Antiviral Research, 22 (1993) 45–75

AVR 00648

Antiviral Research

Molecular approaches for the treatment of hemorrhagic fever virus infections[1]

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(Received 16 April 1993; accepted 11 June 1993)

Summary

Viruses causing hemorrhagic fevers in man belong to the following virus groups: togavirus (Chikungunya), flavivirus (dengue, yellow fever, Kyasanur Forest disease, Omsk hemorrhagic fever), arenavirus (Argentinian hemorrhagic fever, Bolivian hemorrhagic fever, Lassa fever), filovirus (Ebola, Marburg), phlebovirus (Rift Valley fever), nairovirus (Crimian-Congo hemorrhagic fever) and hantavirus (hemorrhagic fever with renal syndrome, nephropathic epidemia). Hemorrhagic fever virus infections can be approached by different therapeutic strategies: (i) vaccination; (ii) administration of high-titered antibodies; and (iii) treatment with antiviral drugs. Depending on the molecular target of their interaction, antiviral agents could be classified as follows: IMP dehydrogenase inhibitors (i.e., ribavirin and its derivatives); OMP decarboxylase inhibitors (i.e., pyrazofurin); CTP synthetase inhibitors (i.e., cyclopentylcytosine and cyclopentenylcytosine); SAH hydrolase inhibitors (i.e., neplanocin A); polyanionic substances (i.e., sulfated polymers); interferon and immunomodulators.

Hemorrhagic fever viruses; Ribavirin; Pyrazofurin; Cyclopentylcytosine; Neplanocin A; Sulfated polymers; Interferon; Immunomodulators

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[1]Presented at the World Health Organization (WHO)/Pan American Health Organization (PAHO) Meeting on Antiviral Drug Treatment of Viral Haemorrhagic Fevers, Washington DC, USA, 15-16 April 1993.

Introduction

Viruses responsible for hemorrhagic fevers in man must be quite ancient. Yellow fever, for example, has been one of the world's major plagues for the last centuries. Other viruses which produce hemorrhagic disease have come to light in the last decades. They have often been labelled as 'new viruses'. However, it is likely that they have existed in nature for years as silent enzootic foci. Man must, by accident, have entered in contact with the natural hosts and become infected.

A classification of viruses causative of hemorrhagic fevers in man is shown in Table 1. Most of these viruses were until recently classified as arthropod-borne viruses (arboviruses), since they multiply in both vertebrates and arthropods. In the cycle of transmission the former serve as reservoir and the latter as vectors. The virus is propagated in the arthropod's gut and if it attains a high titer in the salivary glands, it can be transmitted when a host is bitten. The viruses often cause disease in the vertebrate hosts, but no signs of illness are evident in the arthropods. In the majority of cases, humans are not the major target of virus infection. Increasing knowledge of the chemical and physical characteristics of

TABLE 1

Viral hemorrhagic fevers of man

Family	Genus	Disease	Geographic distribution
Togaviridae	Togavirus	Chikungunya	East Africa, South Africa, Southeast Asia
Flaviviridae	Flavivirus	Dengue	Pacific Islands, South and Southeast Asia, Northern Austria, New Guinea, Caribbean Islands, Nigeria, Central and South America, Republic of China
		Yellow fever	Central and South America, Africa, Trinidad
		Kyasanur Forest disease	India
		Omsk hemorrhagic fever	Siberia
Arenaviridae	Arenavirus	Argentinian hemorrhagic fever (Junin)	Argentina
		Bolivian hemorrhagic fever (Machupo)	Boliva
		Lassa fever	West Africa
Filoviridae	Filovirus	Marburg	Germany, South Africa
		Ebola	Zaire, Sudan
Bunyaviridae	Phlebovirus	Rift Valley fever	Africa
	Nairovirus	Crimean-Congo hemorrhagic fever (CCHF)	Africa, Europe, Asia
	Hantavirus	Korean hemorrhagic fever* (Hantaan virus), severe	Eastern Asia, Eastern Europe
		Hemorrhagic fever* (Seoul virus), mild or severe	Eastern Asia, seaports worldwide
		Nephropathia epidemica (Puumula virus), mild	Scandinavia, Europe, Western Soviet Union

*These clinical entities are also grouped under the title hemorrhagic fever with renal syndrome (HFRS).

arboviruses has revealed great heterogeneity among this group of viruses. The Arenaviridae family was formerly included among the arboviruses albeit without evidence of arthropod transmission, while some hemorrhagic fever viruses (e.g., Rift Valley fever virus) have remained unclassified until very recently.

Their routes of transmission may vary considerably (Table 2); i.e., by mosquito bites in the case of yellow fever, dengue and Rift Valley fever, by tick bite in Kyasanur Forest disease and Omsk hemorrhagic fever, and by contact with rodents and their excreta in the case of arenavirus and Hantavirus infections. Also, some human infections have been acquired by an alternate nonvectored route, that is through contact with either infected animal tissues (i.e., Rift Valley fever and Crimean Congo hemorrhagic fever) or by exposure to infected humans or their body fluids, as evidenced by the occurrence of a number of hospital outbreaks of Ebola, Lassa and Crimean Congo hemorrhagic fevers.

Although relatively rare, the high mortality rates associated with viral hemorrhagic fevers together with the potential ease of air transport from relative remote areas to large population centres have led to coining the term 'exotic' to refer to many of these viruses. These exotic viruses use diverse strategies for their replication, and this implies different approaches for the treatment of acute infections. For example, the genome of flaviviruses and

TABLE 2

Viral hemorrhagic fevers of man classified according their route of transmission

Mechanism of transmission	Disease	Vector	Natural vertebrate host(s)*
Mosquito borne	Chikungunya	*Aedes africanus* *Aedes aegypti*	Primates (acute)
	Dengue	*Aedes spp*	Primates (acute)
	Yellow fever	*Aedes spp*	Primates (acute)
	Rift Valley fever	Mosquitoes (many genera)	Sheep and other domestic animals (acute)
Tick borne	Kyasanur Forest disease	*Haemaphysalis spp*	Small mammals (acute)
	Omsk hemorrhagic fever	*Dermacentor pictus*	Muskrats (acute)
	Crimean-Congo hemorrhagic fever	*Hyaloma spp*	Herbivores (acute)
Zoonotic (aerosolized rodent excreta)	Argentinian hemorrhagic fever	None	*Calomys musculinus* (persistent) *Calomys laucha*
	Bolivian hemorrhagic fever	None	*Calomys callosus* (persistent)
	Lassa fever	None	*Mastomys natalensis*
	Korean hemorrhagic fever (Hantaan virus)	None	*Apodemus agrarius* (striped field mouse) (persistent)
	Hemorrhagic fever (Seoul virus)	None	*Rattus rattus* and *R. norvegicus* (wild urban rats) (persistent)
	Nephropathia epidemica (Puumula virus)		*Clethrionomys glareolus* (bank vole) (persistent)
Unknown	Ebola	Unknown	Unknown
	Marburg	Unknown	Unknown

*Infection of vertebrate hosts is either acute or persistent.

togaviruses consists of one molecule of positive-sense, infectious, ssRNA, while filoviruses contain a single species of negative-sense ssRNA. Yet, the genetic information in Arenaviridae and Bunyaviridae is segregated into, respectively, two or three pieces of ssRNA of different sizes. While most Bunyaviruses have negative-strand genomes, Arenaviruses and the genus *Phlebotomus* have an ambisense genome where mRNAs are transcribed from negative and positive sense forms of the genomic RNA species.

Importance of hemorrhagic fever viruses

Despite the effectiveness of mosquito eradication in reducing the incidence of yellow fever virus infection and the availability of an effective vaccine, yellow fever is still today the most important cause of hemorrhagic fever in man. Yellow fever is primarily a disease of primates and transmitted to man by the mosquitos *Aedes* spp. Even if elimination of the vector has almost eradicated urban yellow fever, sporadic outbreaks still occur throughout much of tropical South America and sub-Saharan Africa. The annual incidence of officially reported yellow fever cases is 50–300 in tropical America and 5–1000 in Africa. However, these data probably represent a significant underestimate of the true morbidity. During an outbreak of yellow fever in Ethiopia about 100 000 cases and over 30 000 deaths were estimated, based on direct investigations of the epidemic (Monath, 1990).

