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Mini-Review

Approaches to antiviral chemotherapy

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Summary

Most of the drugs used today in the treatment of viral infections in man are purine-pyrimidine antimetabolites that interfere with viral replication. Work at Southern Research Institute has identified a number of compounds of this type with promising antiviral activity in both cell culture and rodent test systems. By far the most active and selective agents are carbocyclic nucleoside analogs in which the oxygen of the furanose ring is replaced by a methylene group. The effects of this change on the metabolism and antiviral activity of these compounds is discussed below.

Antiviral development; Carbocyclic nucleoside analog; Drug metabolism; Antimetabolite; Uridine-pyrimidine antimetabolite

Specific antiviral chemotherapy is only one of a number of approaches in our conquest of viral diseases. Others include prevention of the spread of infection by control of vectors, enhancement of the efficacy of natural defense mechanisms by vaccinations, passive immunization, and the use of immunomodulating substances such as the interferons, and symptomatic treatment. Hence the assessment of the potential of any new antiviral drug should consider the availability, safety, effectiveness and cost of the alternative measures listed above. Even so, new or improved drugs are needed for the prevention of treatment of a number of infections caused by viruses that are not at present adequately controlled. These include respiratory tract infections, influenza, chronic hepatitis, gastroenteritis, infectious mononucleosis, measles, rabies, hemorrhagic fevers and warts. They might also be

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useful in the prevention or treatment of virus-associated tumors such as hepatomas, nasopharyngeal carcinomas, Burkitt's lymphoma, Kaposi's sarcoma, cervical carcinomas, and certain human leukemias.

The rational approach to antiviral chemotherapy, in which efforts are directed toward the design and development of compounds that selectively inhibit virus infection and reproduction without causing adverse effects to the host cells, must be based on exploitable biochemical differences that exist between virus-specific processes and cellular biosynthetic processes. This topic has been much talked and written about, but, in truth, none of the drugs that have received FDA approval for the treatment of virus infections in humans was rationally designed. Fig. 1 shows the steps in the virus infection and reproduction process, along with the loci of action of most of the known types of antiviral agents. The clinically used drugs that have received FDA approval are; amantadine, idouridine, trifluridine, vidarabine, ribavirin, acyclovir, and zidovudine. All of these drugs, save amantadine which appears to have an effect on a virus-specific process that takes place between uncoating and primary transcription, are purine-pyrimidine antimetabolites that interfere with viral replication (Shannon, 1984; De Clercq, 1984a). For that reason, the review that follows covers primarily agents of that type that have been studied at Southern Research Institute (Montgomery, 1982a).

Before reviewing our work on antiviral agents it might be useful to discuss briefly the major problems facing antiviral chemotherapy. They are: (1) Selectivity; (2) development of drug resistance; (3) latency; (4) drug delivery; and (5) drug metabolism.

Selectivity is always a problem with chemotherapy, but it is much more serious

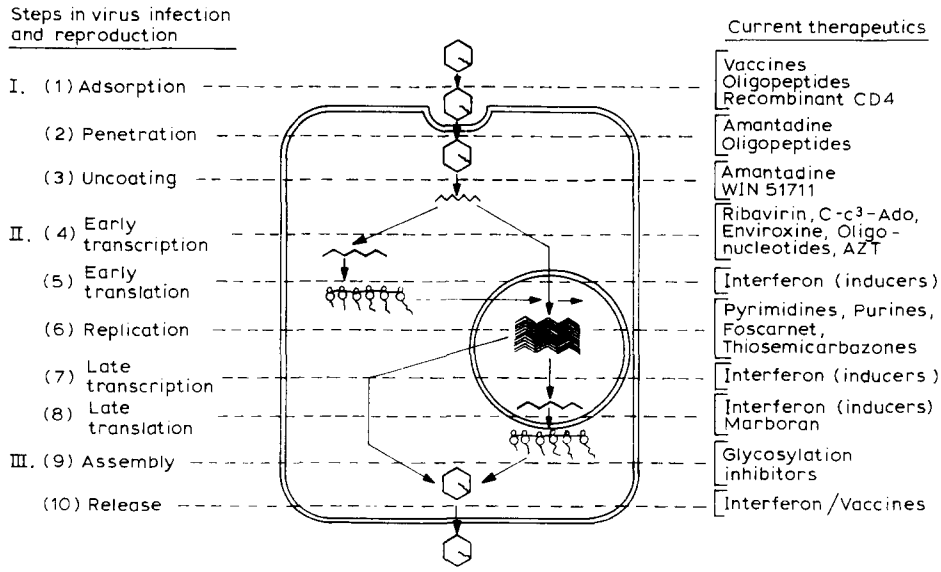


Fig. 1. Specific target areas affected by current antiviral therapeutics.

