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In-vivo activity of antivirals against exotic RNA viral infections

Peter G. Canonico, Meir Kende, Bruno J. Luscri and John W. Huggins

Department of Antiviral Studies, Virology Division,
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick
Frederick, MD 21701, U.S.A.

Infection of humans by viruses belonging to the families of Toga-, Bunya-, and Arenaviridae constitutes a major health problem worldwide and certain of the viruses have the potential to cause widespread epidemics. In the search for effective chemotherapy against these viruses several hundred nucleoside and nucleotide analogues have been screened for antiviral activity. Of the compounds tested, ribavirin, has been shown in laboratory animal models to have significant inhibitory effects against Rift Valley Fever virus, the bunyavirus Punta Toro, Hantaan virus and arenaviruses such as Pichinde, Junin, Machupo and Lassa Fever. Clinical studies with ribavirin in persons infected with Lassa Fever virus are in progress. Ribavirin or certain analogues have no detectable *in vivo* activity against arboviral encephalitic infections caused by Venezuelan or Western Equine Encephalitis or Semliki Forest Viruses. Other compounds including a series of triazolo derivatives, and the thiazole carboxamide nucleoside Tiazofurin appear to have *in-vivo* activity against certain of these exotic RNA viruses.

Introduction

Infection by viruses belonging to the families of Toga-, Bunya-, and Arenaviridae constitutes a major health problem worldwide with the potential for causing devastating epidemics whose circumstances are often poorly understood (Downs, 1976; Casals, 1976). These insect- and rodent-transmitted RNA viruses no longer pose routine health problems in more affluent cultures due to improved sanitation and vector control of insect and rodent hosts. Consequently, the development of prophylaxis and therapy for these exotic diseases has received less attention than the more commercially exploitable infections by herpes, influenza and rhinoviruses.

An ambitious antiviral development programme directed toward exotic diseases currently exists at the U.S. Army Medical Research Institute of Infectious Diseases,

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense. In conducting the research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals', as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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Table I. Exotic RNA viruses causing disease in man

	Pathogenesis in man
Bunyaviridae	
Sandfly fever	Fever
Rift Valley fever	Fever
Oropouche	Fever
Congo Crimean	Haemorrhagic fever
La Crosse viral encephalitis	Encephalitis
California encephalitis	Encephalitis
Arenaviridae	
Lassa	Fever
Junin	Haemorrhagic fever
Machupo	Haemorrhagic fever
Lymphocytic choriomeningitis	Fever
Unclassified	
Hantaan	Haemorrhagic fever
Marburg	Haemorrhagic fever
Ebola	Haemorrhagic fever
Togaviridae	
Alphavirus	
Venezuelan equine encephalitis	Encephalitis
Eastern equine encephalitis	Encephalitis
Western equine encephalitis	Encephalitis
Chikungunya	Fever
O'nyong-nyong	Fever
Flavivirus	
Dengue 1–4	Fever
St Louis encephalitis	Encephalitis
Japanese encephalitis	Encephalitis
West Nile encephalitis	Encephalitis
Yellow fever	Haemorrhagic fever

a component laboratory of the Walter Reed Army Institute of Research in Washington D.C. Viruses of interest include the alpha and flaviviruses of the Togaviridae family which are capable of producing relatively harmless undifferentiated fevers as well as more serious haemorrhagic and encephalitic diseases (Table I) (Fenner & White, 1976). Alphavirus infections by Eastern, Western and Venezuelan Equine encephalitis have caused severe epidemics in man and horses. The 1971 Venezuelan equine encephalitis outbreak in Texas caused the death of thousands of horses and 88 non-fatal human cases. Chikungunya, O'nyong-nyong, Ross river and Mayaro viruses continue to cause epidemics worldwide.

The flaviviruses include members that cause significant human disease. These include dengue virus infections with their characteristic sudden onset of fever, chill, rash and aching pains of the back, muscles and joints; and a number of viruses such as St. Louis, Russian spring-summer, Japanese encephalitis, and West Nile producing mild to severe encephalitic disease. Yellow fever is endemic in forested regions of Africa and the Americas and is capable of causing devastating urban epidemics; it has been a major problem for over two centuries and its severe potential was realized as recently as 1962 in Ethiopia.

