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Inhibitory effect of selected antiviral compounds on arenavirus replication in vitro

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Summary

Several compounds, belonging to different classes of nucleoside analogues and sulfated polysaccharides, were evaluated for their inhibitory effects on the replication of the arenaviruses Junin and Tacaribe in VERO cells. S-Adenosylhomocysteine (AdoHcy) hydrolase inhibitors [i.e. adenosine dialdehyde, carbocyclic 3-deazaadenosine (C-c^{3}Ado), neplanocin A, 3-deazaneplanocin A, 9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA], (*RS*)-3-adenin-9-yl-hydroxypropanoic acid isobutyl ester [(*RS*)-AHPA], the 2′,3′-dihydroxycyclopentenyl derivatives of adenine (DHCA) and 3-deazaadenine (DHCDA)] inhibited arenavirus replication within the concentration range of 1–10 μg/ml, while not being toxic for cell morphology or cellular DNA synthesis at a concentration of 100–400 μg/ml. Based on the ratio of the concentrations required to inhibit cell proliferation and virus replication, only (*S*)-DHPA, DHCA, C-c^{3}Ado and adenosine dialdehyde could be considered as truly selective inhibitors. Tubercidin, cyclopentenyl cytosine, pyrazofurin and ribavirin also inhibited viral cytopathogenicity at concentrations that were well below the cytotoxic threshold. Carbodine (cyclopentyl cytosine) also proved to be a potent inhibitor of arenavirus replication, but it was not as selective as cyclopentenyl cytosine. Very potent and selective inhibitors were the sulfated polysaccharides dextran sulfate, λ-carrageenan, fucoidan, heparin and pentosan polysulfate: they inhibited virus replication at a concentration of 0.1–2.8 μg/ml, whereas the compounds were not inhibitory to cell growth even at a concentration of 200 μg/ml.

Arenavirus; Nucleoside analogue; Sulfated polysaccharide

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Introduction

The Arenaviridae belong to a distinct family of enveloped viruses of which the genetic information is encoded by two segments of single-stranded (-)RNA (Rawls and Leung, 1979). In terms of their geographical distribution arenaviruses are classified into two groups, the 'Old World' arenaviruses (Lassa, lymphocytic choriomeningitis, Mopeia, Mobala) and the 'New World' arenaviruses (Tacaribe, Pichinde, Tamiami, Parana, Machupo, Latino, Junin, etc.). Four members of this family are known to cause significant disease in humans, namely Lassa fever, aseptic lymphocytic choriomeningitis, Bolivian hemorrhagic fever (Machupo virus) and Argentinian hemorrhagic fever (Junin virus) (Johnson, 1985). Tacaribe virus (TACV) is antigenically closely related to Junin virus (JV) but is not pathogenic for humans (Weissenbacher et al., 1975).

The treatment of choice for Argentinian hemorrhagic fever consists of the administration of immune plasma with defined neutralizing antibody titer (Enria et al., 1984; Maiztegui et al., 1979). However, this treatment is only effective when given within the first 8 days after onset of symptoms. It is inefficacious in advanced cases of disease, and, furthermore, approximately 10% of immune plasma-treated patients develop late neurological complications (Maiztegui et al., 1979). In the case of Lassa fever, convalescent plasma fails to improve the recovery from the disease (McCormic et al., 1986).

Ribavirin is the only drug that is known to be of any benefit in the treatment of patients with arenavirus infection. This drug reduces viremia and increases the survival rate of monkeys infected with Lassa virus (Jahrling et al., 1980) or Machupo virus (Stephen et al., 1980) and of guinea pigs and hamsters infected with Pichinde virus (Stephen et al., 1980) or Junin virus (Kenyon et al., 1986). Studies performed in patients with Lassa fever or Argentinian hemorrhagic fever have indicated that early treatment with ribavirin after the onset of symptoms might be effective (McCormick et al., 1986; Enria et al., 1987). However, the drug is not efficacious in patients with advanced disease, and as one of the secondary reactions of the drug ribavirin-treated patients may develop anemia (Canonico et al., 1984; Shulman, 1984). Although many efforts have been made to obtain effective vaccines against pathogenic arenaviruses, they are not yet available. A vaccine trial (double blind, placebo controlled) is currently in progress, but the code has not been broken.