in some diseases than others. Lack of selectivity can arise from similarities between the target, in this case a viral process, and the host, the corresponding cellular process. The polymerization of DNA is a good example and, along with metabolism of the drug, underlies the difference in toxicity of acyclovir (ACV) and 3′-azido-3′-deoxythymidine (AZT). Thus ACV is phosphorylated to the monophosphate by a virus-specified deoxypyrimidine kinase. The monophosphate is converted to the active form, ACV-TP, by cellular enzymes. As a result, very little acyclovir triphosphate is found in uninfected cells. Further, the virus-induced DNA polymerase is sensitive to ACV-TP while the host cell DNA polymerase is not. In contrast, zidovudine (AZT) is phosphorylated to the mono-, di-, and triphosphate by cellular enzymes and, therefore, the active compound, AZT-TP, is found equally in infected and uninfected cells and its selectivity depends entirely on the greater sensitivity to it of the HIV reverse transcriptase relative to the cellular DNA polymerase α (Furman et al., 1986). As a result, AZT is more toxic than ACV and this toxicity (myelosuppression) limits its effectiveness in the treatment of HIV infections (De Clercq, 1987). On the other hand, the lack of selectivity may result from two totally unrelated actions of a drug, one of which produces the desired result and the other a so-called side effect. Almost all drugs show some kind of side effect in some patients. In some cases, such as the painful stocking-glove axonal sensorimotor peripheral neuropathy seen with 2′,3′-dideoxycytidine, these side effects can be severe enough to prevent continued treatment (Yarchoan et al., 1988).

Drug resistance usually results from the selection and overgrowth of a mutant population, in this case again, of the virus. Two mechanisms of resistance that have been observed are alteration in the target for the drug and loss of the enzymatic capacity to metabolically activate the drug. For examples, the herpes simplex virus can acquire resistance to the pyrophosphate analogs, phosphonacetic, and phosphonoformic acid, by changes in its DNA-polymerase gene which leads to the induction of an insensitive polymerase. Resistance to ACV can result from loss of the activating deoxypyrimidine kinase (Field, 1988). Alternative therapies and drug combinations have been successfully employed in cancer treatment to avoid resistance and this approach should be applicable to viral diseases also.

Latency is perhaps the most difficult problem of all and no solution to this problem is in sight, although much effort is being devoted to developing a better understanding of the details of this phenomenon that might lead to new therapeutic approaches.

A number of approaches to drug metabolism and delivery based on principles developed by the medicinal chemist (Montgomery, 1986a,b) are being successfully employed to develop better antiviral agents. One approach that has been successfully applied to this problem is the use of prodrugs. Thus, a number of *O*-acyl derivatives of vidarabine have been shown to be effective in the topical therapy of genital herpes infections in guinea pigs, whereas vidarabine itself, or its monophosphate, is inactive (Shannon, 1984).

In addition to the classical approaches to drug design, however, some new developments give rise to hope for future progress. Rapid strides are being made in

studies on the molecular biology of a number of viruses leading to new targets and new approaches; X-ray crystallographic structural studies look particularly promising (Smith et al., 1986; Wilson et al., 1984), and finally new approaches to drug targeting and transport such as liposomal and microencapsulation may prove useful (Gregoriadis, 1980; Koff and Fidler, 1985).

In the sections that follow the antiviral activity of a variety of purine-pyrimidine antimetabolites studied at Southern Research Institute (Montgomery, 1982a) will be discussed in some detail.

Benzyloxyadenosines and other purine nucleosides

Adenosine-1-oxide has a very high virus rating (>7) and therapeutic index (>300) against vaccinia in VERO cells using the CPE assay, along with some activity against yellow fever, sandfly fever, and Japanese encephalitis. The activity against vaccinia led to the preparation of 1-benzyloxyadenosine and related compounds (Shannon et al., 1974), which showed comparable in vitro activity. More recent work has defined the activity of this series more clearly. Of a number of salts studied, the perchlorates have proven to be most stable and amenable to purification. Fig. 2 summarizes the in vitro results with thirty active structures. Table 1 compares the activity of other structural variations. 2′-Deoxyadenosine-1-oxide (**1**) and its 4-tolyl derivative (**5**) are clearly less efficacious and less potent against vaccinia in cell culture than the corresponding ribonucleosides, although they do have significant activity. Extension of the link X (see Table 1) by one carbon to give the phenylethyl derivative (**3**) decreases both efficacy and potency markedly, but substitution on the benzylic methylene group by a methyl group to give **2** does not. More drastic structural changes such as substituting a methyl or benzyl group for the sugar moiety to give (**6**) or (**7**) resulted in inactive compounds. An examination by HPLC of the metabolism of 1-benzyloxyadenosine in L1210 leukemia cells in culture revealed the formation of the mono- and triphosphates of adenosine-1-

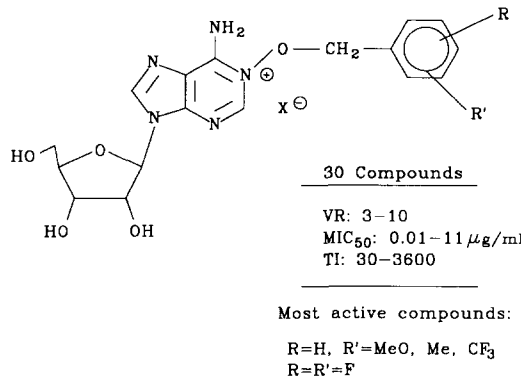
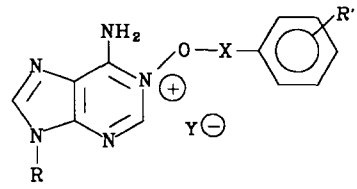


Fig. 2. Summary of in vitro activity of the 1-(substituted benzyloxy)adenosines against vaccinia (CPE).

