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ANTIVIRAL POTENCY OF ADENOSINE ANALOGUES : CORRELATION WITH INHIBITION OF S-ADENOSYLHOMOCYSTEINE HYDROLASE

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SUMMARY For a series of adenosine analogues a close correlation (r = 0.986) was found between their antiviral potency (against vesicular stomatitis virus) and their inhibitory effects (K_i/K_m) on S-adenosylhomocysteine (AdoHcy) hydrolase; thus, in order of increasing inhibitory potency for both virus replication and AdoHcy hydrolase activity : (S)-9-(2,3-dihydroxypropyl)adenine < (RS)-3-adenin-9-yl-2-hydroxypropanoic acid (isobutyl ester) < carbocyclic 3-deazaadenosine < neplanocin A. Our findings point to AdoHcy hydrolase as the target for the broad-spectrum antiviral activity of these adenosine analogues.

Various acyclic and carbocyclic analogues of adenosine have been found to exhibit broad-spectrum antiviral properties : viz. (S)-9-(2,3-dihydroxypropyl) adenine [(S)-DHPA],[1,2] (D)-eritadenine,[3,4] (RS)-3-adenin-9-yl-2-hydroxypropanoic acid [(RS)-AHPA] alkyl esters,[5] 3-deazaadenosine,[4,6,7] carbocyclic adenosine,[4] carbocyclic 3-deazaadenosine (C-c^{3}Ado),[4,8,9] carbocyclic 7-deazaadenosine,[4,10] and neplanocin A.[11,12] These compounds are particularly active against poxviruses (vaccinia), rhabdoviruses (vesicular stomatitis), paramyxoviruses (parainfluenza, measles), and reoviruses (reo, rota).

All these adenosine analogues are also known to inhibit S-adenosylhomocysteine (AdoHcy) hydrolase (EC 3.3.1.1.), a key enzyme in methylation reactions.[8,10,11,13-15] AdoHcy hydrolase is a product inhibitor of S-adenosylmethionine (AdoMet)-dependent methylations. Such methylations are required for the maturation of viral mRNA (5'-terminal cap formation). Hence, inhibitors of AdoHcy hydrolase may be expected to block virus replication by interference with the methylation of viral mRNA.

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Abbreviations : (S)-DHPA, (S)-9-(2,3-dihydroxypropyl)adenine; (RS)-AHPA, (RS)-3-adenin-9-yl-2-hydroxypropanoic acid; C-c^{3}Ado, carbocyclic 3-deazaadenosine; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; Ado, adenosine; Hcy, homocysteine.

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However, the relationship between AdoHcy hydrolase inhibition and antiviral activity has not been clearly established. Various K_i values have been reported for the inhibitory activity of adenosine analogues on AdoHcy hydrolase,[8,10,11,13-15] but because of the differences in the AdoHcy hydrolase preparations and assay systems used by the different authors, a direct comparison of these K_i values is not possible. With a series of adenosine analogues we have now evaluated the K_i values for AdoHcy hydrolase in the same assay system, and found a close correlation between these K_i values and the inhibitory potency of the compounds for virus replication.

MATERIALS AND METHODS

Compounds. (S)-DHPA, (RS)-AHPA and (RS)-AHPA isobutyl ester were provided by A. Holý (Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia). C-c^{3}Ado was provided by J.A. Montgomery (Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama, USA) and neplanocin A was obtained from Toyo Jozo Co. (Mifuku Ohito-Cho, Tagata-Gun, Shizuoka-Ken, Japan) through the courtesy of J. Murase. The structural formulae of the compounds are presented in Fig. 1.

AdoHcy hydrolase assay. AdoHcy hydrolase was prepared from bovine liver by ammonium sulphate precipitation (35-50 %). The specific activity was about 0.1 unit/mg protein (one unit corresponding to the synthesis of 1 µmole AdoHcy per minute). The AdoHcy hydrolase preparation contained less than 2 % adenosine deaminase. AdoHcy hydrolase activity was measured in the direction of AdoHcy synthesis: the assay mixture contained, in a total volume of 0.5 ml, 1 µg enzyme, varying amounts of (8-^{14}C)adenosine (Amersham), 2 mM D,L-homocys-

