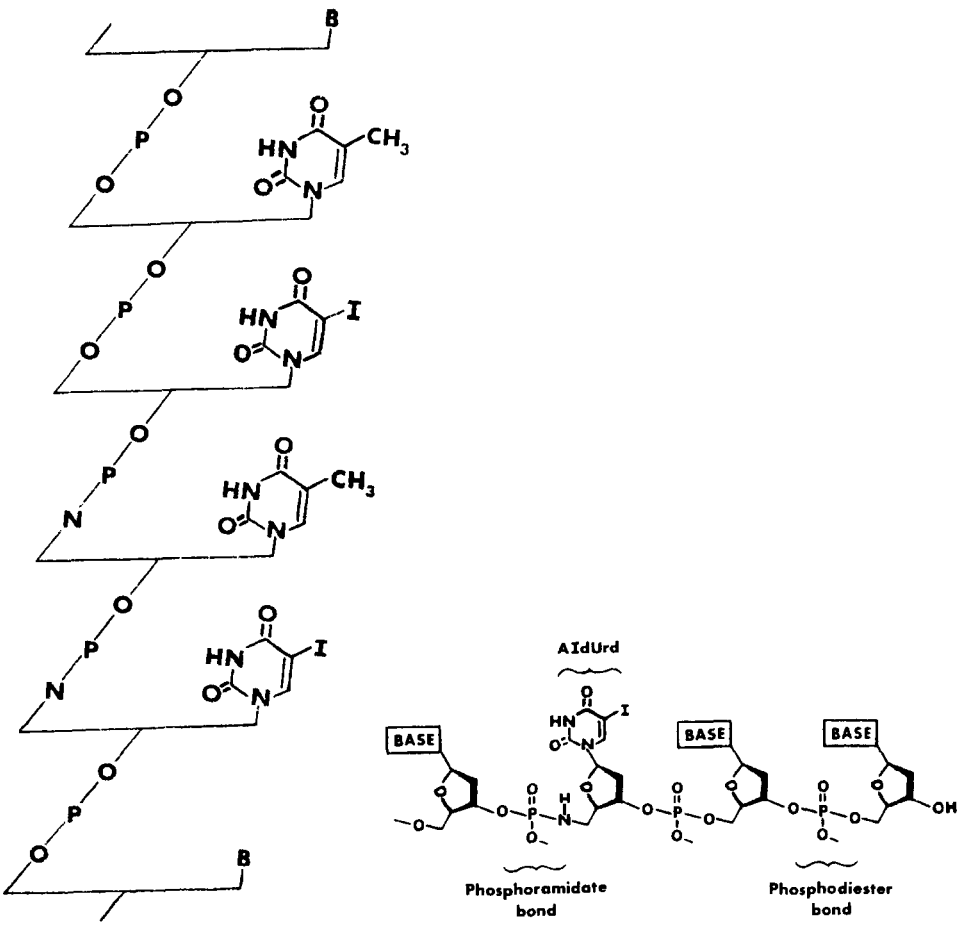


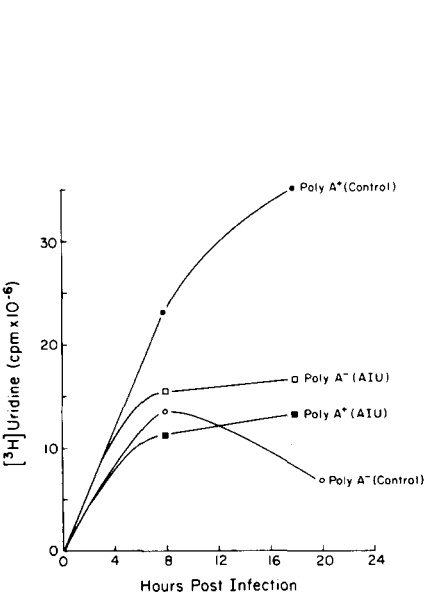
Fig. 3. Comparison of a phosphoramidate bond and a phosphodiester bond in DNA.

RNA along with the decrease in poly A^+ RNA suggests an inhibition of polyadenylation, as well as a possible stabilization of late viral transcripts.