The Bunyaviridae family comprises more than 200 named viruses divided into four genera (Peters & LeDuc, 1984). Most of these viruses are found in tropical areas of Africa and Latin America, but the family is also well represented in temperate climates. Sand fly fever has been recognized as an important febrile illness in the Mediterranean. The clinical syndrome is characterized by sudden onset of fever, severe frontal headache, pain in the eyes, back and joints, photophobia, anorexia and general malaise. Rift Valley fever virus, another member of the family, is pathogenic for domestic animals and is capable of causing serious and fatal infections of man. Since its isolation in 1931, this virus has repeatedly infected domestic herds producing mortality rates of 10 to 30%. The extension of Rift Valley fever virus into Egypt resulted in extensive human and animal disease in 1977–1979. The disease is usually uncomplicated, heralded by the abrupt onset of fever, chills and a sand fly fever type of illness. Incapacitating prostration, myalgia, and fever typically resolve in 2 to 3 days but may last longer. Although Rift Valley fever had been regarded as a non-fatal disease, it was recognized in 1975 that some humans infected with the virus develop fatal haemorrhagic fever rather than a temporarily incapacitating illness and recently, encephalitis has been added to the clinical spectrum of the human disease. Bunyaviruses have also made an impact in the Americas. Oropouche virus, for example, has caused at least eight major epidemics in northern Brazil within the past 20 years, infecting more than 165,000 persons in remote population centres with attack rates of the local residents as high as 40%. La Crosse virus is the most frequent cause of childhood encephalitis in North America and the related Tahyna virus infects humans in many areas of Europe although its disease spectrum is not yet thoroughly understood.

In the Arenaviridae family (Peters & LeDuc, 1984), Lassa fever is the most recently established human pathogen. Severe human Lassa fever progresses from early non-specific symptoms to obvious multi-system involvement to abrupt onset of shock leading to death. From 1977 to 1979, two hospitals in Sierra Leone attributed 12% of their admissions to Lassa fever. Death due to Lassa fever accounted for 30% of all medical deaths at these facilities. Other countries in western Africa have clinical or serological evidence of Lassa fever virus. The actual number of illnesses may be tens of thousands per year and the impact of the disease on human health appears to be considerable. Other severe arenavirus infections include the South American haemorrhagic fever viruses, Junin and Machupo.

Within the last few years a number of related viruses have been identified worldwide which cause severe haemorrhagic fevers with renal syndrome. Hantaan virus, the causative agent of Korean haemorrhagic fever is widely distributed across northern Asia. The disease is characterized by haemorrhage, severe renal failure and high mortality. A milder form of the disease (nephropathia epidemica) is distributed across northern Europe. Finally, Marburg and Ebola, two closely related viruses comprise a new family of African viruses (proposed Filoviridae) causing severe haemorrhagic fever (McKee *et al.*, 1984).

In-vivo efficacy of ribavirin against exotic RNA viruses

In the search for effective chemotherapy against these viruses several hundred nucleoside and nucleotide analogues have been screened for antiviral activity. None has been more efficacious than ribavirin (Figure 1), a relatively non-toxic, non-

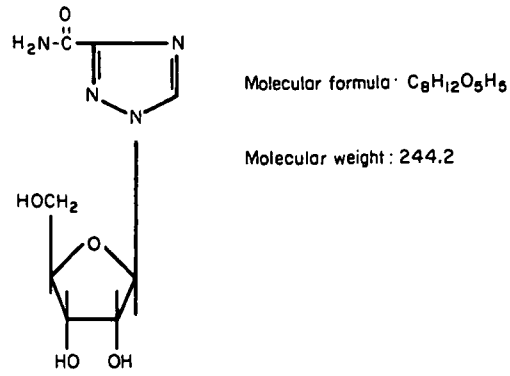


Figure 1. Structure of ribavirin: 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (virazole).

