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COMMENTARY

S-ADENOSYLHOMOCYSTEINE HYDROLASE INHIBITORS AS BROAD-SPECTRUM ANTIVIRAL AGENTS

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Although most of the antiviral agents that are currently in clinical use or considered for clinical use have been discovered serendipitously, i.e. were designed for other purposes, the targets at which selective antiviral drugs may act have been identified with increasing frequency [1, 2]. Foremost amidst these targets are the viral DNA polymerases of the herpetoviridae [herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV)]. This target can be reacted upon either directly, i.e. by phosphonoformate (foscarnet, PFA), or indirectly, after preceding phosphorylation by the virus-encoded deoxythymidine kinase, as has been demonstrated for a number of pyrimidine nucleoside analogues, i.e. (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine, BVDU), and purine nucleoside analogues, i.e. 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV) and 9-(1,3-dihydroxy-2-propoxymethyl)guanine [DHPG, also referred to as BWB759U, BIOLF-62, and 2′-nor-2′-deoxyguanosine (2′NDG)] [3].

The RNA-directed DNA polymerase associated with retroviruses, i.e. human immunodeficiency virus (HIV), has also been considered as an attractive target and the majority of the compounds presently pursued for the chemotherapy of the acquired immune deficiency syndrome (AIDS), i.e. suramin, tungstoantimoniate (HPA-23), PFA, azidothymidine (AZT) and 2′,3′-dideoxynucleosides (ddCyd, ddAdo), are actually targeted at this enzyme [4].

The well established anti-influenza A compounds amantadine and rimantadine inhibit the initiation of virus infection, presumably by interacting with the virus-coded M_2 membrane protein [5], and it would be of interest to examine whether the newly developed cyclononane derivative, which is active in the prevention and treatment of experimental influenza A virus infection in volunteers [6], acts in a fashion similar to that of amantadine or rimantadine. For the antirhinovirus compounds, belonging to such widely different chemical classes as flavans, flavones, chalcones, isoxazoles, pyrazinamines and nitrobenzenes (for a review, see Ref. 7), the principal target may well be the uncoating process, which would be prevented subsequently to the binding of the compounds to the viral capsid.

For ribavirin, the triazole nucleoside that holds promise in the treatment of respiratory syncytial virus (RSV) infections and hemorrhagic fever virus infections (such as Lassa fever), the target of antiviral action has not been rigorously defined. It is clear, however, that ribavirin, through an inhibitory effect on IMP dehydrogenase, interferes with nucleotide pool sizes and, in addition, it also inhibits the initiation of viral mRNA transcription [8, 9].

For a number of adenosine analogues which, according to their chemical structure, could be divided in two classes, (i) acyclic adenosine analogues (Fig. 1) and (ii) carbocyclic adenosine analogues (Fig. 2), the main target of their antiviral action would correspond to *S*-adenosylhomocysteine (AdoHcy, SAH) hydrolase, a key enzyme in transmethylation reactions using *S*-adenosylmethionine (AdoMet, SAM) as the methyl donor. These adenosine analogues are endowed with a unique antiviral activity spectrum, fundamentally different from that of ribavirin and the other compounds mentioned above. They are all potent, reversible or irreversible, inhibitors of SAH hydrolase; and the questions arise as to (i) whether this inhibitory effect on SAH hydrolase is causally related to their antiviral activity, and (ii) how an interference with transmethylation reactions could confer any specificity towards virus replication.

History

SAH hydrolase catalyzes the hydrolysis of *S*-adenosyl-L-homocysteine to adenosine (Ado) and L-homocysteine (Hcy). This reaction is reversible, with the equilibrium far in the direction of synthesis, although physiologically the reaction proceeds in the direction of hydrolysis because Ado and Hcy are readily removed by further metabolism. AdoHcy itself was first isolated and characterized by Cantoni and Scarano [10], and SAH hydrolase was first described in rat liver by de la Haba and Cantoni [11] and later isolated from calf liver by Richards *et al.* [12]. That transmethylation reactions may serve as a pharmacological target became evident from the studies of Lederer and his colleagues [13, 14], who found that 5′-deoxy-5′-*S*-isobutyladenosine (SIBA) and other synthetic SAH analogues inhibited the transformation of chick embryo fibroblasts by Rous sarcoma virus. Further studies [15, 16] showed that SIBA also inhibited the replication of polyoma virus

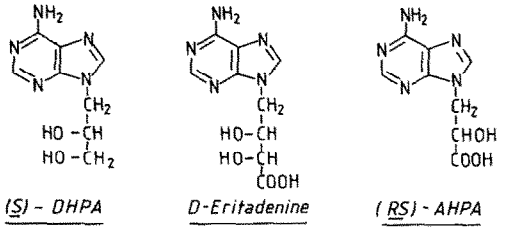

Fig. 1. Representative examples of SAH hydrolase inhibitors among the class of acyclic adenosine analogues: (*S*)-DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine], D-eritadenine [(2*R*,3*R*)-4-adenin-9-yl-2,3-dihydroxybutanoic acid], and (*RS*)-AHPA [(*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid].

and herpes simplex virus. The latter was attributed to an inhibitory effect of SIBA on the methylation of viral mRNA and, specifically, that of the 5'-cap [16].