AIU and AdThd produced altered expression of HSV-1-induced proteins as well as RNA. Infected cell proteins (ICPs) synthesized in the presence of the nucleoside analogs were analyzed [26], and it was found that there was no effect on HSV-1-induced alpha proteins, but beta and gamma proteins were reduced as much as 60%. There are two exceptions: ICP 39 (36 kDa) was not reduced, and ICP 36 (42 kDa) was increased during drug treatment.

Progeny virions, isolated from control cells or drug-treated cells, were analyzed with respect to their polypeptide make-up and for their ability to induce HSV-1 proteins in non-drug-treated Vero cells. The progeny virus from drug-treated cells exhibited altered protein patterns on SDS–polyacrylamide gels with respect to control HSV-1.

The progeny virions from AIU- or IdUrd- but not from AdThd-treated cells, were defective in their ability to induce proteins upon subsequent infection of non-drug-treated Vero cells. Two unusual phosphoproteins were detected: one with an apparent molecular mass of 30 kDa was induced with progeny virus from AIU-treated cells, and another at approximately 69 kDa was induced by progeny virus from AdThd-treated cells. Whereas progeny virus from IdUrd- and AIU-treated cells produced decreased amounts of glycosylated proteins, virions from AdThd-treated cells had no such effect.



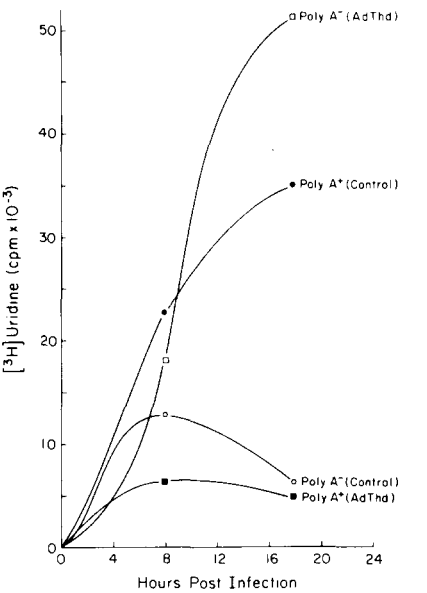


Fig. 4. Effect of 5-iodo-5'-amino-2',5'-dideoxyuridine (AIU, AldUrd) on the synthesis of HSV-1-specific RNA.

Fig. 5. Effect of 5'-amino-5'-deoxythymidine (AdThd) on the synthesis of HSV-1-specific RNA.

Thus IdUrd, AIU and AdThd are all incorporated into herpes simplex progeny DNA in substitution of thymidine. Such incorporation had the following results: (1) Formation of single- and double-stranded breaks in the viral DNA as a consequence of the incorporation of AIU but not of IdUrd or AdThd into the DNA. (2) AIU, IdUrd and AdThd did not affect markedly the synthesis of total HSV-specific late RNA, but the formation of poly A^- HSV-specific RNA was significantly increased. (3) All three analogs reduced the synthesis of most of the late HSV-specific proteins. (4) An overproduction of HSV thymidine kinase was observed. (5) Progeny virus: (a) have abnormal protein composition; (b) are less infective; (c) have a greater particle/pfu ratio with only a slight reduction in total particles; and (d) have the ability to interfere with infection by standard virus.

Trifluoromethyl-2'-deoxyuridine (F_3dThd, TFT)

F_3dThd like IdUrd is phosphorylated by both cellular and viral encoded thymidine kinase. In addition, the monophosphate of F_3dThd is a potent inhibitor of thymidylate synthetase. The triphosphate derivative of F_3dThd has a higher affinity for vaccinia virus DNA polymerase than for cellular DNA polymerase, and its incorporation into viral DNA is probably the critical event for its antiviral activity against vaccinia virus. Thus F_3dThd has been shown to produce non-infectious, morphologically defective virions with fragmented DNA, as well as a 30% reduction in the formation of late vaccinia virus mRNA. It was postulated by Heidelberger and King [21] that the mechanism of action of F_3dThd against HSV-1 and HSV-2 is probably similar to that for vaccinia virus. References to incorporation of F_3dThd into phage, mammalian cells and vaccinia virus as well as the physical and biological consequences are presented in the review by Heidelberger and King [21].