TABLE 1

In vitro activity of other 9-substituted 1-arylalkyloxyadenines against vaccinia[a]



Structure

No.	R	X	R′	VR[b]	MIC_{50}[c]	MTC[d]	TI[e]
1	2′-deoxy-β-D-ribo	–[f]	–	>1.7	3.1	>320	>100
2	β-D-ribo	CH(Me)	H	8.0	0.1	100	1000
3	β-D-ribo	$(CH_2)_2$	H	2.3	55	325	6
4	β-D-ribo	CH_2	4-Me	8.6	0.9	320	3600
5	2′-deoxy-β-D-ribo	CH_2	4-Me	2.1	88	320	4
6	methyl	–[g]	–	0	–	–	–
7	benzyl	CH_2	2-F	0.6	~100	–	–
8	β-D-ribo[h]	CH_2	2-F	5.0	5.0	>320	>64

[a]In VERO cells.
[b]VR=virus rating (see ref. 6).
[c]Minimum concentration (μg/ml) required to inhibit viral cytopathogenicity by 50%.
[d]Maximum tolerated concentration (μg/ml).
[e]In vitro therapeutic index: MTC/MIC_{50}.
[f]2′-Deoxyadenosine-1-oxide.
[g]9-Methyladenine-1-oxide.
[h]Included for comparison.

oxide. Exposure of these cells to adenosine-1-oxide itself resulted in the formation of even larger quantities of these nucleotides (Bennett, Jr., unpublished data). The formation of these phosphates in Ehrlich ascites cells treated with adenosine-1-oxide has been reported (Frederiksen et al., 1968).

Stability studies on adenosine-1-oxide and 1-(3-methylbenzyloxy)adenosine then showed that the benzyloxy compound decomposes ($t_{1/2}$=38 h) at pH= 7.0 and ambient temperature to give adenosine-1-oxide, which is stable to these conditions. It would appear that the 1-benzyloxyadenosines are acting as pro-drug forms of adenosine-1-oxide, the triphosphate of which is the biologically active agent (Frederiksen et al., 1968; Bennett, Jr. et al., 1966).

Adenosine-1-oxide, 1-(3-methylbenzyloxy)adenosine, 1-(2-trifluoromethylbenzyloxy)-adenosine, and 1-(4-fluorobenzyloxy)adenosine all significantly reduced the mean and median tailpox counts in the vaccinia virus-induced tailpox model in CD-1 mice when given IP, qd 1-7 (Hollingshead and Shannon, unpublished data).

6-(Ethylthio)purine ribonucleoside has shown activity against vaccinia, human adenovirus type 2, and perhaps some against three of the so-called exotic RNA viruses: Japanese encephalitis, sandfly fever, and yellow fever. The closely related

(6-allylthio)purine ribonucleoside was active against adenovirus type 2 and Punta Toro virus but not against vaccinia or yellow fever. Although the 6-(alkylthio) purine ribonucleosides have been investigated rather extensively for their anticancer activity (Montgomery et al., 1961), SAR studies in the antiviral area have yet to be carried out. One reason for the limited interest in this class of compounds as antivirals is the toxicity problem that will have to be overcome. This toxicity has even limited their utility as anticancer agents (Bodey et al., 1968), the toxicity of which is generally accepted in the treatment of these life-threatening diseases.

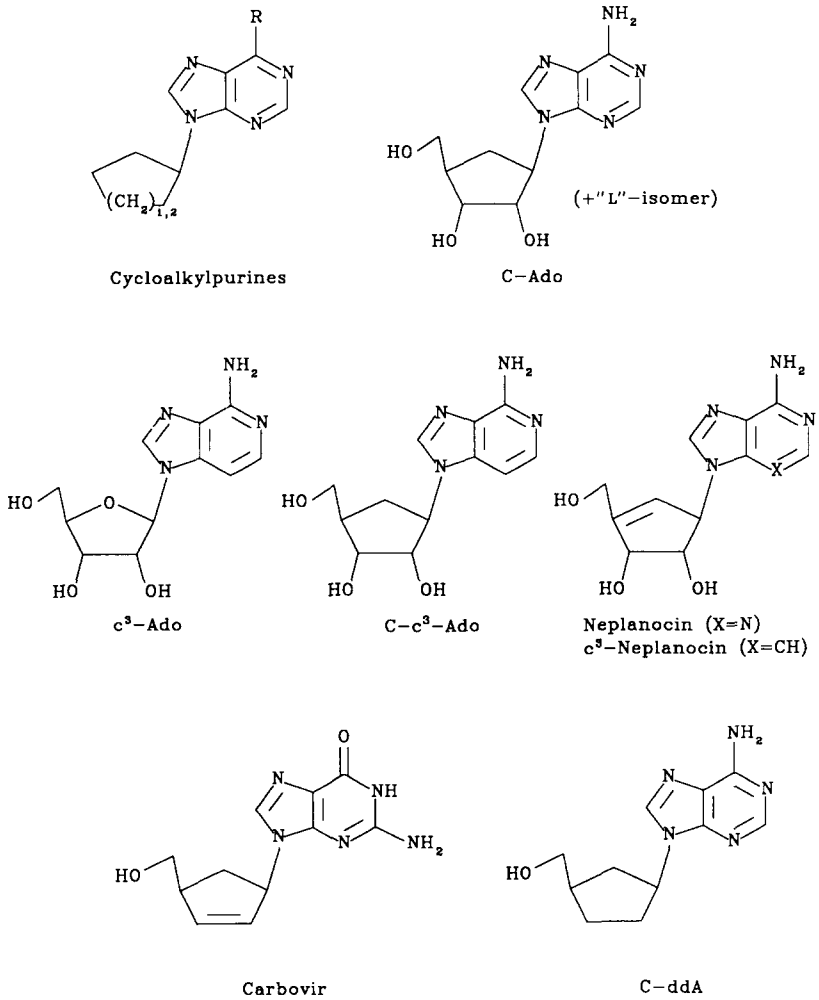
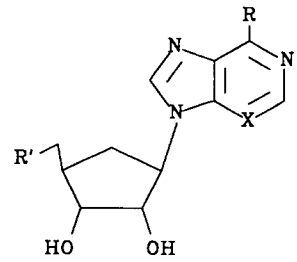


Fig. 3. Carbocyclic analogs and 3-deazaadenosine.