immunosuppressive nucleoside analogue (Huggins *et al.*, 1983). Ribavirin has been used successfully to treat experimental Rift Valley fever infections in several strains of mice when given intraperitoneally, subcutaneously or orally. Ribavirin therapy can result in 90% or greater protection if mice given a potentially lethal dose are treated on days 0 to 4 of infection with 60 to 100 mg of ribavirin/kg body weight (Stephen *et al.*, 1980). A significant increase in survival is still observed if treatment is delayed for as long as 4 days. In the rhesus monkey, Rift Valley fever virus causes a transient viraemia usually without overt clinical signs. Ribavirin virtually eliminates viraemia in this model when administered at 30 mg/kg bid following a 50 mg/kg loading dose (Stephen *et al.*, 1980). At 100 mg/kg/day it increases from 10 to 90% the survival rate of hamsters infected with Punta Toro, another member of the Bunyaviridae. In a limited trial, an effect also has been shown against Hantaan virus in the striped field mouse, the only available animal model for Korean haemorrhagic fever (Lee *et al.*, 1981).

It is against the arenaviruses that ribavirin has shown the most promise. Pichinde virus, which can be inhibited *in vitro* by ribavirin, produces a lethal infection in hamsters and guinea pigs, and serves as a safer model for arenavirus infections. Treatment of Pichinde virus infected hamsters with ribavirin results in an increase in survival from 11 to 100%. Similar results are obtained with guinea pigs. Ribavirin efficacy has been evaluated against Junin virus, the causative agent of Argentine haemorrhagic fever. Infected strain-13 guinea pigs die of a haemorrhagic illness similar to the human disease and have no detectable virus in the brain. Ribavirin treatment at 45 mg/kg/day protects animals from haemorrhagic illness. However, all animals ultimately develop neurological symptoms and finally die with high virus titres in the brain (Kenyon, personal communication). Thus, the neurovirulence of Junin virus revealed in ribavirin treated guinea pigs may be masked in untreated guinea pigs by their early death from haemorrhagic disease. Neurological manifestations are well documented in human and monkey infections. Thus, in Junin virus infected guinea pigs, ribavirin is efficacious in the treatment of haemorrhagic symptoms, but not the ensuing encephalitis.

Bolivian haemorrhagic fever caused by Machupo virus produces a similar disease to Junin. Ribavirin and the triacetyl derivative significantly increase survival of infected guinea pigs. Machupo-infected rhesus monkeys develop a peak viraemia by day 15 and uniformly die with a mean time to death of 26 days (Stephen *et al.*, 1980). Treatment of monkeys with ribavirin [Figure 2(b)] and triacetyl-ribavirin [Figure

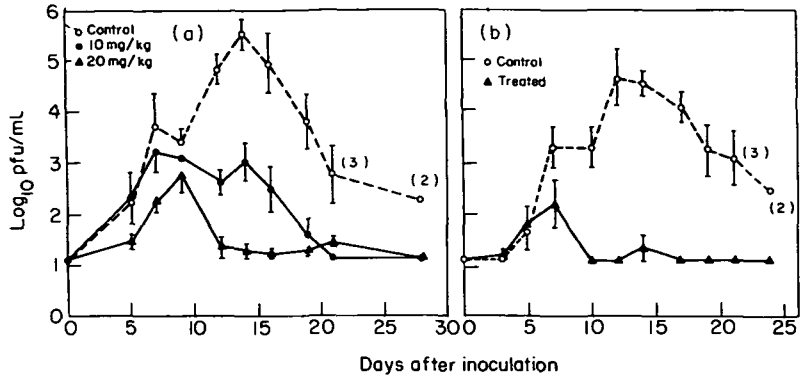


Figure 2. Effect of ribavirin triacetate (a) and ribavirin (b) on Machupo virus-infected rhesus monkeys. Monkeys were inoculated subcutaneously with about 10^3 pfu of the fourth suckling hamster brain passage of Carrallo strain of Machupo virus. Ribavirin triacetate was given at the dosages indicated intramuscularly twice daily on days 0 to 17. Ribavirin was administered intramuscularly at a dosage of 10 mg/kg bid for 10 days. Viraemia is indicated on the ordinate (Stephen *et al.*, 1980).