It would thus seem imperative to search for new antiviral compounds that may be as effective, if not more effective, than ribavirin against arenaviruses. We have now investigated a number of antiviral compounds, belonging to the widely different classes of nucleoside analogues and sulfated polysaccharides, for their inhibitory effects on the replication of JV and TACV.

Materials and Methods

Viruses and cells

Green monkey kidney (Vero) cells (ATCC CCL81) were grown in minimum essential medium (MEM; Gibco, Paisley, Scotland), supplemented with 10% inactivated fetal calf serum (FCS; Gibco), 1% L-glutamine and 0.3% sodium bicarbonate (Gibco).

The attenuated strain of Junin virus (JV), XJ Cl_3, and Tacaribe virus (TACV), TRVL 11573 strain, were propagated in suckling mouse brain and virus stocks were prepared as 10% brain homogenates. Virus titers were determined by the 50% tissue culture-infective dose ($TCID_{50}$) method in the same cell line.

Compounds

The test compounds and their sources were as follows: tubercidin [4-aminopyrrolo-(2,3-di)pyrimidine-β-D-ribofuranoside], Upjohn Co. (Kalamazoo, Mich.); xylotubercidin, M.J. Robins (Chemistry Department, Brigham Young University, Provo, Utah); neplanocin A and neplanocin C, Toyo Jozo Co. (Tagata-Gun, Shizuoka-Ken, Japan); C-c^3Ado (carbocyclic 3-deazaadenosine), J.A. Montgomery (Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala); (*RS*)-AHPA [(*RS*)-3-adenin-9-yl-hydroxypropanoic acid isobutyl ester] and (*S*)-DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine], A. Holy (Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague, Czechoslovakia); 3-deazaneplanocin A, V.E. Marquez (Laboratory of Pharmacology and Experimental Therapeutics, National Cancer Institute, Bethesda, Maryland); DHCA [9-(*trans*-2′,*trans*-3′-dihydroxycyclopent-4′-enyl)adenine] and DHCDA [9-(*trans*-2′,*trans*-3′-dihydroxycyclopent-4′-enyl)-3-deazaadenine], R.T. Borchardt (Department of Biochemistry and Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas); adenosine dialdehyde, Sigma Chemical Co. (St. Louis, MO); carbodine (carbocyclic cytidine, cyclopentyl cytosine), J.A. Montgomery; cyclopentenyl cytosine, Toyo Jozo Co.; ribavirin [1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide], ICN Nutritional Biochemicals (Cleveland, Ohio); pyrazofurin [3-(β-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide], Calbiochem Behring Corporation (Lucerne, Switzerland); dextran sulfate (MW 10,000), Pfeifer & Langen (Dormagen, F.R.G.); heparin, Leo Pharmaceutical Products Ltd. (Ballerup, Denmark); and pentosan polysulfate, fucoidan and λ-carrageenan, Sigma Chemical Co.

Inhibition of virus-induced cytopathogenicity

Confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$, that is 100 times the virus dose needed to infect 50% of the cells. After 1 h of virus adsorption to the cells, residual virus was removed and replaced by cell culture medium supplemented with 2% FCS, 1% L-glutamine and 0.3% sodium bicarbonate containing various dilutions (in duplicate) of the test compounds. In

the tests with the sulfated polysaccharides, the drugs were not added at 1 h, but immediately after virus infection. Viral cytopathogenicity was recorded at 3 to 4 days post infection (p.i.). The antiviral activity of the compounds is expressed as the concentration (or dose) required to inhibit viral cytopathogenicity by 50% (ID_{50}).