Treatment of yellow fever is mostly supportive, although most patients with yellow fever are unable to benefit from the modern intensive care units. A number of compounds with antiviral activity in vitro have been described, including ribavirin. However, trials of ribavirin in experimentally-infected monkeys have yielded conflicting results. Interferon treatment of monkeys resulted in delayed onset of viremia and illness but had no effect on survival (Arroyo et al., 1990).

The development of a safe, attenuated yellow fever virus vaccine (17D strain), grown in chick embryos, could be considered as a real breakthrough. Immunity was demonstrated in over 95% of the vaccinees within 10 days. Various studies have shown persistence of neutralizing antibodies in the vaccinees, for as long as 35 years in some instances, which means life-long.

At present, yellow fever virus vaccines produced may be contaminated with avian leukosis virus. Although undesirable, this contaminant has not been associated with the development of leukemia, lymphoma or other cancers. Recent improvements include the removal of avian leukosis virus by the use of human diploid cell lines as a substitute for chick embryos. However, these vaccines have not yet been thoroughly investigated, for instance for their stability in freeze-dried form. Also, development of alternative vaccines (e.g., recombinant baculoviruses or vaccinia viruses which express different portions of the 17D yellow fever virus) is underway (Shiu et al., 1991; Pincus et al., 1992).

Hundreds of thousands of dengue cases are reported worldwide each year, and because of the difficulty in obtaining full reporting, the actual number of human infections is probably much higher than the number reported (Hayes and Gubler, 1992). The 'classic' form of dengue is usually a nonspecific febrile illness that resolves with supportive therapy and affects mainly adults and older children. However, in the last two decades the incidence of epidemics of a severe disease syndrome caused by dengue viruses has increased, especially in Southeastern Asia, India and the Western Pacific (Howard et al., 1987). It occurs most frequently in young children and is associated with numerous hemorrhagic manifestations often with fatal shock syndrome. The pathophysiology of the severe forms of dengue may be related to sequential infection with different serotypes, variations in virus virulence, interaction of the virus with environmental and host factors or a combination of these factors (Hayes and Gubler, 1992).

The natural reservoir of dengue is now constituted by urban human populations. Travellers are the only dissemination factor of the viruses from one country to another, and all vectors are domestic species of the genus *Aedes* (Howard et al., 1987; Rodhain, 1992). The spreading of the disease is related to human activities, mainly travelling (Lange et al., 1992). The incidence of the disease is increasing, particularly in children. No antiviral treatment or vaccination are available. Control of dengue is primarily dependent on control of the principal vector mosquito, *Aedes aegypti* (Hayes and Gubler, 1992). Treatment of classic dengue is supportive, whereas urgent rehydradation therapy is often required for the most severe forms. Vaccine development programs have been initiated (Schoepp et al., 1991; Blok et al., 1992), but at present, the only way to avoid dengue is to use repellents and mosquito barriers.

Kyasanur Forest disease is limited to Mysore (India), but is gradually spreading. Epizootics occur in wild monkeys. Human infections are reported principally in persons with close contact with forested areas. Thousands of cases have been reported since the recognition of the disease in 1957, the annual incidence of virologically diagnosed cases being between 400 and 500 cases (Howard et al., 1987; Monath, 1990). The case fatality rate is 3–5%. A killed vaccine which produces weak serologic response but confers protection in field trials has been developed. Antiviral treatment is not available.

Most infections with Omsk hemorrhagic fever virus appear in the northern forest-steppe-lake belt of Western Siberia. The case fatality rate of this disease is 0.5–3%. Between 1945 and 1958, 1500 cases were recorded in the Omsk region in Siberia. Small numbers of cases occurred in the 1960s, but in recent years no further cases were notified (Monath, 1990). The disease affected rural populations engaged in field work during the spring and summer. Muskrat hunters were at high risk. No specific Omsk hemorrhagic fever vaccine has been developed. The disease in humans closely resembles Kyasanur Forest disease, except for that sequelae (hearing loss, hair loss, neuropsychiatric complaints) are relatively frequent.

Two or three centuries ago, Chikungunya virus must have occurred in the forests and savannahs of Africa as an infection of primates maintained by sylvatic *Aedes* mosquitos. However, today Chikungunya is also responsible for extensive *Aedes aegypti*-transmitted urban disease in African cities and as well as for major epidemics in Asia. The largest epidemics in recent years have been in cities of India and Southeastern Asia, where Chikungunya virus has been implicated in outbreaks of hemorrhagic fever, often in association with dengue virus (Peters and Dalrymple, 1990). Clinically distinctive symptoms of Chikungunya virus infection are crippling arthritis/arthralgia; and their persistence during convalescence is an important factor in the biomedical impact of the disease. Supportive care is the only treatment indicated.

Crimean Congo hemorrhagic fever (CCHF) virus was originally isolated independently in Asia (Crimean hemorrhagic fever) and Africa (Congo virus). Subsequent laboratory studies showed the two viruses to be identical. Although the virus is found extensively in Africa and occasionally in Europe, recent sporadic outbreaks in the Middle East have raised the interest in this virus. Infection with CCHF virus results in a serious disease, since most cases exhibit hemorrhagic signs, and a 10–50% mortality rate has been observed in different outbreaks. At present, no information is available regarding the potential therapy or prophylaxis of CCHF.

Rift Valley fever (RVF), or enzootic hepatitis, is a viral infection that primarily affects sheep and cattle, causing significant morbidity and mortality in pregnant and newborn animals. RVF virus was first isolated in 1930 after an outbreak of enzootic hepatitis in cattle in the Rift Valley in East Africa. Since then large epizootics have occurred at frequent intervals throughout Eastern, Central and Southern Africa. RVF virus was an unclassified virus for many years after its isolation. In 1973 it was shown to be morphologically similar to the bunyaviruses and its classification was confirmed by basic molecular studies (Schmaljohn and Patterson, 1990). Many infections have occurred in man, particularly through direct contact with infected animals or carcasses. In man, RFV usually presents as a self-limiting, influenza-like illness, although complications of ocular disease, encephalitis and hemorrhagic fever occur (McIntosh et al., 1980; Siam et al., 1980). Because RVF virus can be transmitted by many species of mosquitoes and vertebrates, it has the potential to be spread to distant geographic areas, where epizootics may start. During an epizootic, disease usually occurs first in animals, then in humans. Human infections occur mainly among farmers and others in close contact with infected animals. In the 1950 epizootic in South Africa, as many as 20 000 human cases may have occurred, although there were no deaths. However, there have been several deaths reported during more recent epizootics in South Africa. The virus appeared in 1977–1978 in Sudan and in Egypt, where it was responsible for a very intense epizootic. It was estimated that there were as many as 200 000 human cases and at least 600 deaths. Considering that sheep, cattle and buffalo and other vertebrates act as primarily reservoirs of the virus, it is likely, therefore, that virus spread may be limited by effective

immunization of sheep and cattle within and around endemic areas. Several candidate vaccines have been developed. Formalin-inactivated vaccines have been developed to protect laboratory workers. Vaccinated individuals developed long-lasting levels of neutralizing antibodies (Eddy et al., 1981). Several attenuated virus strains have been isolated and developed as potential live virus vaccines (Rossi et al., 1988; Morrill et al., 1991a). Inactivated RVF vaccine, although not yet licensed, is probably indicated for veterinary and laboratory personnel working in areas where RVF is endemic (Niklasson et al., 1985). Several antiviral compounds have been shown to be partially protective of experimentally infected animals, particularly ribavirin and the interferon inducer, poly (ICLC) (Huggins et al., 1984a,b; Kende et al., 1985, 1987; Peters et al., 1986).

Hemorrhagic fever with renal syndrome (HFRS) has been described under various names (Korean hemorrhagic fever, hemorrhagic nefrosonephritis, Song-go fever) in many countries right across the temperate zone of the northern hemisphere from the Pacific to the Baltic. A similar but less severe disease, nephropathia epidemica (NE), occurs throughout Scandinavia. The striped field mouse *Apodemus agrarius* appears to be the major or sole natural host of Hantaan virus and this rodent probably determines the distribution of the virus. The reservoir of HFRS in the rural endemic areas in Korea is *Apodemus agrarius coreae*. The reservoirs of HFRS in urban and laboratory infections are *Rattus rattus* and *R. norvegicus*, and it was found that urban rats are the natural host of a different virus, Seoul virus, which is related to Hantaan virus. Colonized experimental rats are a dangerous reservoir of HFRS and have been responsible for several outbreaks of HFRS among personnel of laboratory animal facilities at Research Institutes in Korea, Japan and Belgium. HFRS occurs in many countries in Asia and Europe. There are about 600–800 hospitalized cases every year in Korea, over 10 000 cases annually in China and about 100 cases each year in Japan, while in Finland between 10 and 100 cases of NE occur annually (Lee et al., 1989). Although the absolute number of cases is rather small, the real number of people infected must be much higher. Antibodies against Hantaan and Hantaan-related virus have been detected in human sera and urban rat sera in America, Western Pacific and Southeast Asia, Africa, Pacific Islands and Europe. It is evident that house and laboratory rats infected with Hantaan and related viruses has increased recently in Asia, Europe and the American continents where HFRS was not known to exist. Recent findings now suggest that Hantaan or Hantaan-related viruses must exist all over the world where rodents can be found.

