Antiviral Research, 4 (1984) 119–133
Elsevier

AVR 00130

Broad-spectrum antiviral activity of adenosine analogues*

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(Received 23 November 1983; accepted 16 January 1984)

Summary

In recent years certain aliphatic and carbocyclic adenosine analogues have been developed which are of potential clinical importance as antiviral agents. This includes (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA] and carbocyclic 3-deazaadenosine (C-c^{3}Ado). (*S*)-DHPA and C-c^{3}Ado are remarkably similar in their antiviral spectrum in that they are particularly active against (-) RNA viruses such as measles, parainfluenza, respiratory syncytial virus, rabies virus, vesicular stomatitis virus and (±)RNA viruses such as reo- and rotavirus, whereas (+)RNA viruses such as polio, coxsackie and (±)DNA viruses such as herpes simplex are only minimally affected or not inhibited at all. In contrast with (*S*)-DHPA and C-c^{3}Ado which are quite selective in their antiviral action, other adenosine analogues, i.e., 3-deazaadenosine and 7-deazaadenosine (tubercidin), exhibit little, if any, selectivity as antiviral agents. Also, tubercidin has a broader activity spectrum, encompassing (+)RNA viruses as well as herpes simplex in addition to the (-)RNA viruses. Considering the high antiviral potency of tubercidin, attempts have been undertaken to increase its selectivity, i.e., by chemical substitutions at C-5 of the pyrrolo(2,3-*d*)pyrimidine ring. These attempts have been partially successful.

(*S*)-9-(2,3-dihydroxypropyl)adenine; carbocyclic 3-deazaadenosine; 3-deazaadenosine; 7-deazaadenosine (tubercidin); broad-spectrum activity

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

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Introduction

Adenosylhomocysteine (SAH) hydrolase can be considered an important target in the design of antiviral agents with broad-spectrum activity [1]. SAH hydrolase is responsible for the hydrolysis of SAH to homocysteine and adenosine. SAH itself is both the product and a feedback inhibitor of *S*-adenosylmethionine (SAM)-dependent methyltransferase reactions. To the extent that these methylations are involved in the maturation of viral mRNA, i.e., 5' cap formation, they are essential for the synthesis of virus progeny particles. Inhibitors of SAH hydrolase may be expected to lead to an accumulation of SAH, block methyltransferase reactions and suppress the virus replication cycle. Typical examples of SAH hydrolase inhibitors are 5'-deoxy-5'-*S*-isobutyladenosine (SIBA), 3-deazaadenosine (c^3Ado) and 5'-deoxy-5'-*S*-isobutyl-3-deazaadenosine (c^3SIBA). SIBA has been shown to inhibit the replication of Rous sarcoma virus, polyoma virus and herpes simplex virus [2–5] and c^3Ado and c^3SIBA were found to inhibit the replication of Rous sarcoma virus, Gross murine leukemia virus, HL-23 retrovirus, SV40 virus, herpes simplex virus, Sindbis virus, Newcastle disease virus and vesicular stomatitis virus [6–9]. However, neither SIBA nor c^3Ado (or c^3SIBA) proved sufficiently specific in their antiviral action to further evaluate their therapeutic potentials in animal models.

Another example of an adenosine analogue that may act at least partially by inhibition of SAH hydrolase is 9-β-D-arabinofuranosyladenine (Ara-A) (Fig. 1). The antiviral properties of this compound are well known [10]. Ara-A has been pursued primarily as an antiherpes agent and proven efficacious in the topical treatment (as a 3% ointment) of herpetic keratitis and the systemic treatment (as an intravenous infusion at 15 mg/kg/day) of a number of life-threatening herpesvirus infections such as herpetic encephalitis [11,12], neonatal herpes [13] and herpes zoster in immuno-

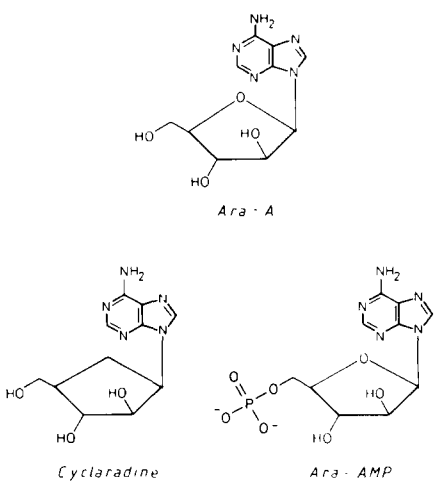


Fig. 1. Structural formulae of 9-β-D-arabinofuranosyladenine (adenine arabinoside, Ara-A, Vira-A^R, Vidarabine^R) and some *ara-A analogues*: carbocyclic ara-A (Cyclaradine) and ara-A 5'-monophosphate (Ara-AMP).

compromised patients [14,15]. Yet, the antiviral activity spectrum of ara-A is not restricted to herpes simplex and varicella-zoster. It also encompasses other DNA viruses, i.e., vaccinia virus, as well as RNA viruses, i.e., retro- and rhabdoviruses. In its mode of action ara-A appears to be directed at a number of targets. Ara-A interferes with SAH hydrolase, but, upon conversion to its 5'-triphosphate, ara-A also interacts with ribonucleotide reductase and DNA polymerase and may even be incorporated into DNA (Fig. 2). Ara-A also inhibits the polyadenylation of mRNA, and from this variety of interactions it is difficult to sort out which is actually responsible for the antiviral effects of the compound.