Cytotoxicity

Cytotoxicity measurements were based on two parameters: (i) alteration of normal cell morphology and (ii) inhibition of host cell DNA synthesis. To evaluate cell morphology, confluent cell cultures which had not been infected but were treated with various concentrations of the test compounds were incubated in parallel with the virus-infected cell cultures and examined microscopically at the same time as viral cytopathogenicity was recorded for the virus-infected cell cultures. A disruption of the cell monolayer, e.g. rounding up or detachment of the cells, was considered as evidence for cytotoxicity.

To determine host cell DNA synthesis, the cells were seeded in 96-well microtest plates at a density of 20,000 cells/well. After 24 h incubation in a humidified, CO_2-controlled atmosphere at 37°C, the cells were refed with fresh MEM containing 2% FCS, 1% L-glutamine, 0.3% sodium bicarbonate and the appropriate concentration (in duplicate) of the test compounds. After 24 h incubation at 37°C, the cells were pulse-labeled for 24 h with [*methyl*-^{3}H]deoxythymidine (specific radioactivity: 25 Ci/mmol) at 10 μCi/well. After the pulse-label period, the cells were washed with phosphate-buffered saline (PBS) and fixed with methanol. Then, the microplates were washed three times with 10% trichloroacetic acid (TCA) and once with methanol; and the TCA pellet was solubilized with 1N NaOH for 2 h at room temperature. The solubilized samples were then transferred to scintillation vials, and, upon addition of scintillation fluid (Instafluor and Soluene, Packard Instruments, Groningen, The Netherlands) analyzed for radioactivity. Inhibition of the radioactivity by 50% was taken as the end point for cytotoxicity (CD_{50}). Selectivity indexes were calculated as the ratio of CD_{50} for cell DNA synthesis to ID_{50} for viral cytopathogenicity.

Cytostatic activity

The cells were seeded at a rate of 3×10^3 cells per well in a volume of 0.1 ml into 96-well microtiter plates and allowed to proliferate for 24 h in MEM, containing 20% FCS, 1% L-glutamine and 0.3% sodium bicarbonate. Twenty-four hours later, 0.1 ml MEM (with 2% FCS, 1% L-glutamine, and 0.3% sodium bicarbonate) containing different concentrations (in duplicate) of the test compounds were added to each well. After 3 days of incubation at 37°C in 5% CO_2, the cell number was determined with a Coulter counter. The minimum cytotoxic dose was expressed as the CD_{50}, or concentration (dose) required to reduce cell growth by 50%. Selectivity indexes were calculated as the ratio of CD_{50} for cell growth to ID_{50} for viral cytopathogenicity.

Inhibition of virus multiplication

Confluent VERO cells grown in trays were inoculated with JV or TACV at a virus input of 100 $CCID_{50}$ per well. After 1 h virus adsorption to the cells, residual virus was removed and replaced by MEM with 2% FCS, 1% L-glutamine, and 0.3% sodium bicarbonate, containing different concentrations (0, 1, 10 and 100 μg/ml) of C-c^{3}Ado, ribavirin and carbodine. In the case of dextran sulfate, the drug was added immediately after virus infection. Supernatants from 8 wells per group were harvested at either 1, 24, 48 or 72 h after infection, stored frozen at −80°C, and titrated for virus content ($CCID_{50}$) in VERO cell cultures.

Results

Inhibitory effects of antiviral compounds on cytopathogenicity of JV and TACV

The antiviral compounds were examined for their inhibitory effect on viral cytopathogenicity. The ID_{50} values of the compounds required for inhibition of the cytopathic effects of JV and TACV are shown in Table 1. In these preliminary experiments, toxicity of the compounds for Vero cells was examined by microscopic examination of cell morphology.

The sulfated polysaccharides dextran sulfate, λ-carrageenan, fucoidan, heparin and pentosan polysulfate were active at concentrations of 0.1–2.8 μg/ml and not cytotoxic up to a concentration of 400 μg/ml.