The importance of SAH hydrolase as a potential pharmacological target was first demonstrated by Chiang *et al.* [17] who tested over fifty analogues of Ado and AdoHcy as inhibitors of SAH hydrolase and found 3-deazaadenosine (c^3Ado) to be nearly 100-fold more potent as an inhibitor of this enzyme than SIBA. 3-Deazaadenosine was then shown to inhibit the transformation of chick embryo cells by Rous sarcoma virus [18], and this inhibitory effect was attributed to an inhibition of SAH hydrolase and ensuing changes in the SAM/SAH levels, Later on, 5'-deoxy-5'-*S*-isobutyl-3-deazaadenosine (3-deaza-SIBA) was synthesized, and, again, this compound proved effective as both an inhibitor of SAH hydrolase [19] and virus replication [19, 20], albeit at rather high concentrations. Among the adenosine analogues, various new congeners, i.e. 2-chloroadenosine, 8-aminoadenosine, 9-β-D-arabinofuranosyladenine (Ara-A), and (±)aristeromycin, the latter being carbocyclic adenosine (C-Ado), were then found to be potent inhibitors of SAH hydrolase [21, 22], and this opened the possibility for further chemical modifications and structure–function relationship studies of adenosine analogues as both inhibitors of SAH hydrolase and virus replication.

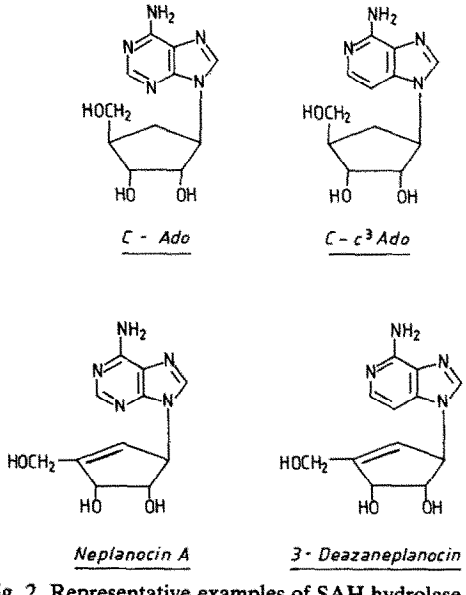

Fig. 2. Representative examples of SAH hydrolase inhibitors among the class of carbocyclic adenosine analogues: C-Ado (carbocyclic adenosine), C-c^3-Ado (carbocyclic 3-deazaadenosine), neplanocin A (cyclopentenyl derivative of adenine), and 3-deazaneplanocin (cyclopentenyl derivative of 3-deazaadenine).

Concept

Our own work in this area, that ultimately led to the concept that acyclic and carbocyclic analogues of adenosine such as those depicted in Figs. 1 and 2 owe their antiviral activity to inhibition of SAH hydrolase [23], started in 1978 with the discovery of (*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA] as a broad-spectrum antiviral agent [24, 25]. The synthesis of this compound had been described previously by Holý [26], and its racemate (*RS*)-DHPA had been reported in 1965 by Schaeffer *et al.* [27] to be a (rather weak) inhibitor of adenosine deaminase. At first, it was not clear, however, how (*S*)-DHPA would exert its antiviral activity, until Votruba and Holý [28] and their colleagues [29] established that (*S*)-DHPA was a rather potent inhibitor ($K_i \sim 1$ μM) of rat liver and murine L1210 leukemia SAH hydrolase.

Whereas (*S*)-DHPA exhibited a reversible inhibitory effect on SAH hydrolase, the carboxylic acid derivative D-eritadenine [(2*R*,3*R*)-4-adenin-9-yl-2,3-dihydroxybutanoic acid] inactivated the enzyme irreversibly [29, 30]. Meanwhile, antiviral studies had indicated that the activity spectrum of D-eritadenine, a hypocholesterolemic substance originally isolated from the edible Japanese mushroom shiitake (*Lentinus edodes*) [31], was remarkably similar to that of (*S*)-DHPA [32, 33].