The pathogenesis of Marburg and Ebola hemorrhagic fevers is similar to that of yellow fever and CCHF. Of all the viral hemorrhagic fevers, these have the highest case-fatality rates (30–90%), the most hepatic involvement and the most severe hemorrhagic manifestations (Murphy et al., 1990). The natural history and origin of Marburg and Ebola viruses remain a mystery. It would seem that the viruses are zoonotic, that is, transmitted to humans from ongoing

life cycles of the virus in animals or arthropods. However, all attempts to backtrack the virus from human cases in Africa or from the monkeys involved in the first Marburg episode in Europe have failed to uncover a reservoir. There is no specific treatment for Marburg and Ebola hemorrhagic fevers. The usefulness of virus subtype-specific immune plasma is unproven, but it has been used in the past, and, if available, its use would be recommended. Similarly, human interferon has been used in the past; its value is unproven, but its use would, again, be recommended. There is no vaccine against Marburg or Ebola virus infection. Preliminary attempts to raise neutralizing antibodies in laboratory animals via injection of crude vaccines have been disappointing. Vaccine development will require better understanding of the viruses, their antigens and the pathogenesis of the infection.

Almost all arenaviruses so far described cause acute or persistent infections of rodents in Africa, Europe and the Americas. Only certain arenaviruses cause severe hemorrhagic disease in man, notably Lassa, Machupo and Junin, responsible for Lassa fever, Argentinian hemorrhagic fever (AHF) and Bolivian hemorrhagic fever (BHF), respectively. Other arenaviruses, such as Pichinde, cause no apparent disease in man despite causing persistent infections in related rodent species. Acute hemorrhagic disease due to Machupo and Junin viruses represent serious health problems and so does Lassa virus because of its association with severe febrile illness among travellers returning from rural parts of West Africa. The morbidity of Lassa and South American hemorrhagic arenavirus infections results from the direct cytolytic action of these viruses. This is in contrast with the immunopathological basis of 'classic' lymphocytic choriomeningitis disease seen in adult mice infected with LCM virus, considered as the prototype of the family (Howard et al., 1987). Studies of hospitalized patients have shown that Lassa fever is a common cause of hospital admissions throughout the years. The death rate is 15–20%, very similar to that described for Junin and Machupo infections. The use of ribavirin therapy had cut this rate to 5% or less. Junin and Machupo are rarely transmitted from person to person; Lassa, however, is frequently transmitted from man to man. This may be correlated with the low titer of circulating virus found in patients with AHF and BHF, in contrast with the high virus titer found in Lassa infections. Inapparent and subclinical infections seem to be quite common for Lassa, but not for Junin and Machupo infections (Howard et al., 1987).

AHF was recognized in the 1950s in a part of the Buenos Aires Province. The total number of cases reported since then is about 21 000 (Maiztegui et al., 1986). AHF is a seasonal disease with yearly peak incidence in May. The annual number of cases range from 100 to 4000. The major group affected is the male working population, the reason being the habit of the rodent vectors. These animals are not peridomestic; they occupy mostly corn, sorghum and sunflower fields. Since the recognition of AHF, the disease has spread from an area of 16 000 km^2 and a quarter of a million persons to an area greater than 120 000 km^2 and one million persons (Maiztegui et al., 1986). Furthermore, the

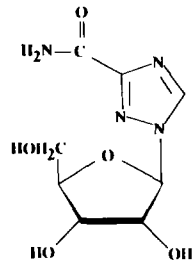
incidence in the older affected areas seems to wane after 5–10 years. BHF was first recognized in 1959, and by 1962 more than 1000 cases were identified. The largest known epidemic occurred in 1963 and 1964 in San Joaquin, due to an increase in the vector (*Callomys callosus*) population (McKenzie, 1965). It appeared that the situation in San Joaquin was unusual, and no increase in the geographic areas affected by BHF in the last decade has been reported.

Significant advances in the therapy of Junin and Lassa virus infections have been made in the last decade. A randomized trial of patients with AHF indicated that convalescent-phase plasma reduces the mortality from 16 to 1% in the patients treated in the first 8 days of illness (Maiztegui et al., 1979). The efficacy of the plasma is directly related to the concentration of neutralizing antibodies. However, a drawback of this therapy is the development of a late neurological syndrome in about 10% of the cases (Maiztegui et al., 1979). In the case of Lassa fever, convalescent plasma fails to improve the recovery from the disease (McCormick et al., 1986). Ribavirin is the only antiviral drug known to be effective in the treatment of patients with AHF and Lassa fever (McCormick et al., 1986; Enria et al., 1987). A live attenuated vaccine against AHF has been extensively evaluated in monkeys and is currently being tested for efficacy in protecting against AHF in the human population at risk (Clegg, 1992). A vaccine against Lassa fever has been made by cloning and expressing the Lassa virus glycoprotein gene into vaccinia virus (Auperin, 1988; Fisher-Hoch, 1989). This vaccine has proven highly successful in preventing severe disease and death in monkeys (Fisher-Hoch, 1989).

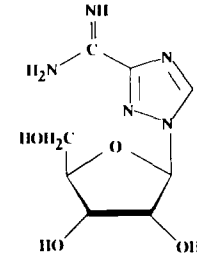
IMP dehydrogenase inhibitors

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, virazole) (Fig. 1) was described two decades ago as a broad-spectrum antiviral agent (Sidwell et al., 1972). Several compounds chemically related to ribavirin (Fig. 1), i.e., tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide), selanazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide), ribamidine (1-β-D-ribofuranosyl-1,2,4-tiazole-3-carboxamide), FICAR (5-fluoro-1-β-D-ribofuranosylimidazole-4-carboxamide) and EICAR (5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide) have been reported (De Clercq et al., 1975, 1991a; Kirsi et al., 1983; Huggins et al., 1984a,b; Sidwell et al., 1985). All these derivatives are markedly active against orthomyxoviruses (influenza A, B), paramyxoviruses (parainfluenza, measles, respiratory syncytial virus), arenaviruses and bunyaviruses. They are also active against pox-, picorna-, toga- and reoviruses (Tables 3 and 4).

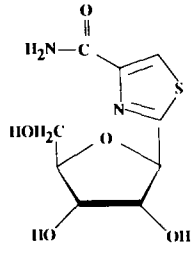
The therapeutic efficacy of ribavirin and some of its derivatives has been evaluated in various animal models of arena- and bunyavirus infections. Ribavirin was shown to reduce viremia and increase the survival rate of monkeys infected with Lassa virus (Jahrling et al., 1980), Junin virus (Weissenbacher et al., 1986) or Machupo virus (Stephen et al., 1980), and of guinea pigs and hamsters infected with Pichinde virus (Stephen et al., 1980; Lucia et al., 1989) or Junin virus (Kenyon et al., 1986). The ribavirin derivative,



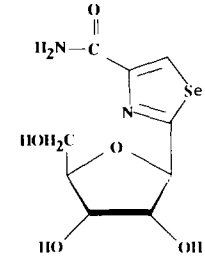
Ribavirin



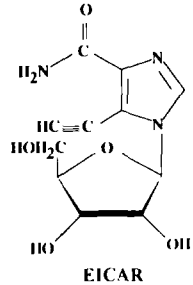
Ribamidine



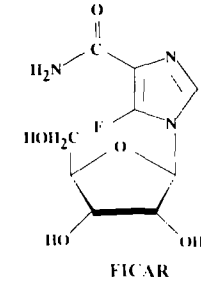
Tiazofurin



Selenazofurin



EICAR



FICAR

Fig. 1. Ribavirin and related compounds. Ribavirin: 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. Tiazofurin: 2-β-D-ribofuranosylthiazole-4-carboxamide. Selenazofurin: 2-β-D-ribofuranosylselenazole-4-carboxamide. Ribamidine: 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine. EICAR: 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide. FICAR: 5-fluoro-1-β-D-ribofuranosylimidazole-4-carboxamide.