The nucleoside analogues C-c^{3}Ado, (*RS*)-AHPA, (*S*)-DHPA, DHCA, DHCDA, 3-deazaneplanocin A, carbodine, cyclopentenyl cytosine, pyrazofurin and ribavirin were inhibitory to the cytopathogenicity of JV and TACV within the concentration range of 0.025–15 μg/ml, while they were not toxic to the host cells at a concentration of 400 μg/ml (Table 1). Although neplanocin A and adenosine dialdehyde were toxic to Vero cells (at a concentration of 10 and 50 μg/ml, respectively), they inhibited viral cytopathogenicity at a concentration that was well below the cytotoxicity threshold (Table 1). Tubercidin and xylotubercidin inhibited viral cytopathogenicity at a concentration that was just below the cytotoxic threshold, whereas neplanocin C showed no antiviral activity at a non-toxic concentration.

Selectivity of the selected compounds as inhibitors of JV and TACV

The cytotoxicity of the compounds for VERO cells was also determined by measurements of [*methyl*-^{3}H]dThd incorporation into host cell DNA and inhibition of cell growth.

As shown in Table 2, none of the compounds inhibited host cell DNA synthesis at a concentration up to 100 μg/ml, except for neplanocin A and tubercidin (CD_{50}: 0.95 and 3.2 μg/ml, respectively). However, xylotubercidin, DHCDA, (*RS*)-AHPA, C-c^{3}Ado, pyrazofurin and ribavirin inhibited cell growth at concentrations ranging from 5 to 30 μg/ml, while neplanocin A, DHCA and cyclopentenyl cytosine were inhibitory to cell growth at lower concentrations (CD_{50}: 0.42, 0.86 and 0.45

TABLE 1

Antiviral activity of several antiviral compounds against Junin virus (JV) and Tacaribe virus (TACV) in VERO cells

Compound	MTC (μg/ml)	ID_{50}	
		JV	TACV
Adenosine analogues			
Tubercidin	0.5	0.05	0.03
Xylotubercidin	20	3.1	5.0
C-c^{3}Ado	> 400	1.2	2.3
(*RS*)-AHPA isobutyl ester	> 400	9.0	9.0
(*S*)-DHPA	> 400	14.0	15.0
DHCA	> 400	1.1	2.3
DHCDA	> 400	2.2	2.8
Neplanocin A	10	0.3	0.3
Neplanocin C	1	> 1	> 1
Adenosine dialdehyde	50	0.6	0.5
3-Deazaneplanocin A	> 400	1.2	1.5
Cytidine analogues			
Carbodine	> 400	0.2	0.3
Cyclopentenyl cytosine	> 400	0.03	0.03
Guanosine analogues			
Pyrazofurin	> 400	2.0	0.4
Ribavirin	> 400	1.0	1.2
Sulfated polysaccharides			
Dextran sulfate	> 400	0.5	0.4
λ-Carrageenan	> 400	0.3	0.2
Fucoidan	> 400	0.3	0.1
Heparin	> 400	1.8	2.0
Pentosan polysulfate	> 400	1.6	2.8

MTC: minimum toxic concentration causing a microscopically detectable change in morphology of normal uninfected cells treated with the compounds and run in parallel with the virus-infected treated cells.
ID_{50}: minimum inhibitory concentration required to reduce virus-induced cytopathogenicity by 50%.
The data represent average values for 2 or 3 experiments.

μg/ml, respectively). Tubercidin, 3-deazaneplanocin A and carbodine were the most inhibitory to cell growth (CD_{50}: 0.006, 0.085 and 0.035 μg/ml, respectively). Neither (*S*)-DHPA nor the sulfated polysaccharides inhibited cell growth at a concentration up to 200 μg/ml (Table 2).

When selectivity indexes were calculated as the ratio of the 50% inhibitory dose for host cell DNA synthesis to the average ID_{50} for TACV and JV, all compounds, except for neplanocin A, showed selectivity indexes greater than 5 (Table 2). Cyclopentenyl cytosine showed the highest selectivity index (>3,333). The sulfated polysaccharides, pyrazofurin, ribavirin, carbodine, C-c^{3}Ado, DHCDA, DHCA, adenosine dialdehyde, 3-deazaneplanocin A, xylotubercidin and tubercidin also showed marked selectivity indexes.