There are two major liabilities in the use of ara-A: (i) a rapid deamination to ara-Hx (9-β-D-arabinofuranosylhypoxanthine) by the ubiquitous adenosine deaminase and (ii) poor solubility in aqueous medium which necessitates the administration of large volumes of water, i.e., for intravenous infusion. In attempts to circumvent these problems, several new ara-A analogues have been synthesized (Fig. 1) i.e., cyclaradine, the carbocyclic analogue of ara-A which is completely resistant to deamination by adenosine deaminase [16], and ara-AMP, the 5'-monophosphate of ara-A which is more soluble in aqueous medium and therefore more amenable for systemic treatment. Cyclaradine exhibits significant therapeutic efficacy in the treatment of herpes simplex virus infections in animals, and ara-AMP has already been the subject of clinical studies, i.e., in the systemic therapy of herpetic encephalitis.

In recent years several new adenosine analogues have been developed which are endowed with interesting antiviral properties. While offering therapeutic promise by themselves, some of these compounds also form the basic structures for the design of more potent or more selective antiviral agents. These newly developed adenosine analogues will now be reviewed. They fall within either of the following classes: (i) acyclic adenosine analogues, (ii) deazaadenosine analogues, (iii) tubercidin analogues, and (iv) carbocyclic adenosine analogues.

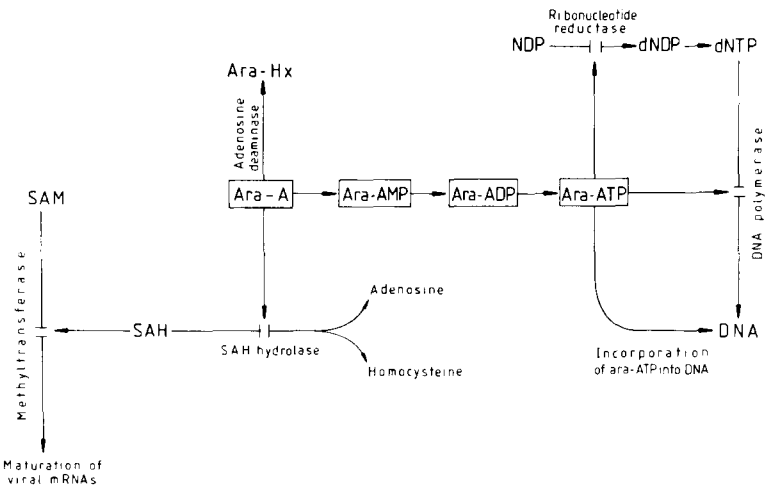


Fig. 2. Mechanism of antiviral action of Ara-A may be based upon an inhibitory effect on several enzymes, i.e., SAH hydrolase, ribonucleotide reductase and DNA polymerase. Abbreviations: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; Ara-Hx, hypoxanthine arabinoside; NDP, (ribo) nucleotide diphosphate; dNDP, deoxy(ribo)nucleotide diphosphate; dNTP, deoxy(ribo)nucleotide triphosphate.

Acyclic adenosine analogues

The prototype of the acyclic adenosine analogues is (*S*)-9-(2,3-dihydroxypropyl)-adenine [(*S*)-DHPA] (Fig. 3). This compound inhibits the replication of a wide variety of viruses, i.e., vaccinia virus [17–19], measles virus [17,20], parainfluenza virus type 3 [20], respiratory syncytial virus (E. De Clercq and R. Bernaerts, unpublished data, 1983), Rous sarcoma virus [21], rabies virus [22,23], vesicular stomatitis virus [17,18,20,24], reovirus type 1 [20], rotavirus [25] and infectious pancreatic necrosis virus (J. Bernstein, personal communication). The antiviral activity of (*S*)-DHPA extends to animal models, as has been demonstrated with vesicular stomatitis virus [17], rabies virus [22] and rotavirus [25] infections in mice.