In 1982 Montgomery *et al.* [34] reported that carbocyclic 3-deazaadenosine (C-c^3Ado) was a potent inhibitor of SAH hydrolase (from hamster liver), and, again, the antiviral activity spectrum of C-c^3Ado was found to correspond closely to that of (*S*)-DHPA [33, 35]. Subsequent studies revealed that (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid [(*RS*)-AHPA], akin to its butanoic acid counterpart D-eritadenine, effected an irreversible inactivation of SAH hydrolase [36], and, once again, (*RS*)-AHPA was found to exhibit an antiviral activity spectrum similar to that of (*S*)-DHPA [37]. In the antiviral experiments, alkyl esters of (*RS*)-AHPA had to be used so as to ensure their uptake by the cells. It is postulated that the alkyl esters of (*RS*)-AHPA are, as such, taken up by the cells and, once inside the cells, hydrolyzed to release the parent compound, (*RS*)-AHPA.

In 1980, Borchardt pointed out the importance of AdoMet-dependent methyltransferases in the methylation, i.e. 5'-cap formation, and hence maturation of viral mRNA, i.e. vaccinia viral mRNA [38], and, following this lead, Borchardt and his coworkers [39] ascertained that neplanocin A, a cyclopentenyl derivative of adenine, which was originally isolated from the culture broth of the actinomycete *Ampullariella regularis* [40, 41], was a potent inhibitor of

both SAH hydrolase and vaccinia virus multiplication. They further postulated that by inhibiting SAH hydrolase neplanocin A would interfere with AdoMet-dependent methylation reactions which are essential in the maturation of viral mRNA and production of new virus particles [39]. In fact, the antiviral activity spectrum of neplanocin A is not limited to vaccinia virus but extends to the same viruses as those found susceptible to the other SAH hydrolase inhibitors, viz. (*S*)-DHPA, C-c^{3}Ado and (*RS*)-AHPA [42]; and for these four compounds a close correlation was found between their antiviral potency (against vesicular stomatitis virus) and their inhibitory effect (K_i/K_m) on SAH hydrolase [23].

Antiviral activity spectrum

The antiviral activity spectrum of (*S*)-DHPA and its congeners [(*RS*)-AHPA, C-c^3-Ado, neplanocin A] is unique in that it includes: (i) *some DNA viruses*, such as vaccinia virus and African swine fever virus, whereas other DNA viruses [herpetoviridae: HSV, VZV, CMV, EBV] are much less sensitive or insensitive to the compounds; (ii) *(−)RNA viruses*, such as the rhabdo (rabies, vesicular stomatitis and infectious hematopoietic necrosis virus) and paramyxo (parainfluenza, measles) viruses, whereas (+)RNA viruses [picornaviridae: entero (polio, Coxsackie, Echo) and rhinoviruses; togaviridae: Sindbis, Semliki forest, tick-borne encephalitis virus], but not the plant (+)RNA viruses (i.e. potex-, poty- and tymovirus), are virtually resistant to the compounds; and (iii) *double-stranded (±)RNA viruses* [reoviridae: reo- and rotavirus, and infectious pancreatic necrosis virus of fish] (Table 1) [23–25, 33, 35, 37, 42–49; and unpublished data from B. Lerch (1980), T. Kimura (1986) and G. De Fazio (1986), cited with permission]. The antiviral activity spectrum of the SAH hydrolase inhibitors has been established empirically. It is not immediately evident why some viruses, i.e. vesicular stomatitis virus, are much more sensitive to SAH hydrolase inhibitors than others, i.e. herpes simplex virus. Presumably, these differential susceptibilities are related to differences in methylation requirements. It should be noted that the transformation of chick embryo fibroblasts by the (+)RNA Rous sarcoma retrovirus is also inhibited by (*S*)DHPA [50], but this inhibition may be atrributed to a direct inhibitory effect of (*S*)-DHPA on the viral protein kinase p60^{src} activity.

Thus, the activity spectrum of the SAH hydrolase inhibitors (*S*)-DHPA, C-c^{3}Ado, (*RS*)-AHPA and neplanocin A is specifically directed towards pox, (−)RNA and (±)RNA viruses. They are inhibitory to these viruses at concentrations that are significantly below the cytotoxic concentrations. In particular, the activity against rabies- and rotavirus is of great medical interest and deserves further examination. As already emphasized above, the SAH hydrolase inhibitors fall into two classes: acyclic adenosine analogues [(*S*)-DHPA, (*RS*)-AHPA] and carbocyclic adenosine analogues (C-c^3-Ado, neplanocin A). Their activity spectrum is fundamentally different from that of other related nucleoside analogues: (i) *acyclic guanosine analogues* (ACV, DHPG), which are specifically effective against those viruses (i.e. HSV) that encode for a virus-specific deoxythymidine kinase which ensures the phosphorylation of the compounds within the virus-infected cell [3]; (ii) *acyclic guanylate and adenylate analogues*, such as 2′-nor-cGMP [9-[(2-hydroxy-1,3,2-dioxaphosphorinan-5-yl)oxymethyl]guanine P-oxide] [51] and (*S*)-HPMPA [(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine] [52], which do not require phosphorylation by a specific virus-encoded deoxythymidine kinase and are effective against a broad variety of DNA viruses [papova viridae (papilloma, SV40), herpetoviridae (HSV, VZV, CMV, EBV), adenoviridae, poxviridae (vaccinia virus) and iridoviridae (African swine fever virus)] but not RNA viruses (except for retroviruses); (iii) *carbocyclic cytidine analogues*, such as the cyclopentyl derivative of cytosine (carbodine) [53] and the cyclopentenyl derivative of cytosine [54], which are, like the SAH hydrolase inhibitors, active against various RNA and DNA viruses, but, unlike the SAH hydrolase inhibitors, inhibitory to (+)RNA (i.e. picorna and toga) viruses and presumably targeted at CTP synthetase [E. De Clercq, unpublished data (1986)]; and (iv) *tubercidin (7-deazaadenosine) analogues* such as sangivamycin, toyocamycin and tuber-