When selectivity indexes were calculated as the ratio of the 50% inhibitory dose for cell growth to the average ID_{50} for JV and TACV, only xylotubercidin,

TABLE 2

Selectivity indexes of selected compounds as inhibitors of JV and TACV

Compound	ID_{50}[a] (μg/ml)	Minimum cytotoxic concentration CD_{50} (μg/ml)[b]		Selectivity index	
	(A)	dThd (B)	Cell growth (C)	B/A	C/A
Tubercidin	0.04	3.2	0.006	80	0.15
Xylotubercidin	1.75	> 100	27.5	> 57	16
Neplanocin A	0.3	0.95	0.42	3.2	1.4
3-Deazaneplanocin A	1.35	> 100	0.085	> 74	0.06
Adenosine dialdehyde	0.55	> 100	1.65	> 182	3.0
DHCA	1.7	> 100	0.86	> 59	0.5
DHCDA	2.5	> 100	18.75	> 40	7.5
(*S*)-DHPA	14.5	> 100	> 200	> 7	14
(*RS*)-AHPA	9.0	> 100	10	> 11	1.1
C-c^3Ado	1.75	> 100	5.85	> 57	3.3
Carbodine	0.25	> 100	0.035	> 400	0.14
Cyclopentenyl cytosine	0.03	> 100	0.45	> 3,333	15
Pyrazofurin	1.1	> 100	25	> 91	23
Ribavirin	1.1	> 100	19.5	> 91	18
Dextran sulfate	0.45	> 100	> 200	> 222	> 444
λ-Carrageenan	0.25	> 100	> 200	> 400	> 800
Fucoidan	0.2	> 100	> 200	> 500	> 1000
Heparin	1.9	> 100	> 200	> 53	> 105
Pentosan polysulfate	2.2	> 100	> 200	> 45	> 91

[a]Average ID_{50} for JV and TACV.

[b]CD_{50}: minimum cytotoxic dose required to cause 50% inhibition of [*methyl*-^{3}H]deoxythymidine incorporation into host cell DNA (B) or host cell growth (C).

The compounds were added after 1 h of virus adsorption to the cells, except for the sulfated polysaccharides, which were added immediately after virus infection.

DHCDA, (*S*)-DHPA, cyclopentenyl cytosine, pyrazofurin, ribavirin and the sulfated polysaccharides exhibited selectivity indexes higher than 5. Neplanocin A, adenosine dialdehyde, (*RS*)-AHPA and C-c^3Ado showed selectivity indexes ranging from 1 to 3.5, while tubercidin, 3-deazaneplanocin A, DHCA and carbodine showed selectivity indexes lower than 1 (Table 2).

Inhibitory effects of dextran sulfate, carbodine, ribavirin and C-c^3Ado on virus growth

Four compounds, namely dextran sulfate, ribavirin, carbodine and C-c^3Ado, as representatives for the sulfated polysaccharides, guanosine, cytidine and adenosine analogues, respectively, were further examined for their inhibitory effects on the growth of JV and TACV in Vero cells. At concentrations below the cytotoxicity threshold (inhibition of host cell DNA synthesis), the compounds inhibited the growth of JV and TACV in Vero cells, as measured at 24, 48 and 72 h after infection (Fig. 1). At a concentration of 10 μg/ml, C-c^3Ado caused about 2 $\log_{10}$ reduction in the 48 h yield of JV and TACV. At 100 μg/ml, C-c^3Ado achieved a greater than 3 $\log_{10}$ reduction in the 48 h yield of JV and TACV (Fig. 1C).