Structurally related to (*S*)-DHPA is (*D*)-eritadenine [(*2S,3S*-4-(adenin-9-yl)-2,3-dihydroxybutanoic acid] (Fig. 3). (*D*)-Eritadenine was originally isolated from the edible Japanese mushroom shiitake (*Lentinus edodes*) and accredited with hypocholesterolemic effects [26–28]. As shown in Table 1, (*D*)-eritadenine is also active as an antiviral agent, and its activity spectrum is remarkably similar to that of (*S*)-DHPA. Both compounds inhibit the replication of vaccinia virus, vesicular stomatitis virus, measles virus, parainfluenza virus type 3 and reovirus type 1 within the concentration range of 10–100 μg/ml. Other viruses such as herpes simplex, polio type 1, Coxsackie B4 and Sindbis are not inhibited by either (*S*)-DHPA or (*D*)-eritadenine. Although not very potent in their antiviral activity, (*S*)-DHPA and (*D*)-eritadenine can be regarded as rather selective since they are not toxic for the host cells at a concentration of 400 μg/ml (Table 1).

The mechanism of antiviral action of (*S*)-DHPA has been elucidated to some extent. Its inhibitory effect on cell transformation by Rous sarcoma virus is attributed to an interaction with the virus-specific protein kinase pp60^{src} [21]. However, the major reason for the broad-spectrum antiviral activity of (*S*)-DHPA would reside in an inhibition of SAH hydrolase, hence accumulation of SAH and shut-off of viral mRNA maturation (5'cap formation) (Fig. 4) [29]. In addition, (*S*)-DHPA may inhibit the deamination of adenosine to inosine [30], and, as outlined in Fig. 4, such inhibition could also lead to an accumulation of SAH, be it indirectly *via* an increase of the adenosine levels. To the extent that the methyltransferases required for virus replication differ, either qualitatively or quantitatively, from the normal cellular

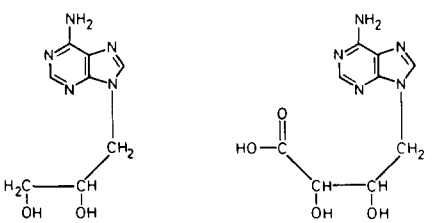


Fig. 3. Structural formulae of *acyclic adenosine analogues*: [(*S*)-DHPA] and (*2S,3S*)-4-(adenin-9-yl)-2,3-dihydroxybutanoic acid [(*D*)-Eritadenine].

TABLE 1

Inhibitory effects of (*S*)-DHPA and (*D*)-eritadenine on virus-induced cytopathogenicity in cell cultures

Virus	Cell culture	Minimum inhibitory concentration[a] (μg/ml)	
		(*S*)-DHPA	(*D*)-Eritadenine
Vaccinia	PRK	70 (> 400)	70 (> 400)
Herpes simplex 1	PRK	>400 (> 400)	>400 (> 400)
Herpes simplex 2	PRK	>400 (> 400)	>400 (> 400)
Vesicular stomatitis	PRK	10 (> 400)	30 (> 400)
Polio 1	HeLa	>400 (> 400)	>400 (> 400)
Coxsackie B4	HeLa	>400 (> 400)	>400 (> 400)
Sindbis	Vero	>400 (> 400)	>400 (> 400)
Measles	Vero	40 (> 400)	40 (> 400)
Parainfluenza 3	Vero	20 (> 400)	150 (> 400)
Reo 1	Vero	50 (> 400)	100 (> 400)

[a] Required to inhibit viral cytopathogenicity by 50%. The data indicated in parentheses refer to the minimum inhibitory concentration (μl/ml) of the compounds causing a microscopically detectable alteration of normal cell morphology (in uninfected cell cultures). PRK, primary rabbit kidney; Vero, African green monkey kidney.

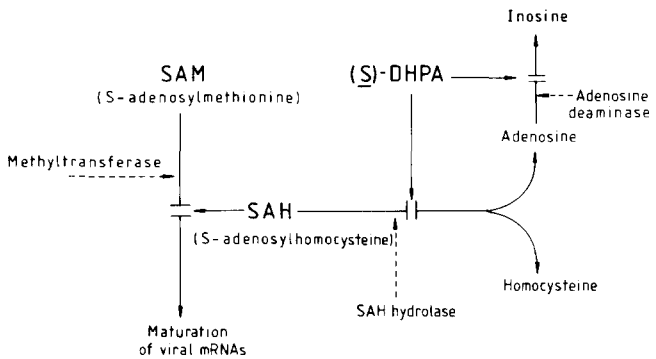


Fig. 4. Mechanism of antiviral action of (*S*)-DHPA would be based primarily on the inhibition of SAH hydrolase, and, possibly, also on an inhibition of adenosine deaminase.

methyltransferases, one may expect some specificity in the antiviral action of those agents that interfere with the methylation reactions. Whether the virus-associated methyltransferases are indeed different from their normal cellular counterparts, remains to be determined, however.

Deazaadenosine analogues

Two deazaadenosine analogues, namely 3-deazaadenosine (c^3Ado) and 7-deazaadenosine (c^7Ado, tubercidin) (Fig. 5) show activity against a broad variety of viruses.