TABLE 2

In vitro activity of C-c[3]-adenosine and related compounds[a]



No.	R	R′	X	Vaccinia[b]		Vesicular stomatitis virus[b]	
				VR	MIC_{50}	VR	MIC_{50}
9	NH_2	OH	CH	3.6	1.7	2.6	2
10	NH_2	H	CH	3.2	2.7	2.9	8
11	NH_2	Cl	CH	0.9	190	1.4	3
12	NH_2	Br	CH	0.6	87	ND	
13	NH_2	NH_2	CH	0.6	38	0.3	–
14	NH_2	i-BuS	CH	ND		0	–
15	H	HO	CH	3.0	7	2.1	49
16	NH_2	HO	N	0.2	–	0	–
17	NH_2	H	N	3.0	2.3	0	–
18	NH_2	H_2NOC	N	3.4[c]	6.5	0.6	160

[a]See footnotes to Table 1.
[b]In L929 cells.
[c]Active vs HSV-1: VR=1.3, MIC_{50}=90.

Carbocyclic analogs of purine nucleosides

The phosphorolytic cleavage of nucleosides such as 6-thioinosine (MPR) by enzymes such as PNP can negate their potential utility as chemotherapeutic agents (Montgomery and Struck, 1973). The concept of enzymatically stable nucleoside analogs and the biologic activity of the 9-cycloalkyl purines (Kelley et al., 1962) led to the synthesis of carbocyclic (cyclopentane) analogs of nucleosides in which the furanose oxygen atom is replaced with a methylene group and the hydroxyl groups occupy the same positions, having the same cis-trans relationships and assuming similar conformations (Shealy and Clayton, 1966; Shealy and Clayton, 1969). Such analogs have the potential to mimic or antagonize the function of the naturally occurring nucleosides and, after phosphorylation, nucleotides; but unlike nucleosides, these analogs have a carbon-nitrogen bond joining the heterocyclic base to the cyclopentane ring comparable in stability to that of a simple alkyl derivative and, therefore, are not susceptible to enzymatic cleavage as is the glycosyl bond of true nucleosides.

The first compound of this type prepared, the racemic carbocyclic analog of adenosine (C-Ado) (Fig. 3), proved to be biologically active as expected (Bennett, Jr. et al., 1968; 1985; Hill et al., 1971). It is highly cytotoxic to both H.Ep.-2 and L1210 cells in culture, but was not effective against L1210 leukemia in vivo at the maximum tolerated dose. C-Ado is a substrate for AK (from H.Ep.-2 cells) and ADA (from calf intestine). Cell lines lacking AK have at best a low level of resistance to C-Ado, indicating that the nucleoside analog itself has potent growth-inhibitory properties (Bennett, Jr. et al., 1986a). The recent finding that this compound is both a potent reversible inhibitor and irreversible inactivator of adenosylhomocysteinase (AdoHcyase) (Chiang et al., 1981; Guranowski et al., 1981) may explain the activity of C-Ado itself, since inhibition of this enzyme is known to cause an accumulation of adenosylhomocysteine which interferes, by feedback, with vital transmethylation reactions involving adenosylmethionine.

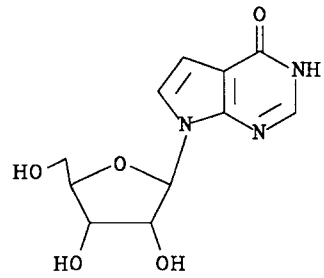
C-Ado has very little in vitro antiviral activity because cellular adenosine kinase rapidly converts it to the monophosphate which is further phosphorylated to the triphosphate, the cytotoxic metabolite with no selectivity for viral replication (or neoplastic cells for that matter) (Bennett, Jr. et al., 1975). 3-Deazaadenosine (c^3-Ado) also inhibits AdoHcyase, although it is much less potent (Guranowski et al., 1981), but more importantly, it is only phosphorylated in cells to a very minor extent (Zimmerman et al., 1983; Bennett, Jr. et al., 1988) and is not deaminated. Combining the two moieties (3-deazaadenine and carbocyclic ribose) to give carbocyclic 3-deazaadenosine (C-c^3-Ado) (Fig. 3) produced a potent AdoHcyase inhibitor that is phosphorylated even less than c^3-Ado and is not measurably deaminated (Montgomery et al., 1982b). C-c^3-Ado is a potent in vitro inhibitor, with a good therapeutic index, of vaccinia and a number of RNA viruses (De Clercq et al., 1984b). It is much more effective than either C-Ado or c^3-Ado. Since our pioneering work on this kind of antiviral agent, other investigators have found that the related carbocycles, neplanocin (Borchardt et al., 1984) and 3-deazaneplanocin (Glazer et al., 1986), are more potent inhibitors of AdoHcyase than C-c^3-Ado, although they have not been shown to have superior antiviral selectivity (i.e., greater therapeutic index). At the same time, antiviral potency has been correlated with inhibition of the enzyme (De Clercq and Cools, 1985). Modifications of these structures have produced less potent compounds (Hasobe et al., 1987).