2(a)] decreases viraemia and in contrast to sham-treated animals, survive the acute phase of illness. Similar results are obtained even when treatment is delayed until the onset of fever. In both instances, however, treated survivors eventually succumb to a chronic secondary neurological syndrome. This phase of the disease is not seen in man. Thus, ribavirin can significantly reduce viraemia and protect monkeys from the acute haemorrhagic phase characteristic of human Bolivian haemorrhagic fever.

Lassa infected cynomolgus monkeys develop high viraemias maximal on day 10 [Figure 3(a)]. Monkeys ultimately die with a mean time to death of 15 days. Ribavirin has been evaluated in this model using a loading dose of 75 mg/kg followed by 15 mg/kg twice daily (Jahrling, 1984). Monkeys treated beginning on day 0 have a significantly reduced viraemia. The longer the delay in the initiation of treatment, the greater the resulting viraemia. All monkeys treated initially on days 0 or 4 survive. However, when treatment is delayed until day 7 only four of eight monkeys survive [Figure 3(c)].

In this study, plasma with high neutralizing antibody titres obtained from rhesus monkeys who survived Lassa infection was used to test the possible benefit of combination therapy (Jahrling, Petus & Stephens, 1984). While immune plasma alone reduced viraemia in all cases it did not prevent death if administered following the third day of infection. In contrast, combined treatment of ribavirin and immune plasma is very successful and saves all treated animals. This combination treatment even when begun as late as day 10, at a point when all monkeys are seriously ill and when some untreated animals begin to die, results in a decrease in viraemia and 100% survival [Figure 3(d)].

Clinical studies with ribavirin

The success of the combination treatment as described above in animal models is striking and has been adopted in clinical trials conducted in Sierra Leone by Dr J. McCormick of the Centres For Disease Control in Atlanta, Georgia (Table II). These studies are still in progress, but results recently reported at the Second Symposium

Table II. Ongoing clinical trials of ribavirin against RNA viruses

Viral illness	Principal investigator	Location	Protocol type
Lassa fever	J. McCormick	Sierra Leone	Open
Respiratory syncytial virus	C. Hall	University of Rochester	DB
Viral respiratory complications	E. Gelfand	University of Toronto	Open
Viral respiratory complications	K. McIntosh	Harvard Medical School	Open
Influenza	V. Knight	Baylor	DB
Influenza	G. Schiff	Cincinnati	DB

DB = Double-blind placebo controlled.

on ribavirin (1983) indicate that ribavirin may be effective in the treatment of Lassa fever in man. High viraemia ($>4{\cdot}0\times10^{-4}$ $TCID_{50}$) and elevated SGOT (>150 μ/ml) effectively predicted lethal outcome in more than 80% of cases during initial studies. Preliminary results of ribavirin treatment show a mortality of only 10% in these high risk patients.

Ribavirin also has been evaluated clinically against other RNA virus infections. Dr Carolyn Hall and co-workers evaluated ribavirin administered orally to adult volunteers against a mild respiratory syncytial virus strain. Ribavirin affected systemic symptoms including fever and reduced viral shedding after day 6, although there was no effect on viraemia. Infants with respiratory syncytial virus pneumonia were also treated in a double blind, placebo-controlled aerosol ribavirin study. This treatment reduced severity of illness by day 3, as well as virus shedding and viral titre. The number of patients treated to date is small and definitive conclusions must await additional results.

Efficacy against virus encephalitis

Although ribavirin has *in-vitro* activity against a number of encephalitic viruses, it has not been possible to demonstrate any *in vivo* efficacy against Venezuelean and Western equine encephalitis, Banzi, Japanese encephalitis, intracerebrally inoculated Semliki Forest, and the encephalitic phase of illness due to Rift Valley fever, Junin and Machupo. The reason for this lack of efficacy is probably the relative inability of ribavirin to cross the blood brain barrier and concentrate in the central nervous system.