ribamidine, also showed anti-Pichinde virus activity in a lethal hamster model (Smee et al., 1993). The potency of ribamidine against this virus infection was between one-third and one-tenth of that of ribavirin. Similar results were observed in studies against the Phlebovirus Punta Toro (Sidwell et al., 1988a,b). Since ribamidine is better tolerated than ribavirin, the therapeutic indices of both compounds may be similar. Inhibition of Phlebovirus infections in vivo by

TABLE 3

Antiviral activity of various classes of compounds against viruses closely related to hemorrhagic fever viruses in man

Compounds	Virus	References
IMP dehydrogenase inhibitors		
Ribavirin	Arena (Lassa, Junin, Machupo, Pichinde)	Enria et al. (1987); McCormick et al. (1986); Stephen et al. (1980); Smee et al. (1993)
	Phlebo (Punta Toro)	Sidwell et al. (1988a,b)
	Hanta (Hantaan)	Huggins et al. (1986), Huggins et al. (1991)
	Toga	
Tiazofurin	Toga, Bunya, Arena	Huggins et al. (1984); Smee et al. (1990c)
30;Selenazofurin	Toga, Bunya, Arena	Kirsi et al. (1983); Huggins et al. (1984); Smee et al. (1990c)
Ribamidine	Bunya, Arena	Sidwell et al. (1988); Sidwell et al. (1993)
EICAR	Arena, Toga	De Clercq et al. (1991)
FICAR		De Clercq (1993)
OMP decarboxylase inhibitors[a]		
Pyrazofurin	Toga, Arena	Descamps and De Clercq (1978); Andrei and De Clercq (1990)
CTP synthetase inhibitors		
C-Cyd and Ce-Cyd	Toga, Flavi, Bunya, Arena	De Clercq et al. (1990); De Clercq et al. (1991b); Marquez et al. (1988)
SAH hydrolase inhibitors[b]		
NpcA, c^{3}NpcA, DHCeA, c^{3}DHCeA C-Ado, C-c^{3}Ado DHCaA, c^{3}DHCaA, 6-C-methyl NpcA, F-C-Ado	Arena	Andrei and De Clercq (1990); Shuto et al. (1992); Patil et al. (1992)
Polyanionic substances[a]		
Sulfated polymers (dextran sulfate, pentosan polysulfate, PAPS, PVAS, PAVAS)	Toga, Arena	Andrei and De Clercq (1990); Witvrouw et al. (1991); Schols et al. (1990a); Witvrouw et al. (1992)
Polyoxometalates		Yamamoto et al. (1992)

[a]Data not available for Bunya- and Flaviviruses.
[b]Togaviruses are not sensitive to SAH hydrolase inhibitors, data for Bunya- and Flaviviruses are not available.

tiazofurin and selenazofurin have been reported (Smee et al., 1990c). Ribavirin has also proven effective in the treatment of suckling mice infected with Hantaan virus (Huggins et al., 1986).

Ribavirin is the only antiviral chemotherapeutic drug that is known to have some benefit in the treatment of some viral hemorrhagic fevers in humans. Studies performed in patients with Lassa fever (McCormick et al., 1986), Argentinian hemorrhagic fever (Enria et al., 1987) or hemorrhagic fever with renal syndrome (Huggins et al., 1991) have indicated that early treatment with ribavirin after the onset of the symptoms might be effective. However, the drug is not effective in patients with advanced disease. The only adverse effect observed in patients treated with ribavirin was the development of anemia, which is a well-recognized side effect of the drug that is fully reversible upon cessation of the treatment (Canonico et al., 1984; Shulman, 1984).

TABLE 4

Antiviral activity spectrum of different classes of compounds[a,b]

Virus family	Virus type	IMP dehydrogenase inhibitors (i.e., ribavirin)	OMP decarboxylase inhibitors (i.e., pyrazofurin)	CTP synthetase inhibitors (i.e., cyclopentenyl cytosine)	SAH hydrolase inhibitors (i.e., neplanocin A)	Sulfated inhibitors (i.e., dextran sylfate)
Herpes	HSV	–	–	–	–	+
	TK⁻ HSV	–	+	+	–	+
	CMV	+	+	+	+	+
Pox	Vaccinia	+	+	+	+	+
Picorna		+	+	+	–	–
Toga		+	+	+	–	+
Flavi		+	? +	+	? –	? +
Bunya		+	? +	+	? +	? –
Arena		+	+	+	+	+
Rhabdo		+	+	+	+	+
Orthomyxo	Influenza A	+	+	+	–	+
	Influenza B	+	+	+	–	–
Paramyxo	Parainfluenza measles	+	+	+	+	–
	Respiratory syncytial	+	+	+	+	+
Reo		+	+	+	+	–
Retro	Murine leukemia sarcoma	+	+	+	+	+
	Human immunodeficiency	–	–	–	–	+

[a]Question marks indicate predictions that should be assessed experimentally.
[b]According to De Clercq (1993) with modifications.

Although ribavirin is highly effective in treating Lassa fever and has some positive effect in hemorrhagic fever with renal syndrome, Congo-Crimean hemorrhagic fever and Argentinian hemorrhagic fever, it has no in vitro effect on Marburg and Ebola viruses and is unlikely to be of any clinical value (Murphy et al., 1990). Ribavirin is also ineffective as a prophylactic drug against dengue viral infection, as based on viremia determinations in Rhesus monkeys (Malinoski et al., 1990).

Recently, a pilot study of ribavirin therapy for chronic hepatitis C was performed (Di-Bisaglie et al., 1992). Therapy was associated with a significant decrease in the geometric mean titer of hepatitis C virus RNA in serum although no patients lost hepatitic C virus RNA from serum during therapy. These findings suggest that ribavirin has a beneficial effect in patients with chronic hepatitis C, although further studies are needed to determine the efficacy of ribavirin against hepatitis C virus and other flaviviruses in vivo.

The primary target for the antiviral action of ribavirin has been originally identified as the enzyme IMP dehydrogenase that converts IMP to XMP (Streeter et al., 1973) (Fig. 2). Thus, GMP, GDP and GTP pool levels are reduced and viral RNA synthesis is suppressed. This inhibition would be dependent on the intracellular conversion of the drug to its 5′-monophosphate form (Streeter et al., 1973). That ribavirin acts via depletion of the intracellular GTP and dGTP pools is supported by the fact that its antiviral and cytotoxic

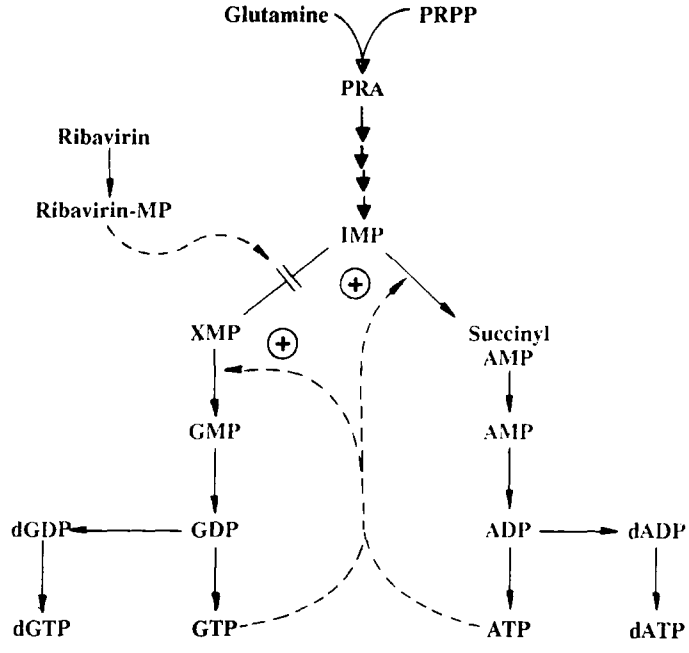


Fig. 2. Mechanism of action of ribavirin. Key enzymes involved in the action of ribavirin: IMP dehydrogenase (IMP → XMP), adenylsuccinate synthetase (IMP → succinyl AMP). PRPP: 5-phosphoribosyl-1-pyrophosphate.

effects can be reversed by the exogenous addition of guanosine, but not by other nucleosides (Scholtissek, 1976; Browne, 1978; Lee et al., 1985). A reduction in the GTP pool levels leads also to a diminished formation of ATP (and dATP), since GTP is an obligatory co-factor for the conversion of IMP to succinyl AMP by adenylsuccinate synthetase. Thus, following ribavirin treatment, intracellular IMP pool levels rise due to (i) a direct inhibitory effect of ribavirin 5'-monophosphate on IMP dehydrogenase and (ii) indirect inhibition of adenosylsuccinate synthetase by the decreased GTP pool levels (Balzarini et al., 1991). Ribavirin can also be phosphorylated to its 5'-triphosphate, and in this form it may interfere directly with viral RNA synthesis (Eriksson et al., 1977), viral mRNA capping guanylylation (Goswami et al., 1979) and primer generation and elongation during viral RNA transcription (Wray et al., 1988).