On the other hand, the same modification (Secrist et al., 1984) of tubercidin (7-deazaadenosine, c^7-Ado) resulted in a less effective antiviral agent (C-c^7-Ado), probably because it is phosphorylated to C-c^7-ATP, which is cytotoxic. Tubercidin itself, although it has a better therapeutic index than C-c^7-Ado, is still much too toxic for use as an antiviral agent in humans.

Since C-c^3-Ado (**9**) itself, and not its nucleotides, appeared to be responsible for its antiviral activity, we prepared the 5′-deoxy analog (**10**) and found its activity against vaccinia and VSV comparable to that of C-c^3-Ado (Table 2). Other variations at 5′ (**11**, **12**, **13** and **14**) reduced both potency and efficacy except in the case of **18**, whose activity against vaccinia was also comparable to that of C-c^3-Ado (Secrist et al., 1989; Montgomery and Secrist, 1986c). 5′-Deoxy-C-Ado (**17**) which, in contrast to C-Ado, cannot be phosphorylated had good activity against vaccinia

TABLE 3

In vitro activity of 7-deazainosine[a]



Virus	Cell	VR	MIC_{50}	TI
HSV-1[b]	VERO	0	–	–
Vaccinia	L929	0.2	–	–
Influenza A_0	MDCK	1.4	2.5	1
Influenza A_2	MDCK	0.7	5.1	
Rhino 1A	MRC5	3.8	1.4	19
Coxsackie A21	MRC5	3.1	2.0	13
Polio 3	VERO	1.1	2.2	4
Parainfluenza 3	H.Ep.-2	0.2	–	–
Respiratory syncytial virus	H.Ep.-2	0	–	–
Vesicular stomatitis virus	L929	0.5	2.2	

[a]See footnotes to Table 1.

[b]Herpes simplex virus type 1; substitution of Br, NO_2, NH_2, CN, CH_2NH_2 at positions 7 and 8 (purine numbering) gave less active or inactive compounds, except 7-Br, which has a VR of 3.4 (100 μg/ml) vs vaccinia.

but was inactive against VSV (Table 3). Its ability to inhibit AdoHcyase has not been determined.

Deamination of c^3-Ado to c^3-Ino, of C-Ado to C-Ino, and C-c^3-Ado to C-c^3-Ino resulted in compounds less efficacious and considerably less potent than the adenines from which they derive (Montgomery and Secrist, 1986c). In contrast, 7-deazainosine is more effective than tubercidin against Rhino 1A and Coxsackie A21 with some activity against influenza A_0 and Polio 3 (Table 3). The enhanced therapeutic index results from the lower toxicity of the c^7-Ino, even though the active antiviral agent is probably c^7-ATP, a metabolite of the cellular enzymes (Mihich et al., 1969; LaFon et al., 1985). The 7-bromo derivative of c^7-Ino has good efficacy but low potency against vaccinia. Other substitutions were detrimental to activity (Montgomery and Secrist, 1986c).

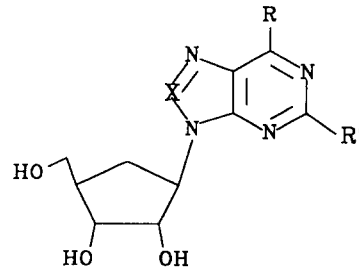
Although C-Ado (**22**) was ineffective as an antiviral agent in vitro, other carbocyclic 6-substituted purine ribonucleosides (**23–28**) have shown some activity against vaccinia (Table 4) and the 6-chloro compound (**28**) was also active against influenza (Bennett, Jr. et al., 1975), but the virus ratings and potency did not suggest in vivo evaluation of these compounds. The principal metabolite of **28** in

mammalian cells is C-GTP (Bennett, Jr. et al., 1986b) but the intermediate metabolite, C-GMP, is a potent inhibitor of hypoxanthine (guanine) phosphoribosyl transferase (Bennett, Jr. et al., 1985). Additionally, there is evidence that **28**, probably as the monophosphate, inhibits thymidylate synthetase (TS) in whole cells (Bennett, Jr., unpublished data). None of these activities have been related to its antiviral activity, but it is likely that its inhibition of TS is responsible for its cytotoxicity.

In contrast to results with the 6-substituted carbocycles, the ribo-carbocycle of 2,6-diaminopurine (**29**) showed good efficacy and potency against vaccinia but was inactive vs HSV-1 (Table 4) (Shealy et al., 1984a). Since carbocyclic guanosine was inactive against vaccinia the rate of deamination of **29** in vitro must be slow. The ribo-carbocycles of the other 2-amino-6-substituted purines (**31**, **32**, **34**, **36** and **37**)

TABLE 4

In vitro activity of carbocyclic analogs of purine ribonucleosides[a]