9-β-D-Arabinofuranosyladenine (ara-A)

Ara-A has a broad spectrum of antiviral activity against DNA viruses. It is phosphorylated to the mono-, di- and triphosphate derivatives by cellular enzymes and is incorporated into both cellular and viral DNA. There are numerous biochemical sites where ara-A or its phosphorylated derivatives have exhibited an inhibitory effect: (1) inhibition of HSV DNA polymerase; (2) incorporation into viral DNA; (3) incorporation into cellular DNA; (4) DNA elongation slowed; (5) inhibition of ribonucleoside diphosphate reductase by ara-ATP; (6) inhibition of RNA polyadenylation by ara-ATP; (7) inhibition of terminal deoxynucleotidyl transferase; (8) inhibition by ara-A of *S*-adenosylhomocysteine hydrolase with resultant inhibition of methylation of mRNA and tRNA.

Therefore it is not clear if the critical site of inhibition of ara-A is incorporation into viral DNA. The HSV DNA polymerase has an associated 3'-exonuclease, which can remove the terminal ara-AMP and this could result in slowing of DNA-chain elongation. However, a terminal ara-AMP that escapes cleavage will appear in internucleo-

tide linkage. The consequences of incorporation of ara-A into viral DNA have not been clarified. See reference [27] for more details.

9-(2-Hydroxyethoxymethyl)guanine (acyclovir, ACV)

ACV inhibits the replication of HSV-1, HSV-2, VZV, and the Epstein–Barr virus. It is preferentially phosphorylated by the virus-encoded thymidine kinase, and then further phosphorylated by cellular enzymes to the di- and triphosphate derivatives of ACV which compete with dGTP for incorporation into DNA [12].

Since the 3′-exonuclease associated with HSV DNA polymerase cannot remove terminal ACV, this analog is responsible for termination of DNA elongation. Furthermore, the ACV-substituted HSV DNA is a potent inhibitor of the HSV DNA polymerase.

E-5-(2-Bromovinyl)-2′-deoxyuridine (BVdUrd, BVdU)

BVdU is one of the most potent inhibitors of HSV-1 and about 200-fold less inhibitory to HSV-2. Initial phosphorylation of BVdU by the virus-encoded thymidine kinase is required for activation, and although the binding affinity by the HSV-1 enzyme is 20-fold greater than by the HSV-2 enzyme, the relative rate of phosphorylation of BVdU by the HSV-2 enzyme is about 1.6-fold greater. These two properties tend to counteract each other. Of possible greater significance is the finding by Fyfe [18] that the HSV-2 thymidine kinase has about 50-fold less associated dTMP–kinase activity, and if the monophosphate of BVdU is a poor substrate, then this may explain the relative insensitivity of HSV-2 to BVdU.

Allaudeen and his colleagues [2,3] found the 5′-triphosphate of BVdU (BVdU–TP) exerted a greater inhibition of HSV-1 polymerase than of cellular DNA polymerase [2], as well as the ability of purified HSV DNA polymerase to utilize the triphosphate of BVdU in substitution of dTTP and to be incorporated into cellular and viral DNA of HSV-1-infected Vero cells [3].

The relationship between incorporation of BVdU into HSV-1 DNA with virus infectivity and DNA integrity was investigated by Mancini et al. [24]. BVdU produced a dose-dependent shift in the density of HSV-1 DNA, and the degree of inhibition of viral replication was related to the amount of BVdU substituted for the thymidine in HSV-1 DNA.

Analysis of the substituted DNA by alkaline sucrose density gradient centrifugation revealed a dose-dependent increase in single-stranded breaks [24]. Whether the strand breakage actually occurs in vivo or is a consequence of the isolation procedure is not clear. However, it is clear that the equivalent handling of control HSV DNA and BVdU-substituted HSV DNA, the latter is more labile. Thus the potent antiviral activity of BVdU correlates not only with its incorporation into HSV-1 DNA, but also with the induction of an altered stability of the substituted DNA.