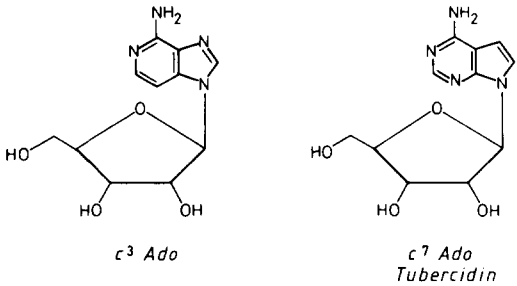


Fig. 5. Structural formulae of *deazaadenosine analogues*: 3-deazaadenosine (c^3Ado) and 7-deazaadenosine (c^7Ado, tubercidin).

The antiviral activity of c^3Ado has been attributed to the inhibition of SAH hydrolase [6–9]. Independently from such inhibitory effect on SAH hydrolase, c^3Ado may directly interfere with viral RNA transcription [31]. Tubercidin would also interfere with viral RNA synthesis, but, in addition, tubercidin is highly cytotoxic because of its ability to suppress a number of cellular processes including mitochondrial respiration, *de novo* purine biosynthesis, rRNA processing, tRNA methylation as well as RNA and protein synthesis [32].

Neither c^3Ado nor c^7Ado are very selective in their antiviral activity. c^3Ado is uniformly toxic to the host cells at 40 μg/ml and active against some viruses, i.e., vaccinia virus, vesicular stomatitis virus, parainfluenza virus type 3 and reovirus type 1, at a concentration which is only 5–20-fold lower than the cytotoxic concentration (Table 2). Tubercidin (c^7Ado) is much more cytotoxic than c^3Ado: it causes a micro-

TABLE 2

Inhibitory effects of deazaadenosine analogues (c^3Ado and c^7Ado) on virus-induced cytopathogenicity in cell cultures

Virus	Cell culture	Minimum inhibitory concentration[a] (μg/ml)	
		c^3Ado	c^7Ado (Tubercidin)
Vaccinia	PRK	7 (40)	0.02 (0.4)
Herpes simplex 1	PRK	≥40 (40)	0.07 (0.4)
Herpes simplex 2	PRK	≥40 (40)	0.2 (0.4)
Vesicular stomatitis	PRK	7 (40)	0.007 (0.4)
Polio 1	HeLa	≥40 (40)	0.007 (0.4)
Coxsackie B4	HeLa	≥40 (40)	0.2 (0.4)
Sindbis	Vero	≥40 (40)	0.2 (0.4)
Measles	Vero	≥40 (40)	0.2 (0.4)
Parainfluenza 3	Vero	2 (40)	0.07 (0.4)
Reo 1	Vero	10 (40)	0.07 (0.4)

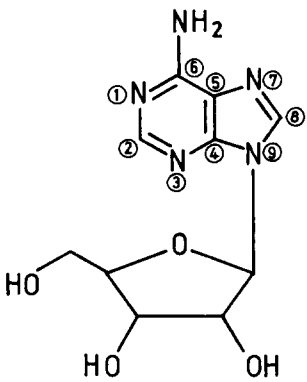
[a] Required to inhibit viral cytopathogenicity by 50%. The data indicated in parentheses refer to the minimum inhibitory concentration (μl/ml) of the compounds causing a microscopically detectable alteration of normal cell morphology (in uninfected cell cultures).

scopically detectable disruption of the cells at a concentration as low as 0.4 μg/ml. For some viruses, i.e., vesicular stomatitis virus and poliovirus type 1, c^7Ado is inhibitory at a concentration which is 50-fold lower than the cytotoxic concentration, but for other viruses it is only inhibitory at concentrations approaching cytotoxicity (Table 2).

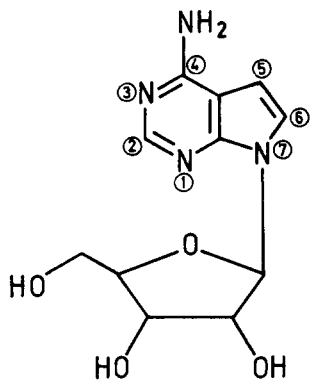
Tubercidin analogues

Since c^7Ado (tubercidin) did not display much selectivity in its antiviral action, various substituents have been introduced at the C-5 position* of c^7Ado in attempts to increase its selectivity: i.e., chlorine, bromine, iodine, cyano, carboxamido, 1-hydroxyethyl, 1-methoxyethyl, 2-bromoethenyl, 2-cyanoethenyl, 2-buten-1-yl, 3-hydroxypropyl or butyl [32]. Three of these tubercidin analogues, namely 5-(2-buten-1-yl) tubercidin, 5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl)tubercidin (Fig. 6), appear particularly interesting. In several instances they achieve an inhibitory effect on virus replication at a concentration that is 100-fold lower than the cytotoxic concentration: i.e., 5-(2-buten-1-yl)tubercidin against vaccinia virus, 5-(1-hydroxyethyl)tubercidin against poliovirus type 1, 5-(2-buten-1-yl)tubercidin against parainfluenza virus type 3 and 5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl)tubercidin against reovirus type 1 (Table 3). Thus, it seems feasible to improve the toxicity to activity ratio of c^7Ado by the appropriate substitution at the C-5 position.