No.[b]	R	R'	X	Vaccinia strain Lederle CA	
				VR	MIC_{50}
22	NH_2	H	CH	0.2	–
23	NHMe	H	CH	1.5	3.2
24	NHOH	H	CH	1.8	3.3
25	OH(C=O)	H	CH	1.6	100
26	OMe	H	CH	1.8	21.0
27	SMe	H	CH	2.9	97.0
28	Cl	H	CH	0.8	13.0
29	NH_2	NH_2	CH	4.6/3.3[c]	0.3/0.9
30	NH_2	NH_2	N	1.4	100
31	NHMe	NH_2	CH	1.3	320
32	Cl	NH_2	CH	1.7/1,7	10.0/8.1
33	Cl	NH_2	N	0.9	81
34	SMe	NH_2	CH	0.1	–
35	SMe	NH_2	N	0	–
36	OMe	NH_2	CH	1.4	260
37	OEt	NH_2	CH	0.3	–

[a]See footnotes to Table 1.
[b]Compounds 22-28 tested in L929 cells; compounds 29-37 in HE.p.-2 cells.
[c]C-Guo, C-8-AzaGuo, and C-6-ThioGuo inactive.

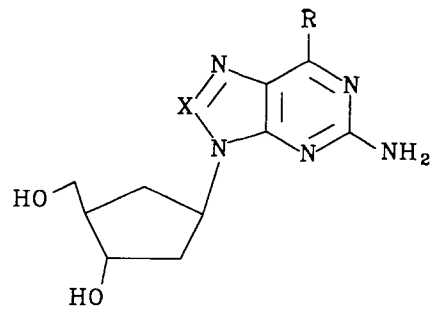
or the 2-amino-6-substituted-8-azapurines (**30**, **33** and **35**) were less efficacious and considerably less potent against vaccinia. The compounds in this series that were tested against HSV-1 and influenza (A_o/PR-8/34) were inactive.

Some of the 3′-deoxyribo carbocycles of the 2-amino-6-substituted purines showed modest antiviral activity. The 6-chloro compound was active against vaccinia, whereas carbocyclic 3′-deoxyguanosine was moderately active against HSV-1 and HSV-2. The 6-amino-8-aza derivative showed activity against influenza, but the MIC_{50} was high. This series, like the ribo series, does not appear to warrant in vivo evaluation.

By far the most promising carbocyclic purine nucleosides for the treatment of herpes infections are in the 2′-deoxyribo series (Table 5) (Shealy et al., 1984b). Of these the most likely compounds appear to be the 2′-deoxyguanosine analog (CDG, **32**) and its prodrug forms (**41** and **44**). The 8-azapurines (**39**, **43**, and **45**) are also quite efficacious but **43** and **44** are significantly less potent as is the thiopurine **40**. Based on this data CDG (**38**) and, to a lesser extent, **41** and **44** have been studied further both in vitro and in vivo. CDG is phosphorylated significantly to the triphosphate in H.Ep.-2 or Vero cells infected with HSV-1 (S-148, TK^+),

TABLE 5

In vitro activity of carbocyclic analogs of 2-amino-6-substituted purine 2′-deoxyribonucleosides[a]



No.	R	X	HSV-1[b]		HSV-2[c]	
			VR	MIC_{50}	VR	MIC_{50}
38	OH (C=O)	CH	>4.6	<0.3	3.7	0.8
39	OH (C=O)	N	4.2	0.6	2.1	7.0
40	SH (C=S)	CH	4.7	2.0	1.7	32
41	OMe	CH	6.0	1.2	3.5	16
42	Cl	CH	1.9	8	2.0	37
43	Cl	N	4.7	1.3	1.9	23
44	NH_2	CH	4.6	0.45	2.9	2.4
45	NH_2	N	4.2	2.6	2.3	10

[a]See footnotes to Table 1; inactive vs influenza (A_o/PR-8/34); adenine and 8-azaadenine compounds inactive vs HSV.
[b]E-377 (TK^+) in primary rabbit kidney cells.
[c]MS strain in VERO cells.

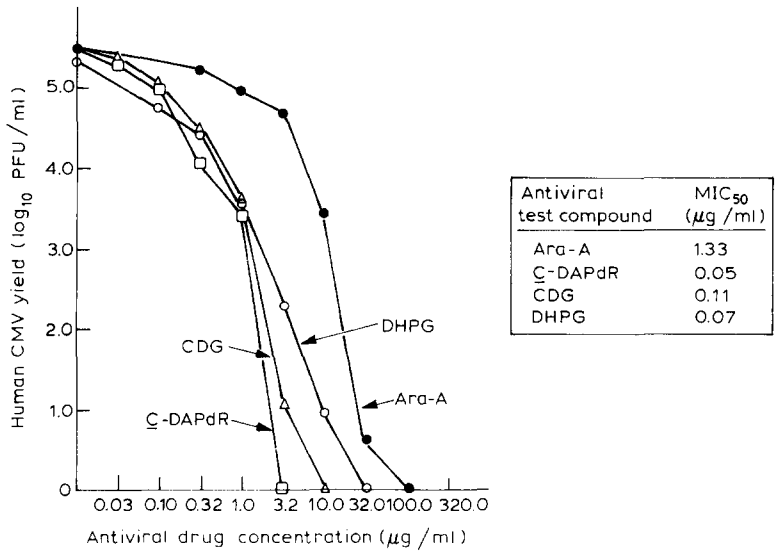


Fig. 4. Activity of 2′-CDG and C-DAPdR vs HCMV in MRC5 cells (virus yield-reduction assay).