OMP decarboxylase inhibitors

Pyrazofurin [3-(β-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide] is a C-nucleoside antibiotic (Fig. 3), which was first isolated from *Streptomyces candidus* and later synthesized chemically, has raised some interest due to its antitumor and broad-spectrum antiviral activity (Sweeney et al., 1973; Gutowski et al., 1975). It is active against both (+)RNA viruses [i.e.,

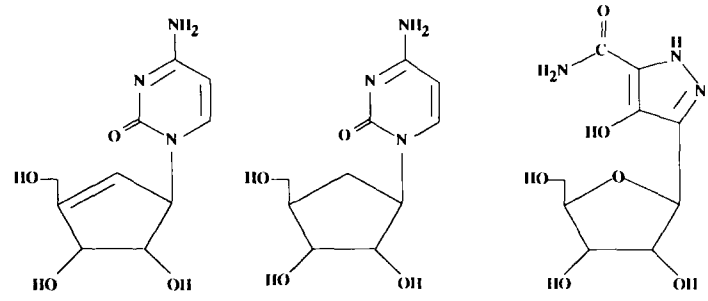


Fig. 3. Pyrazofurin and carbocyclic nucleoside analogues. Pyr: pyrazofurin: 4-hydroxy-3-β-D-ribofuranosylpyrazole-5-carboxamide. C-Cyd: carbocyclic cytidine, cyclopentylcytosine. Ce-Cyd: cyclopentenylcytosine.

picornaviruses (polio, Coxsackie B), togaviruses (Sindbis)] and (−)RNA viruses [i.e., paramyxoviruses (measles, respiratory syncytial virus), orthomyxoviruses (i.e., influenza A, B, C), rhabdoviruses (vesicular stomatitis virus)] as well as some DNA viruses [i.e., poxviruses (vaccinia) and retroviruses (murine leukemia virus)] (Tables 3 and 4) (Shannon, 1977; Descamps and De Clercq, 1978; Kawana et al., 1987; Shigeta et al., 1988; Hosoya et 81., 1989). Pyrazofurin has also shown a marked inhibitory effect on arenavirus replication with a potency and selectivity comparable to that of ribavirin (Andrei and De Clercq, 1990).

Pyrazofurin, upon conversion to its 5′-monophosphate by cellular kinase(s), is assumed to act as an inhibitor of orotidylic acid (OMP) decarboxylase, an enzyme of the de novo pyrimidine biosynthetic pathway responsible for the conversion of OMP to UMP (Fig. 4) (Cadman et al., 1978; Olah et al., 1980). Another compound known to interfere with the conversion of OMP to UMP is 6-azauridine, and this again requires intracellular phosphorylation of the compound to its 5′-monophosphate (Janeway and Cha, 1977). The antiviral activity spectrum of 6-azauridine has not been well defined (Rada and Dragun, 1977). However, if its target enzyme is, as for pyrazofurin, OMP decarboxylase, its activity spectrum should be identical, as well.

CTP synthetase inhibitors

The carbocyclic analogues of the normal nucleoside cytidine, i.e., cyclopentylcytosine (C-Cyd, carbodine) and cyclopentenylcytosine (Ce-Cyd) (Fig. 3) may be also considered as potential chemotherapeutic drugs for the treatment of some viral hemorrhagic fevers. They have proved effective as both antiviral and antitumor agents (Shannon et al., 1981; Lin et al., 1984; Marquez et al., 1988; De Clercq et al., 1990; De Clercq et al., 1991b). The C-Cyd was first described as an anti-influenza virus agent (Shannon et al., 1981) but it has also shown activity against DNA viruses [pox (vaccinia)], (+)RNA viruses [toga

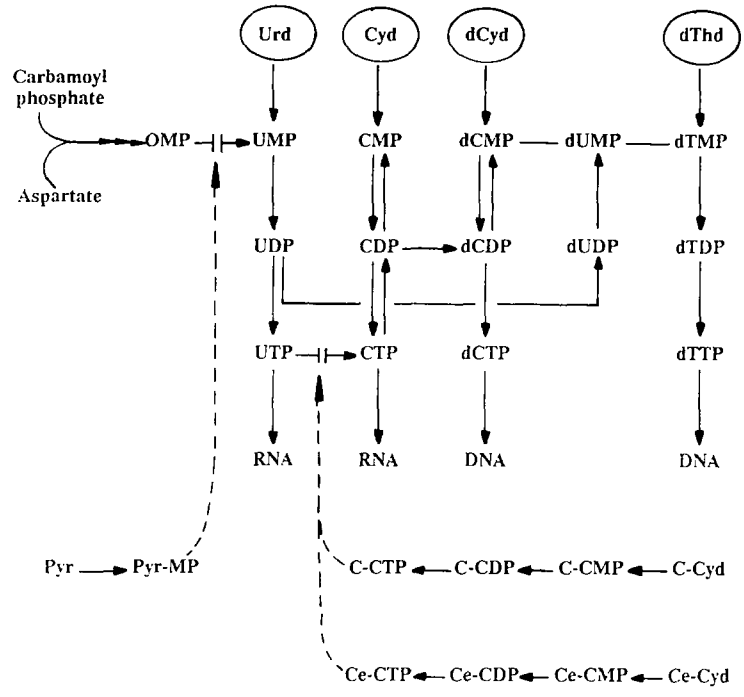


Fig. 4. De novo and salvage pathways for the biosynthesis of pyrimidine nucleoside 5'-triphosphates. Targets for the action of Pyr (pyrazofurin), C-Cyd (cyclopentylcytosine, carbocyclic cytidine) and Ce-Cyd (cyclopentenylcytosine). Target enzyme for Pyr: OMP decarboxylase (OMP → UMP). Target enzyme for C-Cyd and Ce-Cyd: CTP synthetase (UTP → CTP). Pyr-MP, C-CMP and Ce-CMP correspond to the 5'-monophosphate of Pyr, C-Cyd and Ce-Cyd, respectively. C-CDP and Ce-CDP correspond to the 5'-diphosphate of C-Cyd and Ce-Cyd, respectively. C-CTP and Ce-CTP correspond to the 5'-triphosphate of C-Cyd and Ce-Cyd, respectively.

(Sindbis, Semliki forest)], (−)RNA viruses [orthomyxo (influenza), paramyxo (parainfluenza, measles), rhabdo (vesicular stomatitis)] and (±)RNA viruses (reo) (De Clercq et al., 1990). Ce-Cyd is a more potent antiviral agent than its counterpart, C-Cyd (De Clercq et al., 1991b), and, in addition, it has also proved active against picornaviruses (polio, Coxsackie B, rhino) and herpesviruses (i.e., cytomegalo, varicella-zoster, and thymidine kinase-deficient herpes simplex) (Tables 3 and 4) (Marquez et al., 1988; De Clercq et al., 1991b). The antiviral spectrum of Ce-Cyd extends to bunyaviruses (Punta Toro), flaviviruses (Japanese encephalitis) and arenaviruses (Junin, Tacaribe) (Marquez et al., 1988; Andrei and De Clercq, 1990). Ce-Cyd has also proved effective against the replication of the arenaviruses Junin and Tacaribe, the selectivity index (ratio of 50% inhibitory dose for cellular DNA synthesis to the 50% inhibitory dose for virus-induced cytopathicity) being greater than 3000 (Andrei and De Clercq, 1990). C-Cyd also is a potent inhibitor of Junin and Tacaribe virus, although less so than Ce-Cyd.