To improve efficacy against viral encephalitic infections, we screened analogues which might cross the blood brain barrier (Figure 4). These include the acetyl-, valeryl-, butyryl- derivatives of ribavirin as well as the mono-, di- and triphosphate and cyclic phosphate compounds. However, none of these analogues demonstrated any greater efficacy against Venezuelan encephalitis virus in mice than the parent compound. Smee *et al.* (1981) used intraperitoneal administration of ribavirin tri-acetate to treat Colorado tick fever virus-induced encephalitis in mice and reported a significant increase in mean survival time and total survivors. While these data suggest that ribavirin tri-acetate can reach the brain, the dose of drug used in this study was nearly ten times larger than the dose commonly used to treat systemic viral illness in mice.

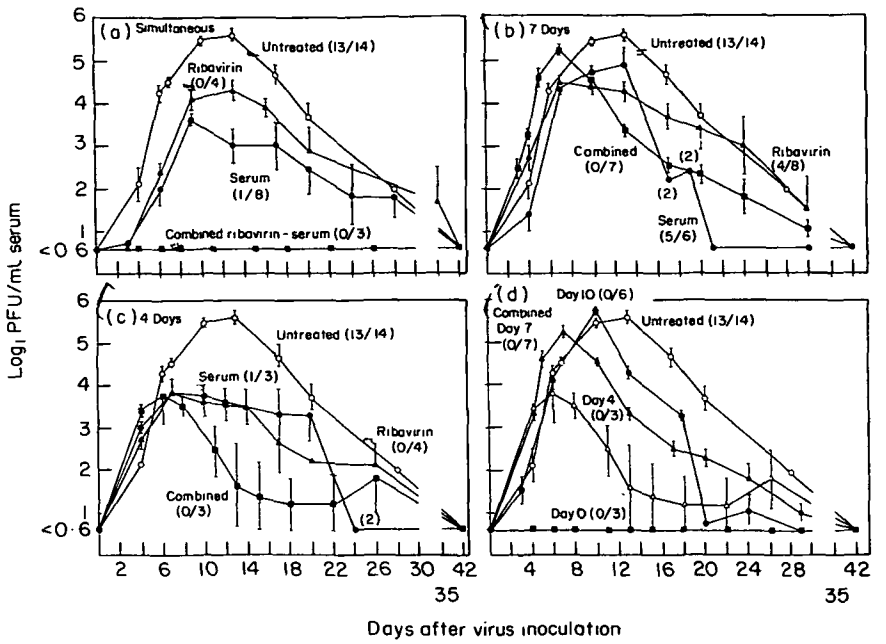


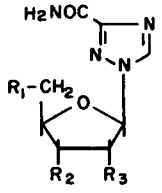
Figure 3. Lassa viraemia and mortality (dead/total) in groups of cynomolgus monkeys treated with immune serum (1 mg/kg), ribavirin (30 mg/kg), or combined serum plus ribavirin treatment, (a) initiated simultaneously with Lassa virus inoculation (day 0), (b) initiated on day 4, or (c) initiated on day 7. Panel 1d compares viraemias in groups of monkeys receiving combined treatment initially on days 0, 4, 7, or 10, versus untreated controls. All points are geometric means (±S.E.M.), based on all monkeys in each group (Jahrling *et al.*, 1984).

Tissue targeting of antiviral agents

Targeting of antiviral agents to specific tissue sites can improve the overall efficacy of a drug. We have found that liposome encapsulated ribavirin is more efficacious against Rift Valley fever infection than free drug. Rift Valley fever replicates in phagocytic cells and by targeting ribavirin to the reticuloendothelial system we can obtain a higher level of efficacy. Ribavirin at concentrations up to 25 mg/kg extends the mean time to death of Rift Valley fever infected mice but does little to improve the survival rate (Figure 5). When the drug is encapsulated in liposomes a 50% survival rate is attained with as little as 12·5 mg/kg of the drug. Since a number of other bunya-, flavi- and most arenaviruses including Lassa fever virus replicate in phagocytic cells, the use of liposomes to target antivirals to the reticuloendothelial system may soon find broader applications in the treatment of these diseases.