TABLE 6

Activity of 2′-CDG and two prodrug forms in random-bred Swiss mice inoculated intracerebrally with 10 LD_{50} of herpes simplex virus type 1

Drug	Drug dose (mg/kg/day)[a]	Uninfected toxicity controls (survivors/total)	Virus-infected animals	
			Survival (21-day survivors/total)	Survival time (days)[b]
None (controls)	–	15/15	0/20	6.4
2′-CDG	20.0	4/4	2/10	9.6**
	10.0	5/5	2/10	8.3
	5.0	5/5	3/10	8.1*
	2.5	5/5	5/10	8.0
	1.25	5/5	1/10	8.0*
C-DAPdR	20.0	2/5 (toxic)	1/10	9.6**
	10.0	5/5	5/10	9.0*
	5.0	5/5	2/10	7.9*
	2.5	5/5	5/10	8.0
	1.25	5/5	4/10	7.3
O^6-Methyl-2′-CDG	20.0	5/5	0/10	8.5**
	10.0	5/5	0/10	8.0*
	5.0	5/5	5/10	7.6*
Ara-A (positive control)	250.0	4/4	6/10	11.3**

[a]Drugs were administered to mice i.p. on a q.d. 1–7 day schedule, beginning 4 h after inoculation with virus.

[b]Only animals dying on or before Day 21 after virus inoculation were considered in calculating the mean survival time (*P <0.05; **P <0.0005).

but with unlabeled CDG no triphosphate was detected in uninfected cells (Bennett, Jr., unpublished data). Presumably its activation is similar to that of acyclovir and the pyrimidines with significant selectivity for cells infected with TK^+ virus (Shannon, 1984; De Clercq, 1984a). It is also active against varicella zoster virus, being somewhat more cytotoxic and about twice as potent as acyclovir, and against human cytomegalovirus (HCMV) in MRC5 cells (Fig. 4). CDG, **41**, and **44** are curative when given to mice inoculated intracerebrally with HSV-1 (Table 6).

CDG is prepared by a long synthetic sequence that gives a racemic mixture of the 'D' and 'L' forms (Shealy et al., 1984b), the 'D' isomer corresponding to the natural nucleoside D-2′-deoxyguanosine and presumably the biologically active form of the compound. Stereospecific syntheses of the amino alcohol precursor to CDG have been developed but are even more lengthy than that used for the racemate, and conventional methods of resolution when applied to carbocyclic nucleosides have failed. These problems led us to develop a simple, enzymatic resolution of the mixture using adenosine deaminase (Secrist et al., 1987). Evaluation of the isomers showed the 'D' isomer to be much more efficacious and potent than the 'L' isomer against both HSV-1 and HSV-2 as expected (Table 7) (Secrist et al., 1987). It is five to six times as potent as acyclovir against HSV-1 and HSV-2 in plaque reduction assays in human foreskin fibroblasts. It is also at least three times as potent as the 'DL' mixture against HCMV and about three times as potent as DHPG (Shannon, unpublished data). The 'L'-CDG was not detectably phosphorylated in vero cells infected with HSV-1 (TK^+) (Bennett, Jr., unpublished data). Although CDG showed no activity against HIV in cell culture, the carbocyclic analog (Fig. 3) of 2′,3′-dideoxy-2′,3′-didehydroguanosine (itself inactive) is quite active and therapeutically synergistic with AZT in cell culture (Shannon, unpublished data). At the same time the carbocyclic analog (Fig. 3) of 2′,3′-dideoxyadenosine is inactive.

In the arabino-carbocyclic series the only compound with significant activity is cyclaradine (Vince et al., 1983; Shannon et al., 1983), the adenosine-deaminase-resistant carbocyclic analog of vidarabine, and even cyclaradine is significantly less active than acyclovir, DHPG (Schwartz et al., 1987), and 'D'-CDG.

TABLE 7

Activity of enantiomers of 2′-CdG against herpes simplex virus type 1 (E-377) and 2 (MS) in VERO cells

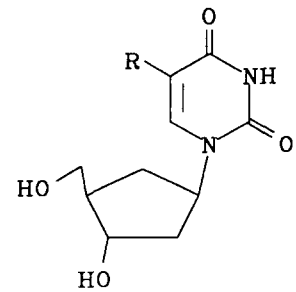
Compound	HSV-1		HSV-2	
	VR	MIC_{50} (μg/ml)	VR	MIC_{50} (μg/ml)
D-2′-CdG	4.8–6.3	0.1–0.3	3.8	0.7
DL-2′-CdG	5.3–7.0	0.2–0.3	3.7	2.6
L-2′-CdG	0.8–2.4	39–257	0	–
Ara-A	1.6–2.5	10–34	1.3	50
Acyclovir	5.7–6.5	1.5–2.9	4.5	5.3

Carbocyclic analogs of pyrimidine nucleosides

Carbocyclic uridine or 3′-deoxyuridine analogs with hydrogen, halogen, or other substituents at the 5-position of the pyrimidine ring (Shealy and O'Dell, 1976) were all inactive against HSV-1, HSV-2, and influenza A_0/PR-8/34, whereas carbocyclic 2′-deoxyuridine when substituted at the 5-position by the bromo (**49**), iodo (**48**), methyl (**50**), ethyl (**52**), or methylamino (**51**) group is quite active against HSV-1 and HSV-2 in cell culture (Table 8) (Shealy et al., 1983). The most efficacious and potent compounds are the thymidine (**50**) and IdUrd (**48**) analogs. In a plaque reduction assay **48** was over twenty times as potent as IUdR and at least as effective against varicella zoster. These analogs are probably activated by the virus-induced deoxynucleoside kinase in infected cells just as the true nucleosides are known to be (Shannon, 1984; De Clercq, 1984a). However, in contrast to IdUrd and BrdUrd, and carbocycles **48** and **49** are not significantly incorporated into DNA as indicated by a lack of shift in the buoyant density in cesium chloride density gradients of DNA from cells exposed to the analogs (Shannon, unpublished data). Further studies with radiolabelled **48** are planned to determine if any detectable incorpo-