The putative target for both the antitumor and antiviral effects of C-Cyd and Ce-Cyd is assumed to be CTP synthetase, the enzyme that catalyses the final step in the de novo pyrimidine biosynthetic pathway, the conversion of UTP to CTP (Fig. 4) (Kang et al., 1989). To interact with CTP synthetase, C-Cyd and Ce-Cyd have to be converted intracellularly to their 5′-triphosphate metabolites (Shannon et al., 1981). The fact that both the antiviral and cytotoxic effects of C-Cyd and Ce-Cyd are readily reversed by Cyd, and, to a lesser extent, by Urd, but not by other nucleosides such as dThd or dCyd, supports the hypothesis that the antiviral and antitumor actions of these carbocyclic cytidine analogues may be mediated by the inhibition of CTP synthetase (De Clercq et al., 1991b).

SAH hydrolase inhibitors

Several acyclic and carbocyclic adenosine analogues, which are potent inhibitors of *S*-adenosylhomocysteine hydrolase (SAH hydrolase), have been found to interfere with the replication of a broad variety of viruses (De Clercq, 1987). These analogues (Fig. 5) encompass such compounds as (*S*)-9-(2,3-dihydroxypropyl)adenine (DHPA) (De Clercq et al., 1978), 3-adenin-9-yl-2-hydroxypropanoic acid (AHPA) (Holy et al., 1985; De Clercq and Holy, 1985), neplanocin A (NpcA) (Borchardt et al., 1984; De Clercq, 1985), 3-deazaneplanocin A (c^3NpcA) (Glazer et al., 1986; De Clercq et al., 1989), the 5′-decapitated derivatives of neplanocin A and 3-deazaneplanocin A (DHCeA and c^3DHCeA, respectively) (Narayanan et al., 1988; De Clercq et al., 1989) and the 5′-decapitated derivatives of carbocyclic adenosine and carbocyclic 3-deazaadenosine (DHCaA and c^3DHCaA, respectively) (De Clercq et al., 1991b), 4′,5′-unsaturated 5′-fluoro adenosine analogues (McCarthy et al., 1989), 6′-fluoro-substituted carbocyclic adenosines (Cools et al., 1991), 5′-nor aristeromycin (5′-norAdo) (Patil et al., 1992), and 6′-C-methylneplanocin A (Shuto et al., 1992).

Inhibitors of SAH hydrolase inhibitors have been shown to have a unique spectrum of antiviral activity, including pox- (vaccinia), paramyxo- (parain-

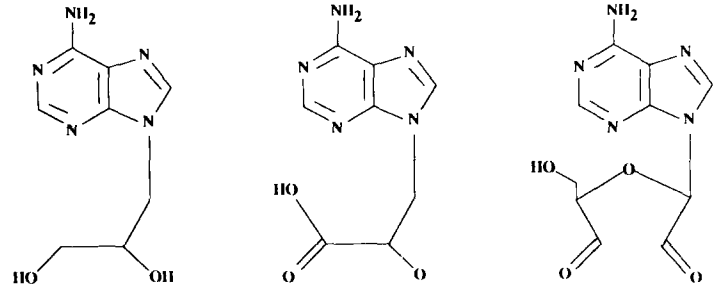


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Fig. 5A. Acyclic adenosine analogues. DHPA: (*S*)-9-(2,3-dihydroxypropyl)adenine. AHPA: 3-adenin-9-yl-2-hydroxypropanoic acid. Ado dialdehyde: adenosine dialdehyde.

fluenza, measles, respiratory syncytial), rhabdo (vesicular stomatitis, rabies), reo- (reo, rota) and some herpesviruses (cytomegalo) (Tables 3 and 4). They

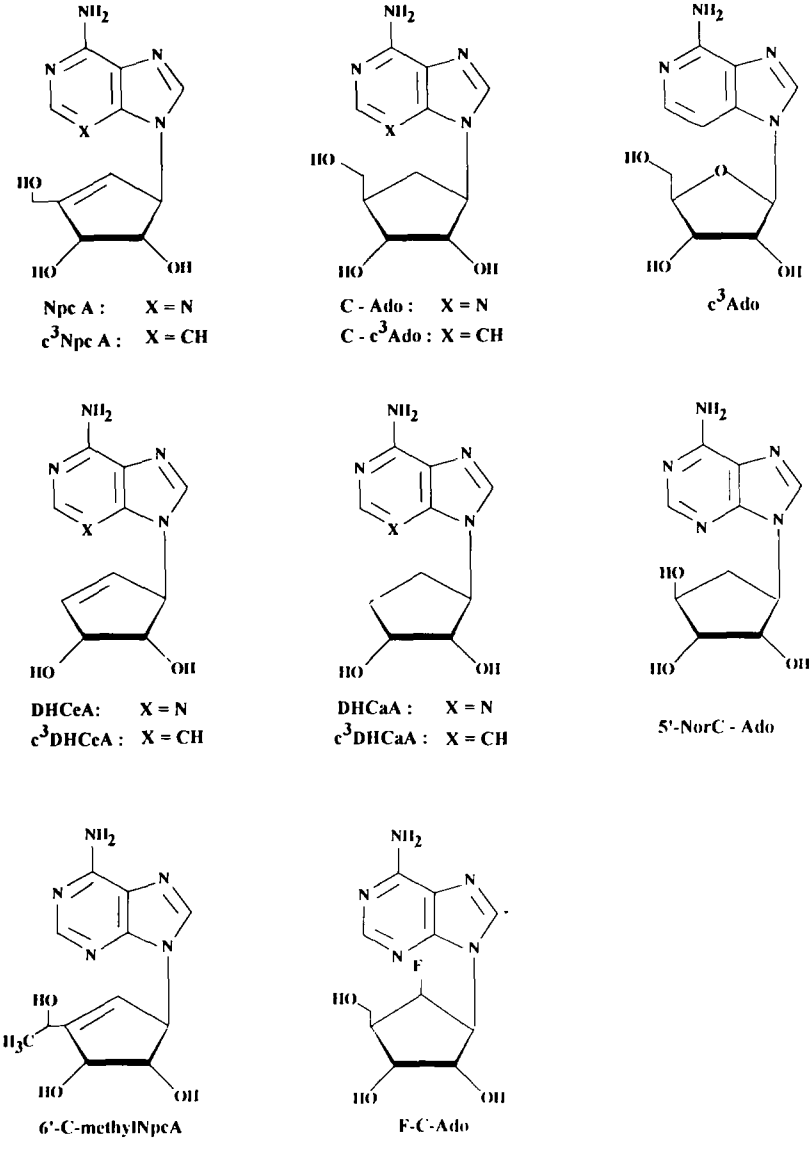

Fig. 5B. Carbocyclic adenosine analogues. Npc A: neplanocin A. c^3NpcA: 3-deazaneplanocin A. C-Ado: carbocyclic adenosine (aristeromycin). C-c^3Ado: carbocyclic 3-deazaadenosine. c^3-Ado: 3-deaazaadenosine. DHCeA: 9-(*trans*-2',*trans*-3'-dihydroxycyclopent-4'-enyl)-adenine. c^3DHCeA: 9-(*trans*-2',*trans*-3'-dihydroxycyclopent-4'-enyl)-3-deazaadenine. DHCaA: 9-(*trans*-2',*trans*-3'-dihydroxycyclopentyl)adenine. c^3DHCaA: 9-(*trans*-2',*trans*-3'-dihydroxycyclopentyl)-3-deazaadenine. 5'norC-Ado: 5'-nor-aristeromycin. 6'-C-methyl NpcA: 6'-C-methylneplanocin A. F-C-Ado: 6'β-fluoroaristeromycin.

have also proved active against arenaviruses (Andrei and De Clercq, 1990; Shuto et al., 1992; Patil et al., 1992), but they have no activity against retroviruses, most herpesviruses (i.e., herpes simplex), and (+)RNA viruses (i.e., toga, picornaviruses). The activity of these compounds against flavi-, filo- or bunyaviruses has not been shown.

The adenosine analogues of both the acyclic and carbocyclic type are assumed to achieve their antiviral activity through an inhibitory effect on SAH hydrolase (Fig. 6) (Cools et al., 1989). SAH hydrolase is responsible for the reversible hydrolysis of *S*-adenosyl-L-homocysteine (AdoHcy) to adenosine (Ado) and L-homocysteine (Hcy) and is a key enzyme in transmethylation reactions using *S*-adenosyl-L-methionine (AdoMet, SAM) as the methyl donor. Such transmethylation reactions are involved in the maturation of viral mRNAs and hence play a critical role in the virus replicative cycle. A close correlation has been found between the antiviral activity of a series of acyclic and carbocyclic adenosine analogues and their inhibitory effects on SAH hydrolase (Cools et al., 1989). Furthermore, within the cells, virus inhibition by these compounds is closely correlated with increases in intracellular AdoHcy levels and elevations in AdoHcy/AdoMet ratios (Hasobe et al., 1989; Cools et al., 1990).