Table 1. Viruses that have proven particularly sensitive to inhibition by SAH hydrolase inhibitors [(*S*)-DHPA, (*RS*)-AHPA, C-c^{3}Ado, neplanocin A]

Poxviridae	:	Vaccinia virus
Iridioviridae (?)	:	African swine fever virus
Paramyxoviridae	:	Parainfluenza virus
		Measles virus
Rhabdoviridae	:	Vesicular stomatitis virus
		Rabies virus
		Infectious hematopoietic necrosis virus (fish)
Reoviridae	:	Reovirus
		Rotavirus
		Infectious pancreatic necrosis virus (fish)
Plant viruses		
Potexvirus	:	Potato virus X
Potyvirus	:	Unidentified potyvirus isolated from *Solanum palinacanthum*
Tymovirus	:	Eggplant mosaic virus
		Belladonna mottle virus
Tobamo	:	Tobacco mosaic virus

Table 2. Inhibitory constants of selected adenosine analogues for SAH hydrolase from different sources

Compound	K_i (nM)	Source	References
(*S*)-DHPA	3500	Rat liver	[28]
	1400	Beef liver	[23]
	900	Murine L1210	[29]
D-Eritadenine	3	Murine L1210	[29]
(*RS*)-AHPA	73	Beef liver	[23]
	40–120	Murine L1210	[29]
C-Ado	5	Beef liver	[22]
C-c^{3}Ado	4	Beef liver	[58]
	13	Beef liver	[23]
	1	Hamster liver	[34]
Neplanocin A	8.4	Beef liver	[39]
	2	Beef liver	[23]
3-Deazaneplanocin	0.05	Hamster liver	[61]

cidin itself, which are also active against (+)RNA viruses [55], quite cytotoxic, and probably targeted at both viral and cellular RNA synthesis. Also, aristeromycin (C-Ado) should be discriminated from the other carbocyclic adenosine analogues (C-c^{3}Ado, neplanocin A), as it is active against a broader array of viruses (including HSV, polio, Coxsackie) than those affected by the "genuine" SAH hydrolase inhibitors (Table 1), and, moreover, C-Ado is, like tubercidin, antivirally active at concentrations which are equal to, or only slightly lower than, the cytotoxic concentrations [33, 56].

Inhibition of SAH hydrolase

Various adenosine analogues, including (*S*)-DHPA [23, 28, 29] and other neutral open-chain analogues [57], D-eritadenine [29, 30], (*RS*)-AHPA [23, 29] and other acid open-chain analogues [36], C-Ado [22], C-c^{3}Ado [23, 34, 58], neplanocin A [23, 39], Ara-A [21] and 2′,3′-dialdehyde derivatives of both Ado [59] and C-Ado [60] have been reported as inhibitors of SAH hydrolase (isolated from either rat, beef or hamster liver, or murine leukemia cells). K_i values for some selected adenosine analogues are presented in Table 2. As these data originate from different laboratories, they do not necessarily permit ranking of the compounds according to relative potency. As the data stand, 3-deazaneplanocin or the cyclopentenyl derivative of 3-deazaadenine [61] could be considered as by far the most potent inhibitor of SAH hydrolase. The inhibitory effects of the adenosine analogues on SAH hydrolase in intact cell systems have been followed up only with a few compounds, i.e. neplanocin A [39], (*S*)-DHPA [62] and Ara-A [63, 64]. Ara-A has even been investigated in patients (with chronic hepatitis B virus infection), where it was found to suppress SAH hydrolase activity in blood mononuclear cells and erythrocytes [65].

Several studies [39, 60] have pointed to a close correlation between the inhibitory effects of the compounds on SAH hydrolase and their antiviral activity. Particularly revealing in this regard were our own studies with the four "genuine" SAH hydrolase inhibitors, (*S*)-DHPA, (*RS*)-AHPA, C-c^{3}Ado and neplanocin A (Fig. 3). The antiviral potencies of these compounds (against five viruses: vesicular stomatitis, vaccinia, measles, reo and rota) closely paralleled their inhibitory effects on SAH hydrolase (from beef liver) [23, 66]. These findings provide compelling evidence for the role of SAH hydrolase, and the therewith associated transmethylation reactions, in the virus replicative cycle, and, conversely, our data suggest that the inhibition of SAH hydrolase may be causally related to the inhibition of virus replication. To further establish the causal nature of this relationship, it would now seem mandatory to directly examine SAH hydrolase activity, i.e. by monitoring SAM/SAH levels, in virus-infected cells exposed to the SAH hydrolase inhibitors.