TABLE 8

In vitro activity of carbocyclic analogs of 2′-deoxyuridine[a]



No.	R	HSV-1 (E-377)[b]		HSV-2 (MS)[b]	
		VR	MIC_{50}	VR	MIC_{50}
46	H	0	–		
47	F	0	–	0	–
48	I	7.2[c]	0.3[c]	3.2[d]	26[d]
49	Br	6.2	0.3	1.5	32
50	Me	5.4	0.8	3.2	7
51	NHMe	4.2[e]	17[e]	1.2	230
52	Et	2.3	57	1.1	200

[a]See footnotes to Table 1.
[b]In VERO cells.
[c]Average of 4 experiments.
[d]Average of 2 experiments.
[e]Average of 3 experiments.

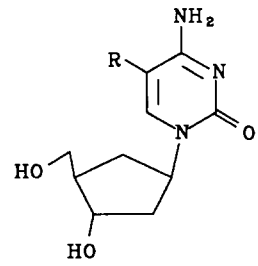
ration occurs. C-IUdR (**48**) and C-thymidine (**50**) produced cures of mice inoculated intracerebrally with 10 LD_{50}'s of HSV-1 (Table 10), but IUdR itself was not effective.

Carbocyclic cytidine (carbodine) (Shealy and O'Dell, 1980) is active against HSV-1, HSV-2, and influenza A_0/PR-8/34 in cell culture, but substitution on, or for, the amino group eliminated the activity. Likewise, substitution at the 5-position eliminated or markedly reduced efficacy and potency (Shealy et al., 1986). Carbodine is also active against a number of (+) and (±) RNA viruses and in Sindbis-infected cells inhibits viral RNA-directed RNA synthesis at a concentration corresponding well with its MIC_{50} (Bernaerts et al., 1984). Although reproducibly active in vitro, carbodine did not exhibit any efficacy against lethal influenza virus infections in mice when administered by either the intraperitoneal or intranasal routes up to dose-limiting toxic levels (Shannon et al., 1981).

Carbocyclic 2′-deoxycytidine (**65**) is about as effective as carbodine against HSV-1 but less effective against HSV-2 and less potent against both. Introduction of chlorine at position 5 (**68**) decreases activity markedly, introduction of bromine does not affect activity greatly (**67**), but introduction of iodine (**66**) greatly enhances activity (Table 9) (Shealy and O'Dell, 1976). Since the activity of C-IdUrd (**48**) is quite similar to that of **66**, deamination of **66** to **48** may explain its activity. On the other hand, **65** must not be deaminated significantly since **46** is inactive. There are data to support the idea that **66** may be deaminated considerably more rapidly than **73** (Kreis et al., 1978), and this may be true of **67** as well, although **67** is less active than **49**. C-ara-C and its 5-iodo derivative are both less active than the 2′-deoxy-

TABLE 9

In vitro activity of carbocyclic analogs of 2′-deoxycytidine[a]



No.	R	HSV-1 (E-377)[b]		HSV-2 (MS)[b]	
		VR	MIC_{50}	VR	MIC_{50}
65	H	2.6	<26.0	1.9	44
66	I	>6.9	< 0.4	2.1	31
67	Br	1.9	30.0	2.6	62
68	Cl	0.3	–	0.7	270

[a]See footnotes to Table 1.
[b]In VERO cells.

TABLE 10

Activity of carbocyclic pyrimidine analogs in random-bred Swiss mice inoculated intracerebrally (ic) with 10 LD_{50} of herpes simplex virus type 1

Drug	Drug dose (mg/kg/day)[a]	Uninfected toxicity controls (survivors/total)	Virus-infected animals	
			Survival (14-day survivors/total)	Survival time (days)[c]
None (controls)	–	15/15	1/20	6.3
C-IdUrd	600	5/5	2/10	10.4***
	400	5/5	3/10	8.9***
	200	5/5	1/10	9.0**
C-IdCyd	32	5/5	0/10	8.1
	10	5/5	1/10	7.3
C-Thymidine	400	4/5	0/10	8.5**
	200	5/5	2/10	8.6*
Ara-A	250	5/5	4/10	8.3**

[a]Drugs were administered i.p. on a q.d. 1–7 day schedule, beginning 4 h after inoculation with virus.
[b]Only animals dying on or before day 14 after virus inoculation were considered in calculating the mean survival time (*P <0.05; **P <0.005; ***P <0.0005).

ribo analogs. C-IdUrd (**48**), C-IdCyd (**66**), and C-thymidine (**50**) are able to effect some cures of mice inoculated intracerebrally with 10 LD_{50}'s of HSV-1 (Table 10).

A variety of carbocyclic pyrimidine nucleoside analogs, including C-AZT, showed no activity against HIV in cell culture (Mitsuya, 1986).

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