Other compounds

Hundreds of other compounds also have been evaluated in the antiviral screening programme at USAMRIID. Candidate compounds submitted for screening may be evaluated against 11 RNA viruses *in vitro* and 8 animal models. A few novel compounds showing promise as potential antivirals are presented in Figures 6 and 7. The series of triazole derivatives in Figure 6 are of interest since compounds [1] and [2] are inactive *in vitro* but show a moderate level of activity against Rift Valley



R_1	R_2	R_3
Valeryl	OH	OH
Caproyl	OH	OH
Butaryl	OH	OH
Acetyl	Acetyl	Acetyl
Monophosphate	OH	OH
Diphosphate	OH	OH
Triphosphate	OH	OH
Monophosphate	Acetate	Acetate
Monophosphate	Butaryl	Butaryl
3'-5'cyclic phosphate		OH
3'-5'cyclic phosphate		Acetyl
'OH	2'-3'cyclic phosphate	

Figure 4. Analogues of ribavirin tested in mice for efficacy against Venezuelan equine encephalitis virus.

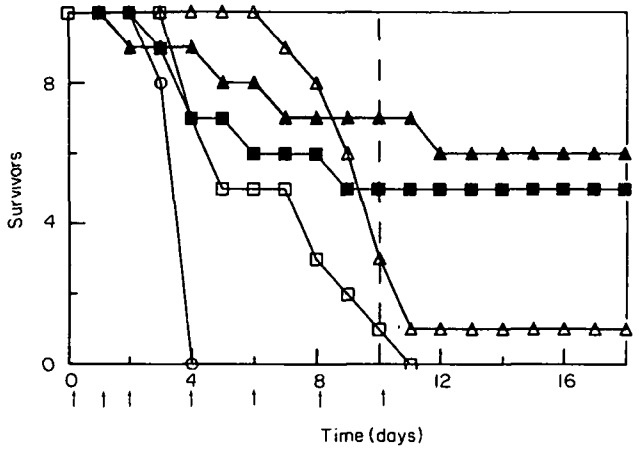


Figure 5. Efficacy of ribavirin/liposome or ribavirin treatment in Rift Valley fever virus-infected mice. Mice were injected subcutaneously with 5000 pfu of virus on day 0 and treated intravenously as indicated on days 0–2, then on alternate days through day 12. Liposomes without drug had no therapeutic value. Nine of ten mice given liposomes alone succumbed to the infection (data not shown). ▲, ■, Drug and liposomes; △, □, ○, drug; △, 25 mg/kg; □, 12·5 mg/kg; ○, placebo.

fever in mice. This suggests that these compounds may be metabolized *in vivo* to an active product. Compound [3] is only slightly active in mice but it is fairly active against Rift Valley fever *in vitro*.

The selenium and sulphur containing compounds in Figure 7 also have shown good *in-vitro* activity. Compound [4] was reported by Bonina *et al.* (1982) to be active against poliovirus 1, echovirus 1 and coxsackievirus B4. We have found compound [5] to be active against several viruses *in vitro* and is yet to be tested in mice. Tiazofurin [6] is a thiazole carboxamide nucleoside which we have found to be active against Yellow fever, Japanese encephalitis, and Korean Haemorrhagic fever. The selenium analogue [7] is exceptionally active against a broader range of viruses. The *in-vivo* evaluation of both tiazofurin and selenazole is in progress. Both