1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC)

The high potency of FIAC as an antiviral agent is similar to that of BVdU, but FIAC is active against both HSV-1 and HSV-2. FIAC also requires the viral-encoded thymidine kinase for activation by phosphorylation. FIAC, per se, is not incorporated into DNA, but after extensive metabolic conversion it appears in cellular DNA as the 2′-fluoroarabinosyl of cytosine (FAC), thymine (FMAU), and 5-iodouracil (FIAU), and in HSV DNA primarily as 2′-fluoroarabinosyl-5-iodouracil (FIAU). See reference [16] for more details.

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

Ribavirin is a broad spectrum antiviral agent against both RNA- and DNA-containing viruses. This compound has multiple sites of potential inhibition including perturbation of nucleotide pools, inhibition of the 5′-capping of viral mRNA, inhibition of animal as well as influenza-A RNA polymerase, and a small uptake into cellular RNA and DNA [37].

Other nucleosides

There are a number of other antiviral agents that have been demonstrated to be incorporated into viral DNA and these include 5-iodo-2′-deoxycytidine [16], 9-(1,3-dihydroxy-2-propoxymethyl)guanine [7], 5-propyl-2′-dUrd [35], 5-ethyl-2′-dUrd (references cited in [14]).

No doubt there will be others; however, the physical and biochemical consequences of their incorporation have not been elucidated as yet.

Future directions for development of antiviral agents have been discussed by Cheng et al. [8], Prusoff et al. [34], and De Clercq [10], and a discussion of some of the problems in the use of antiviral agents has been recently reviewed by Prusoff and Otto [32] and by Darby and Field [9].

Acknowledgements

The research done by our laboratory was supported by Grants CA-05262, T32-CA-09085 and F32-CA-06723 from the National Cancer Institute, United States Public Health Service.

References

1 Aamodt, L. and Goz, B. (1970) An immunological study of an enzyme made by phage containing 5-iodo-2′-deoxyuridine-substituted deoxyribonucleic acid. Biochem. Pharmacol. 19, 2400–2403.

2 Allaudeen, H.S., Kozarich, J.W., Bertino, J.R. and De Clercq, E. (1981) On the mechanism of

selective inhibition of herpesvirus replication by (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. Proc. Natl. Acad. Sci. U.S.A. 78, 2698–2702.

3 Allaudeen, H.S., Chen, M.S., Lee, J.J., De Clercq, E. and Prusoff, W.H. (1982) Incorporation of *E*-5-(2-halovinyl)-2′-deoxyuridines into deoxyribonucleic acids of herpes simplex virus-1 infected cells. J. Biol. Chem. 257, 603–606.

4 Birnbaum, G.I., Lin, T.S., Shiau, G.T. and Prusoff, W.H. (1979) A novel zwitterionic structure and an unusual sugar ring conformation in 5-iodo-5′-amino-2′,5′-dideoxyuridine, an antiviral nucleoside. J. Am. Chem. Soc. 101, 3353–3358.

5 Brockman, R.W. and Anderson, E.P. (1963) Pyrimidine analogues. In: Metabolic Inhibitors, Eds.: Hochster, R.M. and Quastel, J.H. (Academic Press, New York) pp. 239–285.

6 Buettner, W. and Werchau, H. (1973) Incorporation of 5-iodo-2′-deoxyuridine (IUdR) into SV40 DNA. Virology 52, 553–561.

7 Cheng, Y.C., Huang, E.-S, Lin, J.-C., Mar, E.C., Pagano, J.S., Dutschman, G. and Grill, S. (1983) Unique spectrum of activity of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine against herpesviruses in vitro and its mode of action against herpes simplex virus type 1. Proc. Natl. Acad. Sci., U.S.A. 80, 2767–2770.