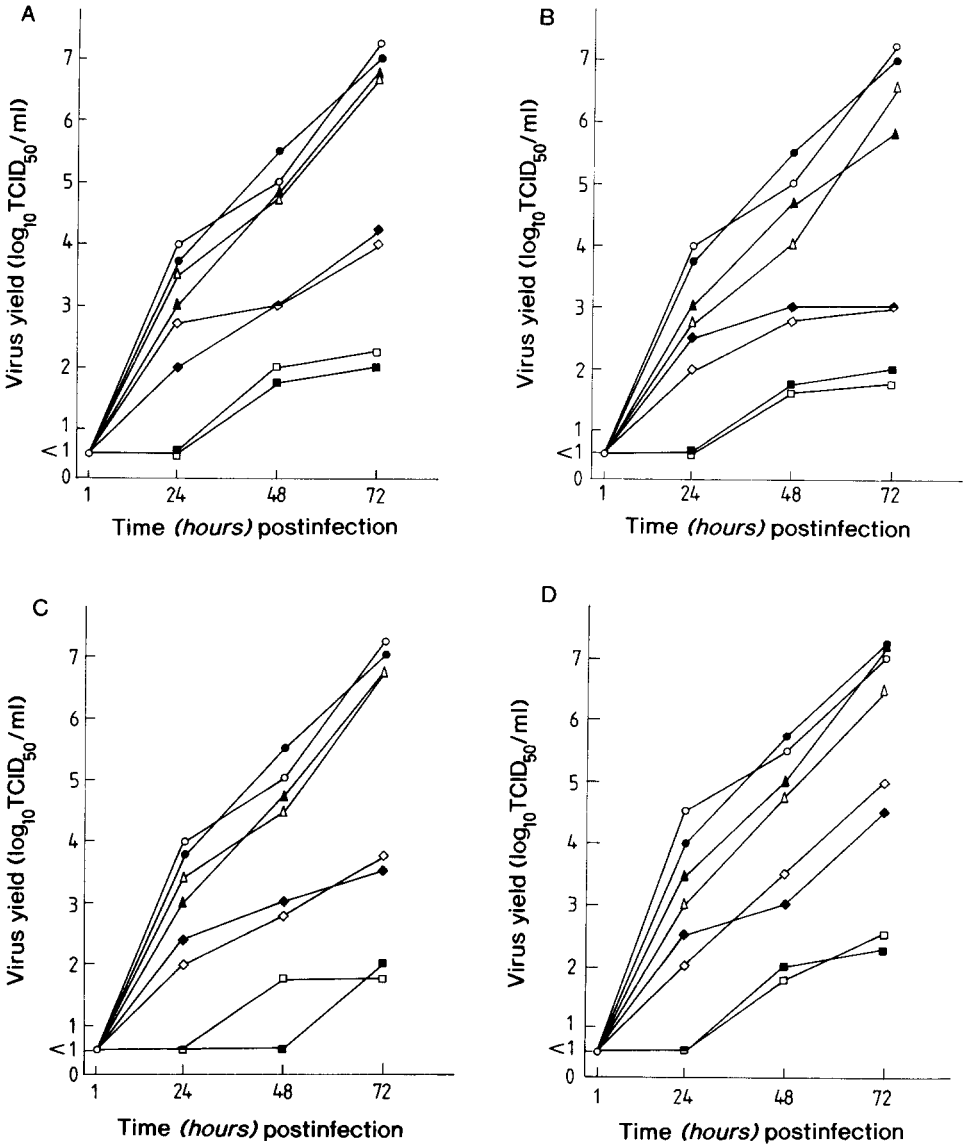

Fig. 1. Effects of ribavirin (panel A), carbodine (panel B), C-c^3Ado (panel C) and dextran sulfate (panel D) on the growth of JV (black symbols) and TACV (white symbols) in VERO cells. •, ○: control (no drug); ▲, △: 1 μg/ml; ◆, ◇: 10 μg/ml; ■, □: 100 μg/ml.

At 72 h after virus infection, the infectious virus titers in the cultures exposed to C-c^3Ado (10 or 100 μg/ml) were reduced by more than 10^3-fold. The results obtained with ribavirin (Fig. 1A), carbodine (Fig. 1B) and dextran sulfate (Fig. 1D) at concentrations of 10 and 100 μg/ml were similar to those obtained with C-c^3Ado

(Fig. 1C). Also, the reductions in virus yield obtained with the four compounds were similar for both arenaviruses examined, TACV and JV.