The increased selectivity of 5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl) tubercidin is also reflected by their activity against Coxsackie B4 virus. Tubercidin is active against Coxsackie B4 only at a concentration 2-fold lower than the cytotoxic



Adenosine



Tubercidin

Thus, substituents at the C-5 position of tubercidin are in the same relative position as N-7 of adenosine because of the difference in the numbering of the purine and pyrrolo[2,3-*d*]pyrimidine ring systems.

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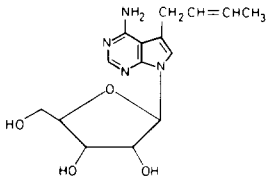
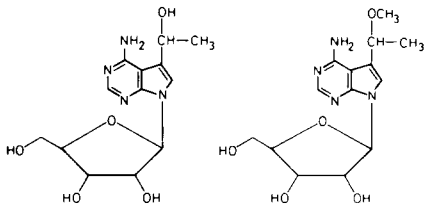
* Strictly speaking the N-7 position of adenosine is equal to the C-5 position of tubercidin (c^7Ado):



5-(2-buten-1-yl) tubercidin



5-(1-hydroxyethyl) tubercidin 5-(1-methoxyethyl) tubercidin

Fig. 6. Structural formulae of *tubercidin analogues*: 5-(2-buten-1-yl)tubercidin, 5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl)tubercidin.

TABLE 3

Inhibitory effects of tubercidin (c^7Ado) analogues on virus-induced cytopathogenicity in cell cultures

Virus	Cell culture	Minimum inhibitory concentration[a] (μg/ml)		
		5-(2-buten-1-yl) tubercidin	5-(1-hydroxyethyl) tubercidin	5-(1-methoxyethyl) tubercidin
Vaccinia	PRK	0.7 (200)	0.7 (10)	0.7 (100)
Herpes simplex 1	PRK	20 (200)	2 (10)	20 (100)
Herpes simplex 2	PRK	150 (200)	7 (10)	40 (100)
Vesicular stomatitis	PRK	40 (200)	0.4 (10)	40 (100)
Polio 1	HeLa	0.2 (1)	0.2 (40)	0.7 (10)
Coxsackie B4	HeLa	0.2 (1)	0.7 (40)	0.7 (10)
Sindbis	Vero	40 (100)	7 (40)	40 (100)
Measles	Vero	>100 (100)	>40 (40)	>40 (40)
Parainfluenza 3	Vero	1 (100)	0.7 (40)	2 (100)
Reo 1	Vero	2 (100)	0.4 (40)	1 (100)

[a] Required to inhibit viral cytopathogenicity by 50%. The data indicated in parentheses refer to the minimum inhibitory concentration (μl/ml) of the compounds causing a microscopically detectable alteration of normal cell morphology (in uninfected cell cultures).

concentration (Table 2), while 5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl)-tubercidin inhibit Coxsackie B4 virus at a concentration which is respectively 50- or 10-fold lower than the cytotoxic concentration (Table 3). These observations have been further extended to an experimental Coxsackie B4 infection in newborn mice (Fig. 7). In this animal model tubercidin is inactive when given as a single dose of 20 μg/mouse and toxic at higher doses [lethal dose-50 (LD_{50}), ≈ 50 μg/mouse]. However,

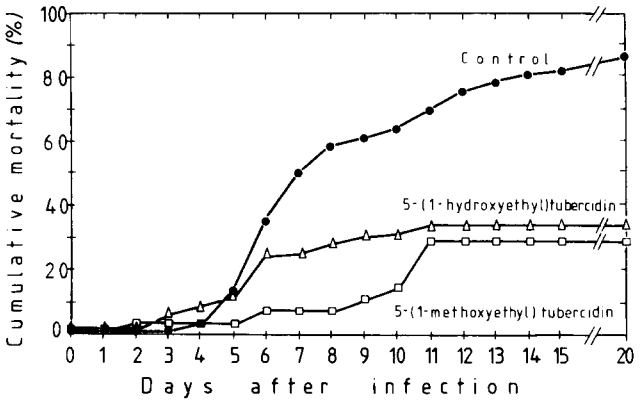


Fig. 7. Effects of tubercidin analogues, 5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl)tubercidin, on mortality of newborn NMRI mice infected subcutaneously with Coxsackie B4 virus. A single dose of 100 μg compound per mouse was administered intraperitoneally 1 h after virus infection. There were 30 mice for each of the treated groups and 100 mice for the control group. The mortality rate of the treated groups was significantly different from the mortality rate of the control group ($P < 0.001$; as assessed by the χ^2-test with Yates' correction).