Role of SAH hydrolase in transmethylation reactions

AdoMet (SAM) serves as the methyl donor for numerous methyltransferase reactions, including those that are involved in the methylation of the 5′-cap of viral mRNA [38]. SAM itself is synthesized from ATP and methionine (Fig. 4) and, upon transfer of its methyl group to the acceptor substrate, SAH is generated which is a product inhibitor of the methyltransferase reaction. To avoid this inhibitory effect and to allow the methyltransferases to proceed with their action, SAH has to be removed by SAH hydrolase. This enzyme catalyzes, as has already been mentioned above, the reversible hydrolysis of SAH to homocysteine and adenosine. Adenosine is, in turn, a product inhibitor of the SAH hydrolase reaction, which means that it has to be removed if SAH hydrolase were to proceed with its catalytic function. This can be achieved by several pathways including deamination to inosine by the ubiquitous adenosine deaminase (Fig. 4). Thus, effective methylation requires the concerted action of at least three enzymes: methyltransferase, SAH hydrolase and adenosine deaminase. Based on this premise, one may envisage at least three types of methylation inhibitors: (i) SAH analogues, such as *S*-aristeromycinyl-L-homocysteine, which interfere directly with the methyltransferase [67, 68]; (ii) the aforementioned acyclic or carbocyclic adenosine analogues (Table 2) which inhibit SAH hydrolase; and (iii) coformycin, EHNA [*erythro*-9-(2-hydroxy-3-nonyl)adenine] and other inhibitors of adenosine

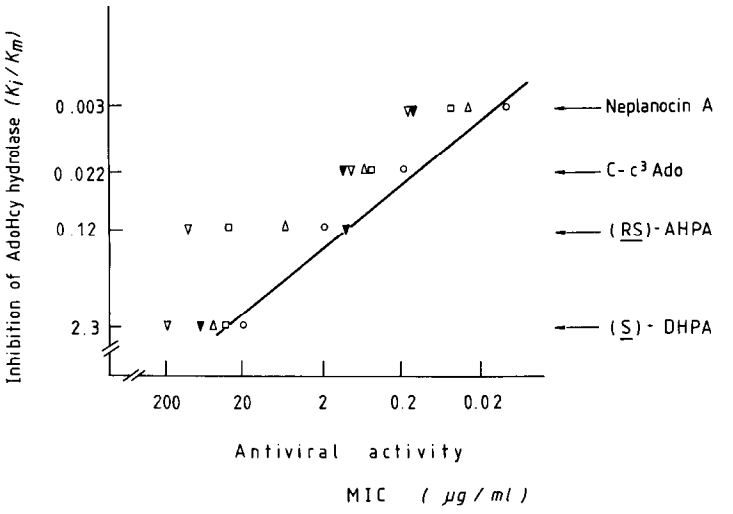

Fig. 3. Correlation between the antiviral activities of the four adenosine analogues and their inhibitory effects on SAH hydrolase. The inhibitory effect on SAH hydrolase is expressed as the K_i/K_m for bovine liver SAH hydrolase activity (measured in the direction of SAH synthesis). The antiviral activity is expressed as the minimum inhibitory concentration (MIC) required to inhibit by 50% the cytopathogenicity of vesicular stomatitis virus (○) [in primary rabbit kidney (PRK) cells], vaccinia virus (△) in PRK cells, measles virus (□) [in African green monkey kidney (Vero) cells], reovirus type I (▽) in Vero cells, or to reduce by 90% the yield of human rotavirus (▼) in embryonic rhesus monkey kidney (MA 104) cells. The linear regression line is shown for K_i/K_m as a function of MIC for vesicular stomatitis virus (○) ($r = 0.986$) [23]. Reprinted from Ref. 66.

deaminase [69, 70]. It is noteworthy that these three classes of methylation inhibitors are by themselves antivirally active, although available data suggest that the SAH hydrolase inhibitors [class (ii)] surpass the others [classes (i) and (iii)] in potency and specificity.