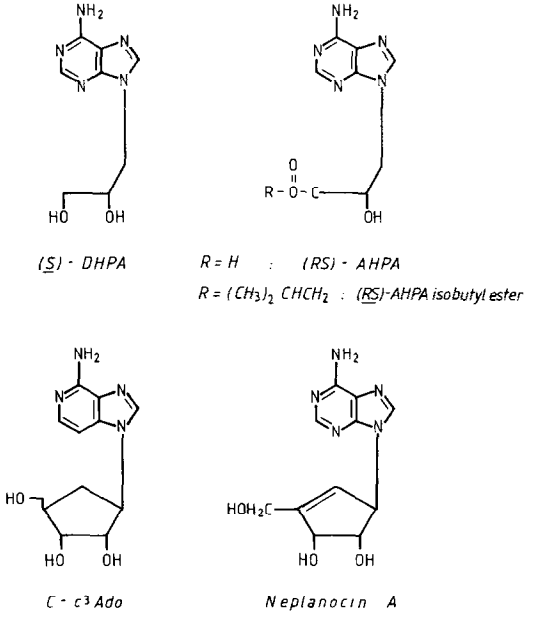

Fig. 1. Structural formulae of (S)-DHPA, (RS)-AHPA (isobutyl ester), C-c^{3}Ado and neplanocin A.

teine, 2 mM dithiothreitol, 1 mM EDTA and test compound at the appropriate concentration. After 2.5 min incubation at 37°C, the reaction was stopped with 4 N perchloric acid; then 4 N KOH was added to pH 7.0, and unreacted adenosine was converted to inosine with 0.1 unit exogenous adenosine deaminase (Sigma). AdoHcy and inosine were separated by ion exchange chromatography on a SP-Sephadex C-25 (Pharmacia). Inosine was eluted with 0.1 N formic acid, and then AdoHcy was eluted with 0.5 N NaOH.[16] The amount AdoHcy formed could be calculated from the radio of the radioactivity counts found in two eluates.

Antiviral assays. The procedure for measuring inhibition of virus-induced cytopathogenicity has been described previously.[1,9,12]

RESULTS

AdoHcy hydrolase catalyzes the reaction Ado + Hcy $\rightleftarrows$ AdoHcy. The enzyme activity was monitored by measuring the synthesis of AdoHcy. The adenosine analogues (S)-DHPA, (RS)-AHPA, C-c^{3}Ado and neplanocin A exerted an inhibitory effect on AdoHcy hydrolase, which was competitive with respect to the substrate (8-^{14}C)Ado (Fig. 2). From the Lineweaver-Burk plots K_i and K_i/K_m values were calculated (Table I), and these indicated the following order of increasing inhibitory potency : (S)-DHPA < (RS)-AHPA < C-c^{3}Ado < neplanocin A.

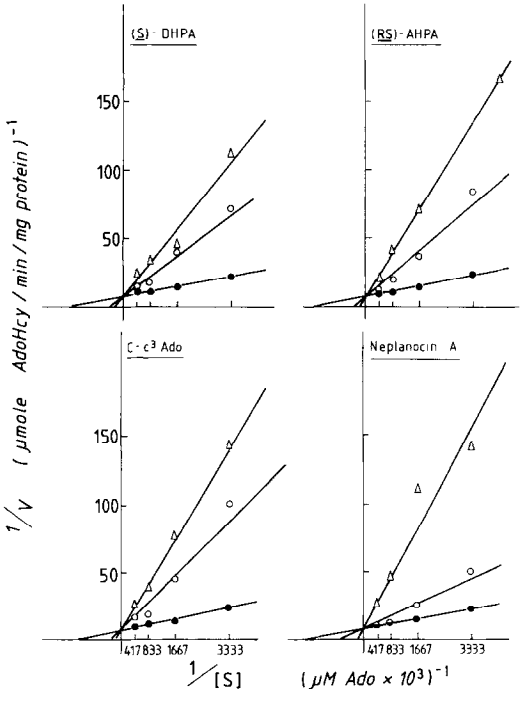

Fig. 2. Lineweaver-Burk plots for AdoHcy hydrolase activity (with (8-^{14}C)Ado and D,L-homocysteine as substrates) in the presence of :
- (S)-DHPA at 0 µM (●), 5 µM (○) or 10 µM (Δ)
- (RS)-AHPA at 0 µM (●), 0.25 µM (○) or 0.5 µM (Δ)
- C-c^{3}Ado at 0 µM (●), 0.05 µM (○) or 0.1 µM (Δ)
- neplanocin A at 0 µM (●), 5 nM (○) or 20 nM (Δ).

Table I. Inhibitory constants of (S)-DHPA, (RS)-AHPA, C-c^{3}Ado and neplanocin A for AdoHcy hydrolase

Compound	K_i (μM)	K_i/K_m
(S)-DHPA	1.4	2.3
(RS)-AHPA	0.073	0.12
C-c^{3}Ado	0.013	0.022
Neplanocin A	0.002	0.003

K_m for adenosine : 0.6 μM.