Discussion

Of the compounds evaluated in this study, only ribavirin has previously been recognized as an inhibitor of arenavirus replication. In our assays the ID_{50} of ribavirin for arenavirus replication was 1.0–1.2 μg/ml. Its CD_{50}, as based on inhibition of cellular DNA synthesis or cell growth, was 100 and 19.5 μg/ml, respectively. Ribavirin has a broad-spectrum antiviral activity, encompassing several viruses other than arenaviruses (Huffman et al., 1973; Sidwell et al., 1972). It has been approved for clinical use as an aerosol in the treatment of acute lower respiratory tract infections caused by respiratory syncytial virus (Hall et al., 1983; McLung et al., 1983). Ribavirin is assumed to interact with a number of target proteins: i.e., IMP dehydrogenase (Streeter et al., 1973), mRNA 5′-capping enzymes (Goswani et al., 1979) and viral mRNA polymerase complex proteins (Wray et al., 1985).

Pyrazofurin can be considered a C-nucleoside antibiotic, originally isolated from a *Streptomyces* sp. (Descamps et al., 1978). It is endowed with both antitumor and antiviral properties (Descamps et al., 1978; Sweeney et al., 1973). As shown here, pyrazofurin also has a marked inhibitory effect on arenavirus replication. Its selectivity index is comparable to that of ribavirin.

The cyclopentyl and cyclopentenyl derivatives of cytosine can, like pyrazofurin, be considered as both antitumor and antiviral agents (Gutowski et al., 1985). They possess a unique spectrum of antiviral activity (Gutowski et al., 1985; Marquez et al., 1988; De Clercq et al., 1990), which apparently extends to arenaviruses. Cyclopentenyl cytosine showed the highest potency against JV and TACV of all the compounds tested. It also proved markedly selective in its inhibitory effect on arenavirus replication. The selectivity index of cyclopentenyl cytosine was greater than 3000, when based on the ratio of the 50% inhibitory dose for cellular DNA synthesis to the 50% inhibitory dose for arenavirus replication. Carbodine (cyclopentyl cytosine) also is a potent inhibitor of arenavirus replication, although it proved less selective in this regard than cyclopentenyl cytosine. It has been described, however, that the antiviral selectivity of carbodine can be markedly increased when combined with cytidine, since addition of cytidine reverses the cytotoxic activity of carbodine to a greater extent than its antiviral activity (De Clercq et al., 1989). The target enzyme of both carbodine and cyclopentenyl cytosine is supposed to be CTP synthetase that converts UTP to CTP (Moyer et al., Glazer et al., 1985).

(*S*)-Adenosylhomocysteine (AdoHcy) hydrolase has been recognized as an important target for the antiviral action of a series of acyclic and carbocyclic adenosine analogs, such as (*S*)-DHPA, (*RS*)-AHPA, C-c^3Ado, neplanocin A, 3-deazaneplanocin A, DHCA, DHCDA and adenosine dialdehyde (De Clercq, 1987). These compounds share a unique antiviral activity spectrum encompassing poxviruses (vaccinia), iridoviruses (African swine fever), (-)RNA viruses [rhabdo

(rabies, vesicular stomatitis), paramyxo (measles, parainfluenza)] and (±)RNA viruses [reo (rota)] (De Clercq, 1985, 1987). Apparently, these viruses heavily depend on transmethylation reactions, i.e. for the maturation (5′-capping) of their mRNA; and this may explain why they are particularly susceptible to inhibitors of AdoHcy hydrolase, a key enzyme in these transmethylation reactions (Borchardt et al., 1984). As clearly indicated by our present findings, the antiviral activity spectrum of the AdoHcy hydrolase inhibitors extends to the arenaviruses. The 50% inhibitory doses of the AdoHcy hydrolase inhibitors for arenavirus replication ranged from 0.3 to 15 μg/ml (Table 1). A close correlation has been found between the inhibitory effects of the AdoHcy hydrolase inhibitors on murine L929 cell AdoHcy hydrolase and their inhibitory effects on the replication of vaccinia virus and vesicular stomatitis virus in murine L929 cells (Cools and De Clercq, 1989). In terms of their (increasing) inhibitory effects on both virus replication and AdoHcy hydrolase activity the compounds rank as follows: (*S*)-DHPA < (*RS*)-AHPA < 3-deazaneplanocin A ~ C-c^{3}Ado < adenosine dialdehyde < neplanocin A (Cools and De Clercq, 1989). The same order of (increasing) activity has now been found when the compounds were evaluated against JV and TACV. It may be inferred, therefore, that AdoHcy hydrolase is the target enzyme for the antiviral activity of these compounds against Arenaviridae.