5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl)tubercidin suppress the mortality rate of newborn mice infected with Coxsackie B4 virus when administered at a dose (100 μg/mouse) well below their LD_{50} (≈ 300 μg/mouse) (Fig. 7). 5-(1-Hydroxyethyl)-tubercidin and 5-(1-methoxyethyl)tubercidin should therefore be further pursued for their potentials in the treatment of Coxsackie and other (i.e., parainfluenza and reo) virus infections.

Carbocyclic adenosine analogues

The carbocyclic adenosine analogues are represented by C-Ado (carbocyclic adenosine), C-c^3Ado (carbocyclic 3-deazaadenosine) and C-c^7Ado (carbocyclic 7-deazaadenosine) (Fig. 8). C-Ado and C-c^7Ado do not show much selectivity in their antiviral action: these compounds inhibit virus replication at a concentration which is equal to or only slightly (at the most 5–10-fold) lower than the cytotoxic concentration (Table 4). C-c^7Ado can be considered as the carbocyclic analogue of tubercidin (c^7Ado). It is neither more potent nor more selective than its parent compound (Table 2).

However, a dramatic improvement in both potency and selectivity is demonstrated by C-c^3Ado relative to c^3Ado (Tables 2 and 4) [33]. While not cytotoxic at 400 μg/ml, C-c^3Ado inhibits the replication of vaccinia virus, vesicular stomatitis virus, measles virus, parainfluenza virus type 3 and reovirus type 1 at a concentration of 0.2–1 μg/ml (Table 4), thus achieving a selectivity index of 400–2000. The activity spectrum of C-c^3Ado is similar to that of c^3Ado (Table 2), but C-c^3Ado is about 10–100 times more potent as an antiviral agent and at least 10 times less cytotoxic.

Like (*S*)-DHPA, C-c^3Ado is a potent inhibitor of SAH hydrolase [34] and the

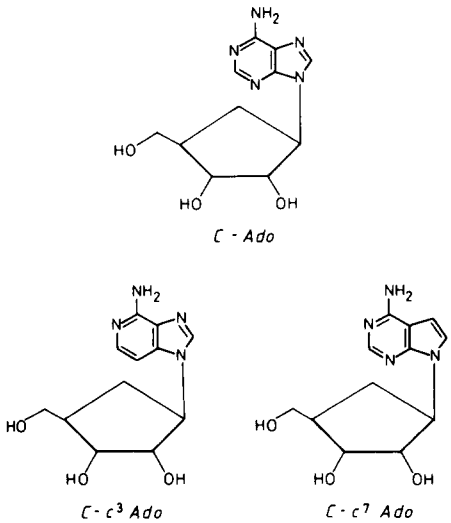


Fig. 8. Structural formulae of *carbocyclic adenosine analogues*: carbocyclic adenosine (C-Ado), carbocyclic 3-deazaadenosine (C-c^{3}Ado) and carbocyclic 7-deazaadenosine (C-c^{7}Ado).

TABLE 4

Inhibitory effects of carbocyclic adenosine analogues (C-Ado, C-c^{3}Ado and C-c^{7}Ado) on virus-induced cytopathogenicity in cell cultures

Virus	Cell culture	Minimum inhibitory concentration[a] (μg/ml)		
		C-Ado	C-c^{3}Ado	C-c^{7}Ado
Vaccinia	PRK	2 (10)	0.8 (> 400)	2 (10)
Herpes simplex 1	PRK	7 (10)	≥200 (> 400)	≥10 (10)
Herpes simplex 2	PRK	7 (10)	≥300 (> 400)	7 (10)
Vesicular stomatitis	PRK	4 (10)	0.2 (> 400)	≥10 (10)
Polio 1	HeLa	≥10 (10)	>400 (> 400)	≥10 (10)
Coxsackie B4	HeLa	≥10 (10)	>400 (> 400)	7 (10)
Sindbis	Vero	≥ 4 (4)	20 (> 400)	≥10 (10)
Measles	Vero	≥ 4 (4)	0.4 (> 400)	4 (10)
Parainfluenza 3	Vero	≥ 4 (4)	0.2 (> 400)	2 (10)
Reo 1	Vero	0.4 (4)	1 (> 400)	4 (10)

[a] Required to inhibit viral cytopathogenicity by 50%. The data indicated in parentheses refer to the minimum inhibitory concentration (μl/ml) of the compounds causing a microscopically detectable alteration of normal cell morphology (in uninfected cell cultures).

activity of both compounds is directed at the same viruses, i.e., vaccinia, vesicular stomatitis, measles parainfluenza and reo (Tables 1 and 4). Neither (*S*)-DHPA nor C-c^{3}Ado are subject to deamination or phosphorylation. Thus, their antiviral activity seems to depend exclusively on an inhibition of SAH hydrolase.