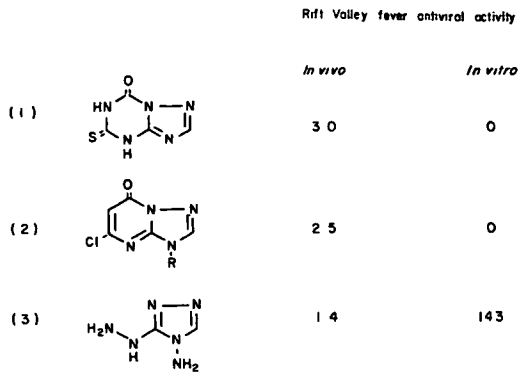


Figure 6. Antiviral activity of (1) 5-thio-s-triazole [1,5-2]-s-triazin-5, 7-dione; (2) 5-chloro-3-(β-D-ribofuranosyl)-s-triazole [1,5-a] pyrimidin-7-one; (3) 4-amino-3-hydrazino-1,2,4-triazole dihydrochloride. The *in-vitro* antiviral activity is given as the geometric mean time to death of experimental group/geometric mean time to death control group. *In-vitro* therapeutic index is given as the ratio of minimum toxic concentration/inhibitory dose$_{50}$. The *in-vitro* ID$_{50}$ of the latter compound was 1·4 mg/l.

compounds are thought to cross the blood brain barrier raising the possibility that they may be efficacious in treating encephalitic viral illnesses. Both drugs, however, appear to be rapidly excreted and appropriate treatment schedules must be established before their full potential can be realized.

Combination chemotherapy

In our search for more effective treatment approaches, we have tested combinations of antiviral compounds. The use of two drugs with different modes of action has the potential for synergistic drug interactions. *In vitro* studies were performed on binary combinations of selenazole or tiazofurin with ribavirin (Huggins *et al.*, 1983). Drug interactions were evaluated graphically with the use of isobolograms (Figure 8). The isobologram is constructed by plotting the amount of drug A and B alone or in combination that produces the same effect (50% plaque reduction). Points which fall on the diagonal indicate additive effects, whereas, synergy is indicated when points fall substantially below the additive line. The strongest synergy was found with ribavirin and selenazole against Japanese encephalitis and Venezuelan encephalitis virus (Figure 9). The ED$_{50}$'s of the drugs in combination were a small fraction of the concentration of either drug alone. Synergy was shown for tiazofurin in combination with ribavirin against yellow fever but not for other viruses. The inhibition of the bunyaviruses, Rift Valley fever and Korean haemorrhagic fever, by combinations of these drugs was simply additive. This differential inhibition of viruses in the same cell line is evidence that the synergistic inhibition of the flaviviruses is a virus specific effect. These observations provide the basis for the design of combination chemotherapy in experimental animal models of encephalitic flavivirus infections.

Macrophage activation

Activated macrophages can demonstrate intrinsic or extrinsic resistance to viral proliferation. The intrinsic resistance is probably mediated by soluble intracellular factors such as enzymes and oxygen metabolites. Extrinsically, macrophages can

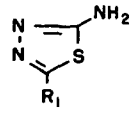
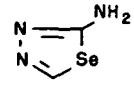
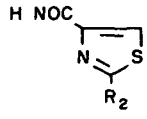
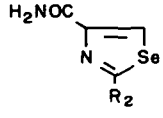
		In-vitro therapeutic index			
		Venezuelan encephalitis	Japanese encephalitis	Rift valley fever	Pichinde
(4)		NT	NT	NT	NT
(5)		380	719	1110	919
(6)		0	71	0	10
(7)		149	312	357	232

Figure 7. *In-vitro* antiviral activity of (4) 2-amino-5-(2-sulphanoylphenyl)-1,3,4-thiadiazole; (5) 2-amino-1,3,4-selenazole; (6) 2-β-D-ribofuranosyl thiazole-4-carboxamide (tiazofurin); (7) 2-β-D-ribofuranosyl-selenazole-4-caboxamide (selenazole). Therapeutic indexes were calculated as described in Figure 6. The *in-vitro* minimum toxic concentration for compounds 5, 6 and 7, respectively, were >441, >500, and >1000 mg/l. NT = not tested.