The AdoHcy hydrolase inhibitors affected the replication of arenaviruses at concentrations that were well below their cytotoxicity threshold, whether the cytotoxicity measurements were based on alteration of cell morphology or inhibition of cellular DNA synthesis (Tables 1 and 2). When the compounds were examined for their inhibitory effects on the proliferation of VERO cells in their exponential growth phase, some compounds (i.e. neplanocin A, 3-deazaneplanocin A, adenosine dialdehyde and DHCA) proved quite cytostatic (Table 2). When the selectivity index was based on the ratio of the 50% inhibitory dose for cellular DNA synthesis to the 50% inhibitory dose for virus replication, all AdoHcy hydrolase inhibitors proved selective in their anti-arenavirus activity. However, when the selectivity index was based on the ratio of the 50% inhibitory dose for cell growth to the 50% inhibitory dose for virus replication, only (*S*)-DHPA, DHCDA, C-c^{3}Ado and adenosine dialdehyde could be considered to be truly selective arenavirus inhibitors.

The sulfated polysaccharides emerged as potent and selective inhibitors of arenavirus replication. The ID_{50} of the sulfated polysaccharides ranged from 0.1 to 2.8 μg/ml, whereas the compounds were not inhibitory to host cell proliferation even at a concentration of 200 μg/ml (Tables 1 and 2). Thus, based on the ratio of the CD_{50} for host cell proliferation to the ID_{50} for viral cytopathogenicity, the selectivity index of λ-carrageenan, fucoidan, dextran sulfate, heparin and pentosan polysulfate was > 800, > 1 000, > 444, > 105 and > 91, respectively. As previously demonstrated (Baba et al., 1988b), sulfated polysaccharides are potent and selective inhibitors of enveloped DNA and RNA viruses. The mechanism of action of these compounds is due to the inhibition of virus binding to the host cells (De Somer et al., 1968). For that reason the sulfated polysaccharides were added immediately (and not at 1 h) after infection. Although sulfated polysaccharides are notorious for their anticoagulant activity, it has been previously demonstrated that they achieve

their virus-inhibitory effects at concentrations that are well below the anticoagulant threshold (Baba et al., 1988a).

In contrast with tubercidin, which showed a lower ID_{50} for host cell growth than for JV or TACV replication, xylotubercidin reduced viral cytopathogenicity at a concentration that was not inhibitory to host cell growth (Table 2). Tubercidin is a very potent but non-specific antiviral agent: it is inhibitory to a variety of DNA and RNA viruses but only at concentrations that are slightly lower than or similar to the cytotoxic concentrations (De Clercq et al., 1987). It has previously been demonstrated that conversion of tubercidin to its xylo derivative is accompanied by an increase in antiviral selectivity, which, as proven by the present findings, also extends to the arenavirus family.

In summary, our findings thus indicate that, in addition to ribavirin, various other compounds including pyrazofurin, cyclopentenyl cytosine, AdoHcy hydrolase inhibitors [i.e. (*S*)-DHPA, DHCDA, C-c^{3}Ado], sulfated polysaccharides (heparin, dextran sulfate, pentosan polysulfate, fucoidan and λ-carrageenan), and xylotubercidin can be considered as selective anti-arenavirus agents. Because of their promise as candidate drugs for the treatment of arenavirus infections, they should be further pursued for this purpose.

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