8 Cheng, Y.C., Ostrander, M., Derse, D. and Chen, J.-Y. (1979) Development of antiherpes virus agents on the basis of virus induced enzymes. In: Nucleoside Analogs: Chemistry, Biology and Medical Application, Eds.: Walker, R.T., De Clercq, E. and Eckstein, F. (Plenum Press, New York) pp. 319–335.

9 Darby, G. and Field, J.H. (1983) Latency and acquired resistance-problems in chemotherapy of herpes infections. Pharmacol. Ther. 16, 217–251.

10 De Clercq, E. (1982) Specific targets for antiviral drugs. Biochem. J. 205, 1–13.

11 Easterbrook, K.E. and Davern, C.I. (1963) The effect of 5-bromodeoxyuridine on the multiplication of vaccinia virus. Virology 19, 509–520.

12 Elion, G.B. (1982) Mechanism of action, pharmacology and clinical efficacy of acyclovir. In: Herpesvirus, Clinical, Pharmacological and Basic Aspects, Eds.: Shiota, H., Cheng, Y.-C. and Prusoff, W.H. (Excerpta Medica, Amsterdam) pp. 229–235.

13 Fischer, P.H., Chen, M.S. and Prusoff, W.H. (1980) The incorporation of 5-iodo-5′-amino-2′,5′-dideoxyuridine and 5-iodo-2′-deoxyuridine into herpes simplex virus DNA: A relationship to their antiviral activity and effects on DNA structure. Biochim. Biophys. Acta 606, 236–245.

14 Fischer, P.H. and Prusoff, W.H. (1982) Pyrimidine nucleosides with selective antiviral activity. In: Chemotherapy of Viral Infections, Eds.: Came, P.E. and Caliguiri, L.A. (Springer-Verlag, Berlin, Heidelberg, New York) pp. 95–116.

15 Fox, J.J., Watanabe, K.A., Lopez, C., Philips, F.S. and Leyland-Jones, B. (1982) Chemotherapy on herpesvirus, particularly the effect of antiviral amino acid analogues. In: Herpesvirus, Clinical, Pharmacological and Basic Aspects, Eds.: Shiota, H., Cheng, Y.-C. and Prusoff, W.H. (Excerpta Medica, Amsterdam) pp. 135–147.

16 Fox, L., Doberson, M. and Greer, S. (1983) Incorporation of 5-substituted analog of deoxycytidine into DNA of herpes simplex virus-infected or -transformed cells without deamination to the thymidine analog. Antimicrob. Agents Chemother. 23, 465–476.

17 Fujiwara, U. and Heidelberger, C. (1970) Fluoroinated pyrimidines XXXVII. Effect of 5-trifluoromethyl-2′-deoxyuridine on the synthesis of deoxyribonucleic acid of mammalian cells in culture. Mol. Pharmacol. 6, 281–291.

18 Fyfe, J.A. (1982) Differential phosphorylation of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine monophosphate by thymidylate kinases from herpes simplex viruses types 1 and 2 and varicella zoster virus. Mol. Pharmacol. 21, 432–437.

19 Goz, B. and Prusoff, W.H. (1968) The ability of phage containing 5-iodo-2′-deoxyuridine-substituted DNA to induce enzymes. J. Biol. Chem. 243, 4750–4756.

20 Goz, B. and Prusoff, W.H. (1970) The relation of antiviral activity of IUdR to gene function in phage. Ann. N.Y. Acad. Sci. 173, 379–389.

21 Heidelberger, C. and King, D.H. (1979) Trifluorothymidine. Pharmacol. Ther. 6, 427–442.

22 Kan-Mitchell, J. and Prusoff, W.H. (1979) Studies on the effect of 5-iodo-2′-deoxyuridine on the formation of adenovirus type 2 virions and the synthesis of virus induced polypeptides. Biochem. Virol. 28, 1819–1829.

23 Kaplan, A.S., Ben-Porat, T. and Kamiya, T. (1965) Incorporation of 5-bromo-deoxyuridine and 5-iodo-deoxyuridine into viral DNA and its effect on the infective process. Ann. N.Y. Acad. Sci. 130, 226–239.