As has been shown previously for (*S*)-DHPA [17], C-c^{3}Ado is also effective in protecting mice against a lethal vesicular stomatitis virus infection. In newborn mice, a single dose of 20 μg C-c^{3}Ado per mouse suffices to reduce the mortality rate (due to vesicular stomatitis virus infection) from 90% to 48%; and with a single dose of 500 μg C-c^{3}Ado the mortality rate is further reduced to 20% (Fig. 9). C-c^{3}Ado is also efficacious in protecting older mice against an intranasal vesicular stomatitis virus infection (Fig. 10), although the increase in survival rate achieved with a single dose of 5 mg C-c^{3}Ado in these older mice is barely significant. To obtain a significant increase in the survival rate, repeated doses of 5 mg C-c^{3}Ado have to be administered daily, starting immediately after virus infection (Fig. 10).

C-c^{3}Ado is also effective in suppressing the formation of tail lesions in mice infected intravenously with vaccinia virus (Table 5). Following a single dose of 5 mg C-c^{3}Ado the number of pox tail lesions is reduced by approximately 25%; and when given as repeated doses of 5 mg per mouse, C-c^{3}Ado reduces the number of pox tail lesions by almost 60%. Similar inhibitory effects on vaccinia virus tail lesion formation have been described for other nucleoside analogues such as 5-iodo-2′-deoxyuridine (IDU), 1-β-D-arabinofuranosylcytosine (Ara-C) and 1-β-D-ribofuranosyl-1,2,4,-triazole-3-carboxamide (Ribavirin) [35].

As could be predicted from the cell culture data (Table 4), C-c^{3}Ado fails to protect newborn mice against a lethal Coxsackie B4 virus infection, even if administered at a dose of 500 μg/mouse (data not shown). In this regard, C-c^{3}Ado diverges from 5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl)tubercidin which are both effective in the therapy of Coxsackie B4 virus infection in newborn mice (Fig. 7). On the other hand, the tubercidin analogues fail to protect newborn mice infected with vesicular stomatitis virus (data not shown). It thus appears as though the in vivo activity spectrum of C-c^{3}Ado, while similar to that of (*S*)-DHPA, is totally different from that of the tubercidin analogues.

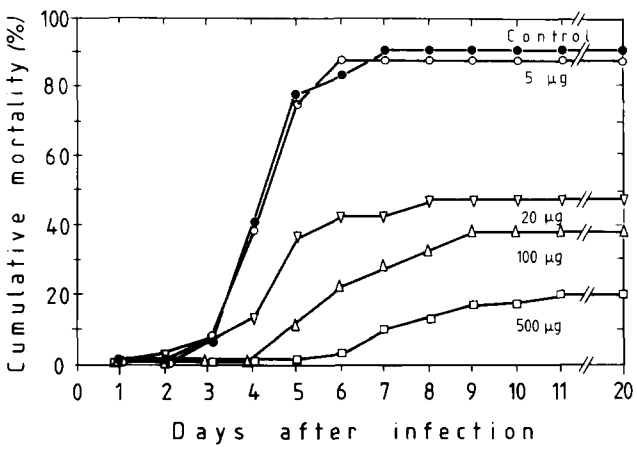


Fig. 9. Effects of C-c^{3}Ado on mortality of newborn NMRI mice infected subcutaneously with vesicular stomatitis virus. A single dose of either 5, 20, 100 or 500 μg C-c^{3}Ado per mouse was administered intraperitoneally 1 h after virus infection. There were 30 mice per group. The mortality rate of the groups treated with 20, 100 or 500 μg C-c^{3}Ado per mouse was significantly different from the mortality rate of the control group ($P < 0.001$; as assessed by the χ^2-test with Yates' correction).