How then could an inhibition of SAH hydrolase impart any specificity towards virus replication? SAH hydrolase is functionally linked to all SAM-dependent methyltransferases, and there is no evidence for a virus-specified SAH hydrolase that would be more susceptible to inhibitors than cellular SAH hydrolase. This leaves us with two alternative explanations for the selective antiviral activity of SAH hydrolase inhibitors: (1) quantitative differences in the methylation requirements between virus-infected and uninfected cells, i.e. a greater demand on methyl groups in the virus-infected cells, which would make their methyltransfer reactions more vulnerable to perturbations in the SAH levels, and (2) qualitative

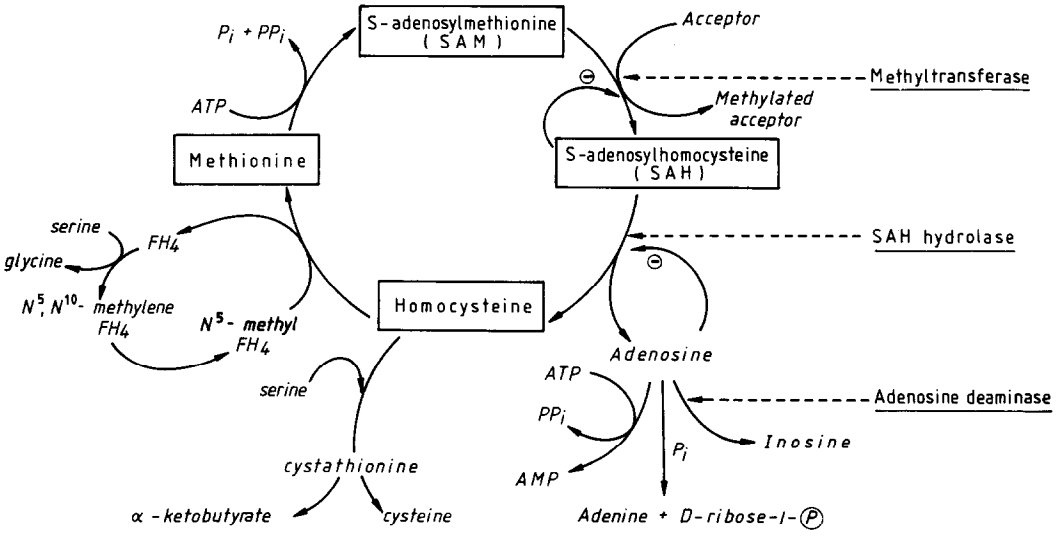

Fig. 4. Key intermediates in the SAM/SAH cycle. For effective methylation (acceptor → methylated acceptor), concerted action of three enzymes (methyltransferase, SAH hydrolase and adenosine deaminase) is required.

differences between the methyltransferases of virus-infected and uninfected cells. The first hypothesis can be readily approached experimentally by measuring the SAM/SAH levels in virus-infected and uninfected cells exposed to the SAH hydrolase inhibitors. The second hypothesis is borne out by available data in the literature: i.e. reovirus [71], vaccinia virus [72], vesicular stomatitis virus [73] and Newcastle disease virus (a paramyxovirus related to parainfluenza virus) [74], thus all viruses which fall within the activity spectrum of SAH hydrolase inhibitors (Table 1), have been shown to contain their own methyltransferases. Vaccinia virus [75–79] and vesicular stomatitis virus [80–82] contain two distinct methyltransferases, a (guanine-7)methyltransferase and a (nucleoside-2′)methyltransferase. Both methyltransferases are encoded by the virus and involved in the 5′-capping of viral mRNA. As SAH hydrolase is closely associated with methyltransferase, SAH hydrolase inhibitors may confer their antiviral specificity via inhibition of the virus-specified methyltransferases involved in the capping process.

Metabolic disposition

The SAH hydrolase inhibitors exert a multitude of biological effects which may or may not be linked to their interaction with methyltransferases. For example, (*S*)-DHPA has proven to be embryotoxic for chicks [83], to inhibit spermatogenesis in mice [84], to induce sterility in insects [85] (even if administered via the host plant [86]), and to suppress the formation of roots in plants (*Vicia faba*) [87]. At the cellular level, SAH hydrolase inhibitors, i.e. C-c^{3}Ado and neplanocin A, have generally been accredited with cytostatic and cytotoxic properties [88–90]. This raises the question as to whether the adenosine analogues themselves or metabolites thereof are responsible for their multiple biological effects. To interact with SAH hydrolase, adenosine analogues do not need any metabolic conversion, as, akin to adenosine itself, they can, as such, inhibit the SAH hydrolase reaction (Fig. 4).