24 Mancini, W.R., De Clercq, E. and Prusoff, W.H. (1983) The relationship between incorporation of *E*-5-(2-bromovinyl)-2′-deoxyuridine into herpesvirus type 1 DNA with virus infectivity and DNA integrity. J. Biol. Chem. 258, 792–795.

25 McCrea, J.F. and Lipman, M.B. (1967) Strand-length measurements of normal and 5-iodo-2′-deoxyuridine treated vaccinia virus deoxyribonucleic acid released by the Kleinschmidt method. J. Virology 1, 1037–1044.

26 Otto, M.J., Lee, J.J. and Prusoff, W.H. (1982) Effects of nucleoside analogues on the expression of herpes simplex type 1 induced protein. Antiviral Res. 2, 267–282.

27 Park, N.H. and Pavan-Langston, D. (1982) Purines. In: Chemotherapy of Viral Infections, Eds.: Came, P.E. and Caliguiri, L.A. (Springer-Verlag, Berlin, Heidelberg, New York) pp. 117–136.

28 Pennington, T.H. (1976) Effect of 5-bromodeoxyuridine on vaccinia virus-induced polypeptide synthesis: Selective inhibition of the synthesis of some post-replicative polypeptides. J. Virol. 18, 1131–1133.

29 Prusoff, W.H., Bakhle, Y.S. and McCrea, J.F. (1963) Incorporation of 5-iodo-2′-deoxyuridine into the deoxyribonucleic acid of vaccinia virus. Nature 199, 1310–1311.

30 Prusoff, W.H., Bakhle, Y.S. and Sekely, L. (1965) Cellular and antiviral effects of halogenated deoxyribonucleosides. Ann. N.Y. Acad. Sci. 130, 135–150.

31 Prusoff, W.H. and Goz, B. (1975) Halogenated pyrimidine deoxyribonucleosides. In: Handbook Experimental Pharmacology Bd. 38 A, Eds.: Sartorelli, A. and Johns, D. (Springer-Verlag, Berlin, Heidelberg, New York) pp. 272–347.

32 Prusoff, W.H. and Otto, M.J. (1983) Problems in the pharmacology and pharmacokinetics of antivirals. In: Problems of Antiviral Therapy, Eds.: Stuart-Harris, G.H. and Oxford, J. (Academic Press, New York) pp. 125–148.

33 Prusoff, W.H., Chen, M.S., Fischer, P.H., Lin, T.-S., Mancini, W.R., Otto, J.J., Shiau, G.T., Schinazi, R.F. and Walker, J. (1984) Antiviral iodinated pyrimidine deoxyribonucleosides: 5-iodo-2′-deoxyuridine; 5-iodo-2′-deoxycytidine; 5-iodo-5′-amino-2′,5′-dideoxyuridine. In: Viral Chemotherapy, Vol. 1, Ed.: Shugar, D. (Pergamon Press, Oxford, New York) pp. 341–383.

34 Prusoff, W.H., Lin, T.-S., Mancini, W.R., Otto, M.H., Siegel, S. and Lee, J.J. (1984) Overview of the possible targets for viral chemotherapy. In: Targets for the Design of Antiviral Agents, Eds.: De Clercq, E. and Walker, R.T. (Plenum Press, New York and London) pp. 1–27.

35 Ruth, J. and Cheng, Y.C. (1982) Selective antiviral agents. J. Biol. Chem. 257, 10261–10266.

36 Smith, K.O. and Dukes, C.D. (1964) Effect of 5-iodo-2′-deoxyuridine (IDU) on herpes virus synthesis and survival in infected cells. J. Immunol. 92, 550–554.

37 Smith, R.A. and Kirkpatrick, W. (Eds.) (1980) Ribavirin, A Broad Spectrum Antiviral Agent (Academic Press, New York) pp. 1–237.

38 Szybalski, W. (1962) Properties and applications of halogenated deoxyribonucleic acids. In: The Molecular Basis of Neoplasia (University of Texas Press, Austin, Texas) pp. 147–171.

39 Wigand, R. and Klein, W. (1974) Properties of adenovirus substituted with iododeoxyuridine. Arch. Ges. Virusforsch. 45, 298–300.