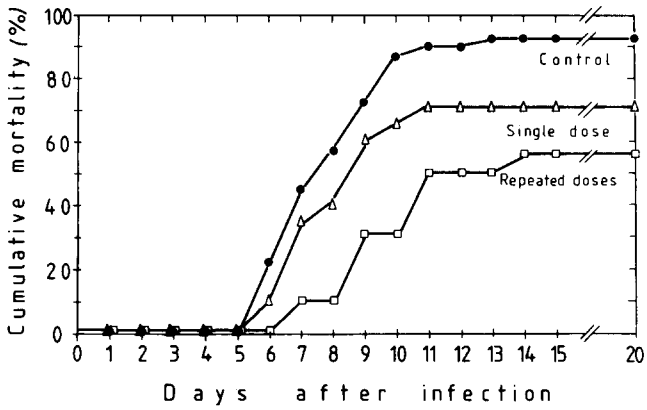


Fig. 10. Effects of C-c³Ado on mortality of young NMRI mice (weighing 11–13 g) infected intranasally with vesicular stomatitis virus. C-c³Ado was injected intraperitoneally either as a single dose of 5 mg per mouse at 1 h after virus infection or as repeated doses of 5 mg per mouse at 1 h, 1, 2, 3 and 4 days after virus infection. There were 20 mice for each of the treated groups and 40 mice for the control group. The mortality rate of the group treated repeatedly with C-c³Ado was significantly different from the mortality rate of the control group ($P < 0.005$), whereas the difference in the mortality rates of the control group and the group treated with a single dose of C-c³Ado was barely significant ($P \approx 0.06$; as assessed by the χ^2-test with Yates' correction).

Table 5

Effects of C-c³-Ado on the formation of vaccinia virus tail lesions in mice

Treatment	N. of lesions		*P* (compared with control group)
	Per individual mouse	Average	
Single dose	12,11,29,19,24,8,6,18,21, 39,17,7,19,17,16,7,6,12 9,5	15.1	<0.05
Control	18,14,23,33,16,33,8,18,12, 29,11,27,4,17,28,35,18,16, 20,15	19.75	-
Repeated doses	11,3,3,2,0,14,4,26,10,9	8.2	<0.025
Control	42,23,27,16,0,29,30,5,13, 40,28,1,6,17,20,4,38,4,40, 27,40,18,7,5,3,20,4,5,56, 19	19.6	-

Young NMRI mice (weighing 11–13 g) were inoculated intravenously (in a tail vein) with vaccinia virus and treated intraperitoneally with a single dose of C-c³Ado (5 mg/mouse) at 1 h after virus infection or with repeated doses of C-c³Ado (5 mg/mouse) at 1 h, 1, 2, 3 and 4 days after virus infection. Tail lesions were counted 7 days after infection. Statistical significance of the results was assessed by Student's *t* test.

Conclusion

Several adenosine analogues yield great promise as antiviral agents. One of these agents, ara-A (Vira-A^R, Vidarabine^R), is in clinical use for the systemic treatment of severe herpes simplex and zoster infections. Two structural analogues of ara-A, cyclaradine and ara-AMP, are being pursued for their antiherpes potentials. Various other adenosine analogues that have been developed recently differ in their activity spectrum from ara-A, cyclaradine and ara-AMP. Foremost among these new adenosine analogues are (*S*)-DHPA and C-c^{3}Ado.

(*S*)-DHPA and C-c^{3}Ado show remarkable similarities in their spectrum of antiviral activity. They are inactive against (+)RNA strand viruses [picornaviridae (i.e., polio, Coxsackie) and togaviridae (i.e., Sindbis)] but quite effective against (–)RNA strand viruses [paramyxoviridae (i.e., measles, parainfluenza, respiratory syncytial virus), rhabdoviridae (i.e., rabies, vesicular stomatitis virus) and reoviridae (i.e., reo-, rotavirus)]. Some DNA viruses [poxviridae (i.e., vaccinia)] are sensitive to (*S*)-DHPA and C-c^{3}Ado, whereas other DNA viruses [herpetoviridae (i.e., herpes simplex)] are only minimally affected or not inhibited at all. The peculiarities in the activity spectrum of (*S*)-DHPA and C-c^{3}Ado may be innate to their mechanism of action, namely the inhibition of SAH hydrolase. The importance of this enzyme in methyltransferase reactions and virus replication, i.e., viral mRNA maturation, has been addressed in the Introduction. For the orthomyxo-, arena- and bunyaviruses the antiviral potentials of (*S*)-DHPA and C-c^{3}Ado remain to be evaluated. In as far as these (–)RNA strand viruses require specific methylations for their maturation, they may, like the other (–)RNA strand viruses, prove susceptible to the inhibitory effects of (*S*)-DHPA and C-c^{3}Ado.

Tubercidin and its 5-substituted derivatives encompass both (+)RNA strand and (–)RNA strand viruses, as well as DNA viruses, in their activity spectrum. Although tubercidin is a potent antiviral agent, it exhibits little, if any, selectivity in its antiviral action. As demonstrated by 5-(1-hydroxyethyl)tubercidin and 5-(1-methoxyethyl)tubercidin, it seems feasible to increase the selectivity of this class of compounds by appropriate modifications at the C-5 position.

Acknowledgements

This work was supported by Krediet No. 3.0040.83 from the Belgian F.G.W.O. (Fonds voor Geneeskundig Wetenschappelijk Onderzoek) and Conventie No. 81/86-27 of the Belgian G.O.A. (Geconcerteerde Onderzoeksacties). The excellent technical assistance of Anita Van Lierde and Willy Zeegers is gratefully acknowledged.

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