The compounds were also evaluated for their antiviral activity in a broad range of assay systems.[4,5,12] The minimum virus-inhibiting concentrations for a number of viruses, i.e. vesicular stomatitis, vaccinia, measles and reo (type 1) (which are representative for the rhabdo-, pox-, paramyxo- and reoviridae, respectively), are presented in Table II. Again, the order of increasing potency was (S)-DHPA < (RS)-AHPA isobutyl ester < C-c^{3}Ado < neplanocin A. In the antiviral experiments the isobutyl ester of (RS)-AHPA, rather than (RS)-AHPA itself, was used, to enable the compound to penetrate into the cells. It is assumed that within the cell the isobutyl ester of (RS)-AHPA is hydrolyzed to release the parent compound.[5]

When then the K_i/K_m values of the compounds for AdoHcy hydrolase were plotted in function of their minimum inhibitory concentrations for virus (i.e., vesicular stomatitis virus) replication (Fig. 3), a close correlation emerged ($r = 0.986$). This close correlation points to a causal relationship between the antiviral activities of the four adenosine analogues and their inhibitory effects on AdoHcy hydrolase.

Table II. Antiviral activity of (S)-DHPA, (RS)-AHPA isobutyl ester, C-c^{3}Ado and neplanocin A

Compound	MIC_{50}* (μg/ml)			
	VSV	VV	MV	RV-1
(S)-DHPA	20	50	40	200
(RS)-AHPA isobutyl ester	2	7	40	150
C-c^{3}Ado	0.2	0.7	0.7	1
Neplanocin A	0.01	0.03	0.04	0.2

*Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity by 50 % in either primary rabbit kidney cells (VSV (vesicular stomatitis virus), VV (vaccinia virus)) or African green monkey kidney (Vero) cells (MV (measles virus), RV-1 (reovirus type 1)).

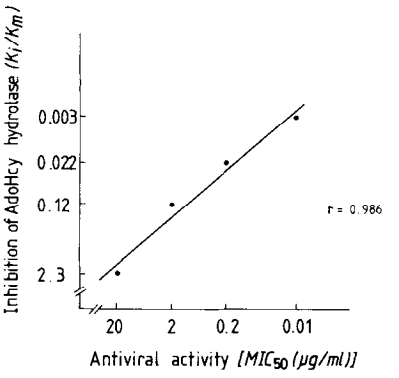

Fig. 3. Linear regression for K_i/K_m of (S)-DHPA, (RS)-AHPA (isobutyl ester), C-c^{3}Ado and neplanocin A for AdoHcy hydrolase as a function of their MIC_{50} for vesicular stomatitis virus replication (data taken from Tables I and II).

DISCUSSION

The adenosine analogues that were subject of the present study have been evaluated previously for their inhibitory effects on AdoHcy hydrolase,[8,11,13,14] but these previous studies, conducted by different investigators using different methods and sources of AdoHcy hydrolase, do not allow a proper comparison of the relative inhibitory activities of the compounds on AdoHcy hydrolase. Thus, Borchardt et al.[11] reported a K_i of 8.39 nM of neplanocin A for the beef liver enzyme; Montgomery et al.[8] found a K_i of 1 nM of C-c^{3}Ado for the hamster liver enzyme but for the beef liver enzyme the K_i of C-c^{3}Ado was 3 μM. For the rat liver enzyme, Votruba and Holý[13] noted a K_i of (S)-DHPA of 3.5 μM; for the L1210 leukemia cell enzyme, the K_i of (S)-DHPA was 0.9 μM. With the latter enzyme, Merta et al.[14] found K_i values of 0.04 and 0.12 μM with the R and S enantiomers of AHPA, respectively.

The present studies clearly demonstrate the relative potencies of (S)-DHPA, (RS)-AHPA, C-c^{3}Ado and neplanocin A as inhibitors of AdoHcy hydrolase and, furthermore, establish a close correlation between the inhibitory effects of the compounds on AdoHcy hydrolase and their antiviral potencies. These findings implicate AdoHcy hydrolase as the target for the antiviral action of the adenosine analogues. To unequivocally demonstrate that neplanocin A and its congeners achieve their antiviral action by the inhibition of AdoHcy hydrolase and concomitant shut off of viral mRNA methylation, it would appear necessary to directly determine AdoHcy hydrolase activities, AdoMet/AdoHcy ratios and the extent of viral mRNA methylation (5'-terminal cap formation) in the virus-infected cells.

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