A novel adenosine analogue, 3'-fluoro-3'-deoxyadenosine (3'F3'Ado), has recently been described (Van Aerschot et al., 1989). This adenosine analogue is active against both DNA viruses [pox (vaccinia)], single-stranded (+) RNA viruses [picorna (polio, Coxsackie B), toga, (Sindbis, Semliki Forest)] and double-stranded RNA viruses (reo). In its antiviral spectrum 3'F3'Ado clearly

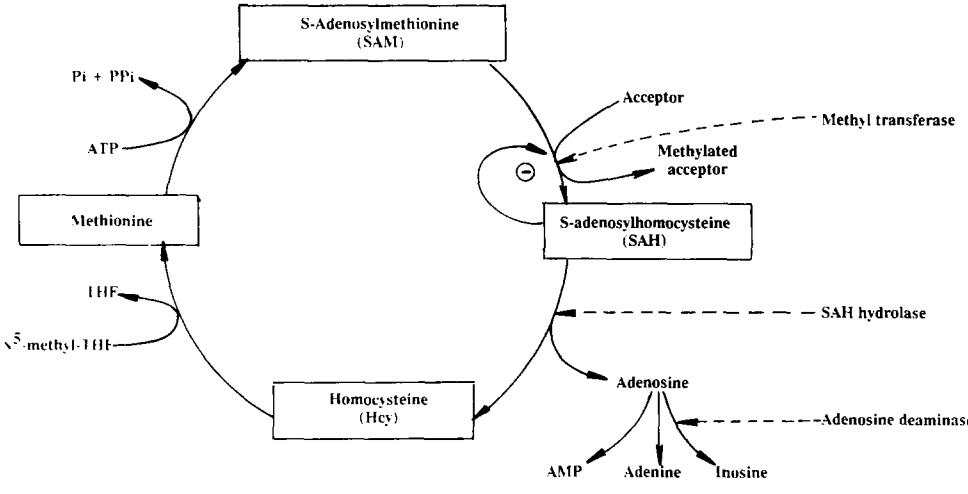

Fig. 6. Mechanism of action of acyclic and carbocyclic adenosine analogues as inhibitors of SAH hydrolase. Key enzymes involved in methylation reactions: methyltransferases (SAM → SAH + Methyl); SAH is a feedback inhibitor of all transmethylation reactions in which SAM is the methyl donor. – SAH hydrolase (SAH → Hcy + Ado). – Adenosine deaminase (Ado → Ino).

differs from that of the SAH hydrolase inhibitors and when it was evaluated as an inhibitor of SAH hydrolase it was found inactive (Van Aerschot et al., 1989). Recently, 3'F3'Ado was evaluated for activity against several arthropod-borne and arenaviruses and it proved active against togaviruses (Semliki Forest, Venezuelan equine encephalitis, flaviviruses (banzi), reoviruses (Colorado tick fever), arenaviruses (lymphocytic choriomeningitis, but not Pichinde) and bunyaviruses (San Angelo but not Punta Toro) (Smee et al., 1992). Lymphocytic choriomeningitis virus belongs together with Lassa fever virus to the 'Old World' arenavirus group, whereas Pichinde, like Junin, Tacaribe and Machupo, is a member of the 'New World' group (or Tacaribe complex) of arenaviruses (Bishop, 1990a). Bunyaviruses represent an heterogenous collection of different virus subgroups, all having a tripartite genome and a lipid envelope. Punta Toro virus belongs to the Phlebovirus group, whereas San Angelo virus falls in the Bunyavirus group. The mode of genome replication is different for these two groups (Bishop, 1990b). The target for the antiviral action of 3'F3'Ado appears to be viral RNA synthesis. To interact at this level the compound must be converted intracellularly to its 5'-triphosphate. Viral RNA synthesis is preferentially inhibited over cellular RNA synthesis in Semliki Forest virus-infected cells (Smee et al., 1992).

Sulfated polysaccharides

Polyanionic substances, particularly sulfated polysaccharides, have been known for some time to interfere with the virus adsorption process two decades ago (De Somer et al., 1968a,b). The inhibitory effects of sulfated polysaccharides (i.e., dextran sulfate, pentosan polysulfate and heparin) (Fig. 7) on the replication of several enveloped viruses including retroviruses (human immunodeficiency virus), herpesviruses (herpes simplex, cytomegalovirus), poxviruses (vaccinia virus), togaviruses (Sindbis, Semliki forest), arenaviruses (Junin, Tacaribe), paramyxoviruses (respiratory syncytial virus) and rhabdoviruses (vesicular stomatitis virus) have been well established (Tables 3 and 4) (Baba et al., 1988a,c). As a rule, non-enveloped viruses are insensitive to inhibition by the sulfated polysaccharides. More recently, it has been shown that also sulfated cyclodextrins (Schols et al., 1991), low-molecular-weight dextran sulfate (Witvrouw et al., 1991), sulfated polymers [i.e., sulfated polyvinyl alcohol (PVAS) and the co-polymer of acrylic acid with sulfated vinylalcohol (PAVAS) (Schols et al., 1990a)], polyoxometalates (Yamamoto et al., 1992), polyacetal polysulfate (termed PAPS) (Witvrouw et al., 1992) are inhibitory to various enveloped viruses. These findings indicate that the polysaccharide backbone is not essential, since sulfated polymers (i.e., PVAS, PAVAS, PAPS), containing an undegradable C-C-C-C backbone or C-C-O-C backbone are as active as the sulfated polysaccharides.

The mechanism of action of the sulfated polymers against HIV can be attributed to an inhibition of HIV attachment to $CD4^+$ cells, as has been demonstrated by monitoring virus-cell binding with radiolabeled virus particles (Baba et al., 1988b; Mitsuya et al., 1988), radioimmunoassay (Nakashima et

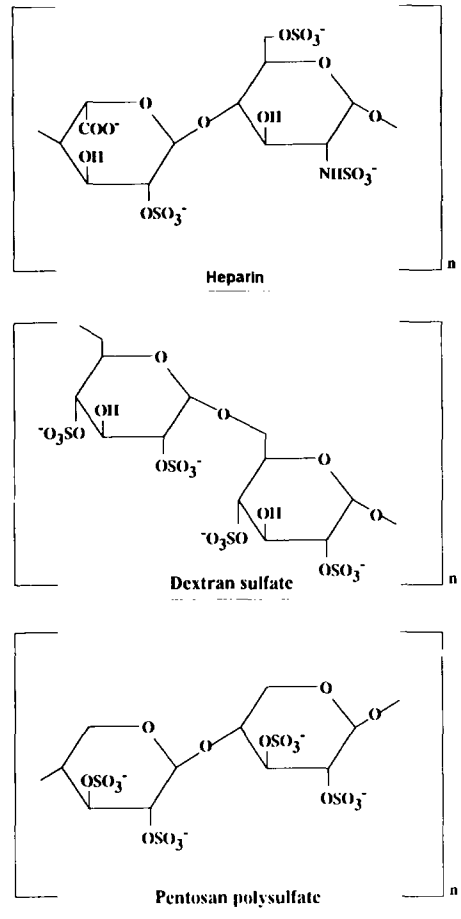

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Fig. 7A. Sulfated polysaccharides: heparin, dextran sulfate, pentosan, polysulfate.

al., 1989) and flow cytometry (Schols et al., 1989a, 1990b, 1991). The site of attack of these polyanionic substances is the interaction between the viral envelope glycoprotein gp120 and the cellular CD4 receptor. Since this interaction is needed not only for HIV adsorption to the cells but also for syncytium formation between HIV-infected cells and uninfected $CD4^+$ cells, dextran sulfate and other sulfated polysaccharides are indeed potent inhibitors of HIV-induced syncytium formation (Mitsuya et al., 1988; Bagasra and Lischner, 1988; Baba et al., 1990b,c). Syncytium formation is accompanied by the destruction of the target $CD4^+$ cells (Schols et al., 1989c, 1990b).