From Fig. 4 it is also clear that, like adenosine, the adenosine analogues may be processed (i) via phosphorylation to the corresponding 5′-mono-, 5′-di- and 5′-triphosphates (and the latter may, if acting as substrates for the ATP: L-methionine *S*-adenosyltransferase, even be converted to the corresponding SAM analogues), (ii) via deamination to the corresponding inosine analogues, and (iii) via the synthetic route of SAH hydrolase to the corresponding SAH analogues. (*S*)-DHPA and (*RS*)-AHPA would not undergo any of these reactions, and, similarly, C-c^{3}Ado is neither phosphorylated nor deaminated. Nor is it an efficient substrate for SAH hydrolase [34]. Other adenosine analogues are readily metabolized, however: i.e. C-Ado is converted to its 5′-triphosphate [91] and tubercidin has been shown to undergo metabolism both to *S*-tubercidinyl-L-methionine and *S*-tubercidinyl-L-homocysteine [92]. Neplanocin A is metabolized efficiently to neplanocin 5′-triphosphate and *S*-neplanocylmethionine [89, 91, 93–95]. Yet, the finding that neplanocin A is converted to a number of metabolites does not necessarily imply that these metabolites, rather than neplanocin A itself, account for the biological (viz. antiviral) effects of the compound. As clearly pointed out by Keller *et al.* [95], the metabolic conversion of neplanocin A to *S*-neplanocylmethionine may have little, if any, consequence on transmethylation reactions.

To further delineate the role of SAH hydrolase as target enzyme for the antiviral activity of neplanocin A and its congeners, we have evaluated recently the antiviral activity of various adenosine analogues in a tandem system of adenosine kinase-deficient (AK^-) and adenosine kinase-positive (AK^+) cell lines [96]. It was reasoned that those adenosine analogues that require phosphorylation and are targeted at sites other than SAH hydrolase would be much more effective in AK^+ cells than in AK^- cells. Conversely, the "genuine" SAH hydrolase inhibitors among the adenosine analogues would be expected not to be much less active in AK^- than in AK^+ cells (as they do not require phosphorylation). In fact, those adenosine analogues (i.e. tubercidin, toyocamycin, sangivamycin, xyloadenosine) that we assumed *not* to be targeted at SAH hydrolase were found to inhibit virus replication in AK^+ cells at a concentration 10^3- to 10^4-fold lower than the concentration at which they inhibited virus replication in AK cells. In contrast, (*S*)-DHPA, (*RS*)-AHPA, C-c^{3}Ado and neplanocin A, the "genuine" SAH hydrolase inhibitors, were equally or only slightly (10-fold) less active in AK^- than in AK^+ cells [96]. These findings are consistent with their action at the SAH hydrolase level.

In the context of the "genuine" SAH hydrolase inhibitors, Ara-A has a place apart. There is no question that Ara-A is an efficient inhibitor of SAH hydrolase [21]; it even causes "suicide" inactivation of the enzyme [97]. However, Ara-A is also metabolized to Ara-Hx (9-β-D-arabinofuranosylhypoxanthine) by adenosine deaminase, and onto Ara-AMP, Ara-ADP and Ara-ATP by cellular nucleoside and nucleotide kinases. Ara-ATP can act as both substrate and inhibitor of the DNA polymerase and also interacts with ribonucleotide reductase. Considering the variety of metabolic conversions Ara-A is undergoing [33], it is at first glance difficult to sort out which metabolite is actually responsible for the antiviral activity of the compound. With the "genuine" SAH hydrolase inhibitors Ara-A shares an activity against poxviridae (vaccinia virus) and rhabdoviridae (rabies virus and vesicular stomatitis virus). With the viral thymidine kinase-dependent drugs (ACV, BVDU, DHPG) Ara-A shares an activity against the herpetoviridae (HSV and VZV). It may be postulated, therefore, that the activity of Ara-A against rhabdoviruses is due to an inhibition of SAH hydrolase, that its activity against herpesviruses is due to an inhibition of DNA polymerase, and that its activity against poxviruses is due to an inhibition of both SAH hydrolase and DNA polymerase.

Leads for further research

Although it has become increasingly clear that SAH hydrolase inhibitors are endowed with antiviral properties, it is not obvious yet how inhibition of the SAH hydrolase may impart antiviral specificity. It

would thus appear necessary to directly monitor SAH hydrolase activity in the cells after they have been exposed to SAH hydrolase inhibitors, and to dissect the effects of SAH hydrolase inhibitors on the normal cellular methylation processes from those associated with the virus replicative cycle. This would allow us to conceive how a specific antiviral activity can be achieved without concomitant cytotoxicity.

Equally important would seem the design of new adenosine analogues with modifications in either the adenine moiety or sugar moiety or both, in attempts to obtain more potent and/or more selective inhibitors of SAH hydrolase. The carbocyclic analogues of tubercidin and 6-methyltubercidin [98, 99] are examples of such double-modified adenosine analogues, but neither compound exhibited much activity as either SAH inhibitor or antiviral agent. On the contrary, 3-deazaneplanocin (the cyclopentenyl derivative of 3-deazaadenine) proved exquisitely potent as an inhibitor of SAH hydrolase [61], and this observation will undoubtedly prompt the synthesis of other adenosine analogues combining the cyclopentenyl moiety of neplanocin A with modifications in the purine ring.

To further assess the role of SAH hydrolase inhibition, and the concomitant accumulation of SAH, in the antiviral activity of SAH hydrolase inhibitors, we attempted to reverse the antiviral action of the SAH hydrolase inhibitors by adding L-homocysteine to the cell culture medium. It was rationalized that, through the exogenous supply of L-homocysteine, L-methionine and, hence SAM, may be generated (Fig. 4), and that, by increasing the intracellular levels of SAM, the inhibitory effect of SAH on the SAM-dependent methyltransferases may be overcome. Surprisingly, L-homocysteine was found not to antagonize but to potentiate the antiviral activity of the SAH hydrolase inhibitors [E. De Clercq, unpublished data (1986)]. For example, in the presence of 10^{-3} M L-homocysteine, the minimum inhibitory concentration of C-c^{3}Ado for vaccinia and vesicular stomatitis virus in primary rabbit kidney (PRK) cell cultures was reduced from 2 μg/ml to 0.2 and 0.1 μg/ml respectively. Similarly, L-homocysteine potentiated the antiviral activity of the other SAH hydrolase inhibitors, c^{3}Ado, (*S*)-DHPA, (*RS*)-AHPA and neplanocin A. The antiviral activity of those compounds that are not supposed to act via SAH hydrolase inhibition, viz. tubercidin, acyclovir and ribavirin, was not potentiated by L-homocysteine, and, furthermore, L-homocysteine potentiated the activity of the SAH hydrolase inhibitors only against those viruses that are intrinsically susceptible to their antiviral action (Table 1). Thus, for those viruses (such as HSV) that do not belong to the activity spectrum of the SAH hydrolase inhibitors, no increased activity was noted upon addition of L-homocysteine. The exact mechanism by which L-homocysteine increases the antiviral activity of the SAH hydrolase inhibitors remains to be elucidated. Presumably, L-homocysteine helps the SAH hydrolase inhibitors in generating higher intracellular levels of SAH, hence resulting in a greater inhibitory effect on SAM-dependent transmethylation reactions. Our observations made with L-homocysteine also indicate that the antiviral activity of the SAH hydrolase inhibitors can be modulated, i.e. potentiated, if combined with the proper regulatory substances; this should form the basis for more extensive combination studies.

Finally, the possibility should be envisaged that SAH hydrolase inhibitors may be effective against those viruses that are normally not sensitive to the antiviral action of the SAH hydrolase inhibitors, provided these viruses have undergone some mutation that makes their replication more dependent on methylation reactions. Thus, thymidine kinase-deficient (TK^-) HSV mutants that are resistant to the viral TK-dependent drugs (ACV, DHPG, BVDU) appear to be more sensitive to SAH hydrolase inhibitors such as neplanocin A than are wild-type HSV strains [E. De Clercq, unpublished data (1986)]. They are also much more sensitive to other antiviral agents, i.e. the cyclopentyl and cyclopentenyl derivatives of cytosine which are assumed to interact with CTP synthetase. This implies that when viruses become deficient in one or another particular pathway, i.e. the thymidine salvage pathway for herpesviruses, they may also become more dependent on other metabolic pathways. Consequently, they may also become vulnerable to a number of compounds which interfere with these metabolic pathways and which would not inhibit the parent wild-type viruses. This opens new interesting perspectives from a therapeutic viewpoint.

Conclusion

S-Adenosyl-L-homocysteine (SAH) hydrolase has been recognized as an important target for the antiviral activity of acyclic and carbocyclic analogues of adenosine, i.e. (*S*)-9-(2,3-dihydroxypropyl)adenine, (*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid, D-eritadenine, carbocyclic 3-deazaadenosine, neplanocin A, cyclopentenyl 3-deazaadenine, and carbocyclic 2′,3′-adenosine dialdehydes. SAH being a product inhibitor of *S*-adenosyl-L-methionine (SAM)-dependent methyltransferases, including those that are involved in the maturation (i.e. 5′-capping) of viral mRNAs, adenosine analogues, which inhibit SAH hydrolase and thereby increase the intracellular levels of SAH, may be expected to exert their antiviral effects through interference with methylation processes. Their antiviral activity spectrum is unique in that it encompasses poxviruses (vaccinia), negative-stranded RNA viruses (paramyxo: parainfluenza, measles; rhabdo: rabies, vesicular stomatitis), and double-stranded RNA viruses (reo, rota), whereas herpesviruses and positive-stranded RNA viruses (picorna: entero, rhino; toga) are virtually resistant to the compounds. Although there is a close correlation between the inhibitory effects of the compounds on SAH hydrolase and their antiviral potency, the basis for their antiviral specificity remains to be elucidated. Other interesting leads that should be pursued further include the synthesis of more potent and/or specific inhibitors of SAH hydrolase, the therapeutic potential of SAH hydrolase inhibitors in combination with L-homocysteine or other substances, and the sensitivity to SAH hydrolase inhibitors of virus mutant strains that have become resistant to other antiviral drugs.

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