In their effect on the viral gp120/cellular CD4 interaction, it is assumed that aurintricaboxylic acid specifically binds to the CD4 receptor (Schols et al., 1989b) whereas dextran sulfate and other sulfated polysaccharides specifically

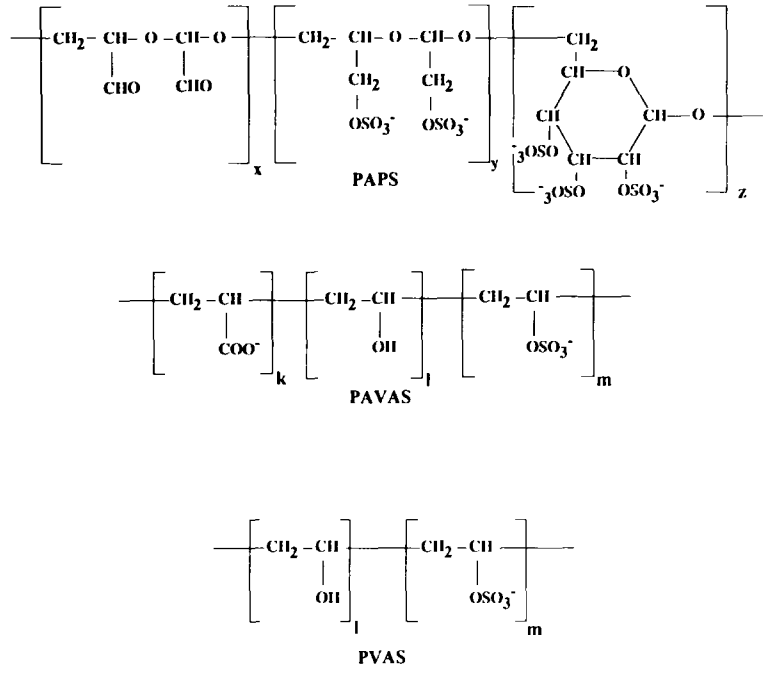

Fig. 7B. Sulfated polymers: polyacetal polysulfate (PAPS), polyvinylalcohol sulfate (PVAS) and co-polymers of acrylic acid with sulfated vinylalcohol (PAVAS) (x, y, z, k, l and m represent portions of the different components).

bind to, and thus shield off, the gp120 glycoprotein (Schols et al., 1990c).

Recently, it has been demonstrated that the mechanism of action of the sulfated polymers against cytomegalovirus (CMV) may be attributed to an inhibition of binding of virus particles to the host cells (Neyts et al., 1992). Herpes simplex virus and cytomegalovirus were shown to interact with heparan sulfate at the cell surface (WuDunn and Spear, 1989; Neyts et al., 1992). It may be assumed that sulfated polymers achieve their anti-cytomegalovirus or anti-herpes simplex virus activity by occupying the sites on the virion envelope that are necessary for attachment of the virus particles to cell surface heparan sulfate (Lycke et al., 1991; Neyts et al., 1992).

In analogy with other enveloped RNA viruses, the attachment of arenaviruses to the surface of the host cell probably occurs through the interaction of the viral envelope glycoprotein(s) with a receptor protruding from the cell membrane (Bishop, 1990a). Which viral glycoprotein is responsible for the attachment to the cellular receptor is unknown. Two glycoproteins (G1 and G2) have been identified in arenaviruses, which are present in essentially equimolar proportions on the surface of the virions. Yet, only one particular size has been observed for the glycoproteins of Junin, Tacaribe and Tamiami viruses (Bishop, 1990a). These viruses may thus possess

two glycoprotein species of the same size.

A phase I/II trial of orally administered dextran sulfate for the treatment of the acquired immunodeficiency syndrome (AIDS) has indicated little clinical efficacy (Abrams et al., 1989). This is not surprising since it was found that the oral bioavailability of dextran sulfate is less than 1% (Lorentsen et al., 1989). Although the in vitro toxicity of the sulfated polymers is very low, these compounds are known to interfere with the blood coagulation process. This anticoagulant activity might hamper their practical usefulness as antiviral agents against hemorrhagic fever viruses. Nevertheless, the sulfated polymers have proved inhibitory to various enveloped viruses in vitro at concentrations that are far below their anticoagulant threshold (Schols et al., 1990a). In addition, as demonstrated with chemically modified heparin derivatives, anti-HIV activity can be dissociated from antithrombin activity (Baba et al., 1990a).

Interferon and immunomodulators

The study of potential immunomodulators as antiviral agents for the treatment of some viral hemorrhagic fevers is of particular importance since some viral infections are associated with a rapid immunosuppression of the host. Different substituted pyrimidine immunomodulators have proved effective when they were evaluated in mice against the hepatotropic and immunosuppressive Punta Toro virus, a Phlebovirus related to Rift Valley fever virus (Sidwell et al., 1990, 1992). The derivative 5-bromo-2,3-dihydro-2-imino-6-phenyl-4(1H)-pyrimidine (ABPP) emerged as the most active of this series of pyrimidinones. ABPP was able to prevent death and decrease the severity of hepatitis, not only when it was administered prior to virus exposure, but also as late as 24 or 48 h after virus infection. It is possible that the activity of these pyrimidinone immunomodulators primarily results from interferon induction. Several of these compounds are known to be rapid interferon inducers (Nichols et al., 1976; Stringfellow, 1977, 1981) and on the other hand, it has been shown that Punta Toro virus-resistant mice treated with anti-interferon antibody are rendered strikingly sensitive to infection by the virus (Pifat and Smith, 1987). However, these substituted pyrimidones have a spectrum of other immunomodulatory properties, including macrophage activation, augmentation of natural killer cell cytotoxicity, induction of polyclonal B-cell response, induction of IL-1 and IL-2, enhancement of antigen-mediated antibody formation and stimulation of bone marrow proliferation (Wierenga, 1985). Through some of these immunomodulating properties, the compounds may limit the viral infection. ABPP, a potent interferon inducer, has proved efficacious in the mouse model for Punta Toro virus infection while another substituted pyrimidone, ABMP (5-bromo-2,3-dihydro-2-imino-6-methyl-4(1H)-pyrimidine, which is also a good interferon inducer, was only weakly effective in this animal model (Sidwell et al., 1990). Some of these pyrimidinones have also proved to be effective in the treatment of a variety of DNA and RNA virus infections in mice, including infections induced by Semliki Forest virus, encephalomyocarditis virus, herpes simplex

virus types 1 and 2, cytomegalovirus, and pseudorabies virus. Also, influenza and parainfluenza virus infections of hamsters have responded to ABPP therapy (Wierenga, 1985).

Recently, the nucleoside analogue 7-thia-8-oxo-guanosine (TOGuo) has been reported to have broad-spectrum antiviral activity in vivo against several DNA and RNA viruses (Smee et al., 1989, 1990a). It has immunoenhancing properties: it induces interferon and activates natural killer cells (Smee et al., 1990b), however, it does not inhibit virus replication in vitro even at high concentrations. The activity against Semliki Forest and Punta Toro virus infections in mice appears to be due to interferon induction (Smee et al., 1990b, 1991b). When ribavirin was combined with TOGuo to treat Punta Toro virus infection in mice, TOGuo was shown to increase the therapeutic index of ribavirin by decreasing toxicity and increasing antiviral activity in this model (Smee et al., 1991a). If combined, the ribavirin and the interferon inducer poly(ICLC) showed enhanced therapeutic efficacy against Rift Valley fever virus infection in mice (Kende et al., 1987). Also, TOGuo and ribavirin, when combined, may show enhanced potency against Rift Valley fever virus infection in vivo.

Studies in experimentally RVF virus-infected rhesus monkeys have demonstrated that recombinant human interferon-αA or recombinant human interferon-γ reduces viremia and clinical disease (Morrill, 1989, 1991b). In contrast, arenaviruses are known to be relatively insensitive to the action of interferons (Stephen et al., 1977; Canonico et al., 1984). A Pichinde virus infection in a guinea pig model did not respond to therapy with interferon or a known interferon inducer, CL246,738 [3,6-bis(2-piperidinoethoxy)acridine trihydrochloride] (Lucia et al., 1989), while this compound protected mice against the lethal encephalitis produced by Semliki Forest virus and the flavivirus Banzi (Sarzotti et al., 1989). It is assumed that the induction of interferon may be directly responsible for the antiviral action of the drug (Sarzotti et al., 1989). Ampligen, an interferon-inducing mismatched double-stranded RNA molecule (Montefiori and Mitchell, 1987; Sidwell et al., 1992) was found inactive against Pichinde virus infection in hamsters (Smee et al., 1993).

Acknowledgements

The dedicated editorial assistance of Christiane Callebaut is gratefully recognized. We thank the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (FGWO) for support of the original investigations.

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