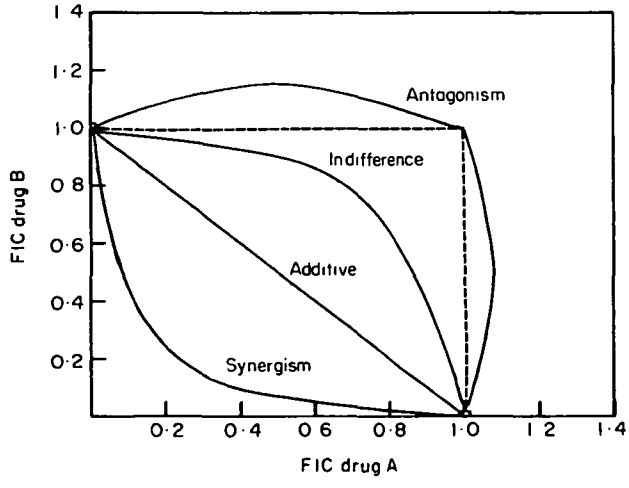


Figure 8. Isobologram of possible drug interactions.

affect viral replication in host cells via cytolysis. In view of the macrophage's role in protection of the host against microbial infection, one should recognize that non-immune activation of macrophages may be usefully manipulated for the treatment of acute viral infections. The non-specific activation of mononuclear phagocytes *in vivo* is gaining attention as the number of agents capable of effective stimulation increases (Table III).

Table III. Activators of host resistance to viral infections

Macrophage activators	Virus	Reference
Muramyl tripeptide	Rift Valley fever	Kende *et al.* (1983)
	Parainfluenza	Detrick *et al.* (1983)
	Influenza A & B	
	Vesicular stomatitis	
	Herpes simplex	
	Vaccinia	
	Encephalomyocarditis	
Glucan	Herpes simplex	Di Luzio (1984)
	Rift Valley fever	Reynolds *et al.* (1980)
	Hepatitis	Williams & Di Luzio (1980)
MVE-2	Encephalomyocarditis	Munson (1984)
	RNA tumor viruses	Chirigos *et al.* (1965, 1967)
Lipoidal diamine (CP-20,961)	Venezuelan equine encephalitis	Anderson & Reynolds, J. (1979)
	Rhinovirus	Niblack (1977)
Lentinan	Vesicular stomatitis	Chang (1984)
Biostim	Influenza	Rudent *et al.* (1982)
Pyran	Herpes simplex	Breinig *et al.* (1978), Morahan *et al.* (1972)
	Encephalomyocarditis	
	Friend leukaemia	
Poly(I). Poly(C)	Japanese encephalitis	Stephen *et al.* (1977)
	Yellow fever	

MVE-2: Copolymer of meleic anhydride and divinyl ether.

In a recent study, we sought to elicit the intrinsic virucidal activity of liver macrophages with one such macrophage activator, muramyl dipeptide. Since muramyl dipeptide is rapidly cleared from the body, liposome encapsulated muramyl dipeptide was used to effectively treat Rift Valley fever infection in mice (Kende *et al.*, 1983). Prophylactic treatment of mice with liposome encapsulated muramyl dipeptide initiated 2 h prior to virus challenge resulted in a 40% survival rate (Figure 10). None of the mice treated prophylactically with a mixture of empty liposomes and liposome encapsulated muramyl dipeptide survived. When administered therapeutically starting on day 3, there was a 60% survival rate. Even when treatment was delayed until day 5 of infection, 50% of the mice survived, while all untreated mice died by day 8.

Interferon

The antiviral effects of interferon are well documented. A survey of the *in-vitro* sensitivity of several RNA virus families to fibroblast interferon is shown in Table IV. Results are reported as relative sensitivity of viruses compared to Vesicular Stomatitis virus in order to standardize for the various cell lines used in these assays. Results show that alphaviruses are quite sensitive to beta-fibroblast interferon. Only Venezuelan equine encephalitis is comparatively less sensitive. The flaviviruses are relatively insensitive with the exception of Japanese encephalitis. This is consistent with published studies which successfully used the interferon inducer poly(I). poly(C) stabilized with carboxymethylcellulose and poly-l-lysine [poly (ICLC)]

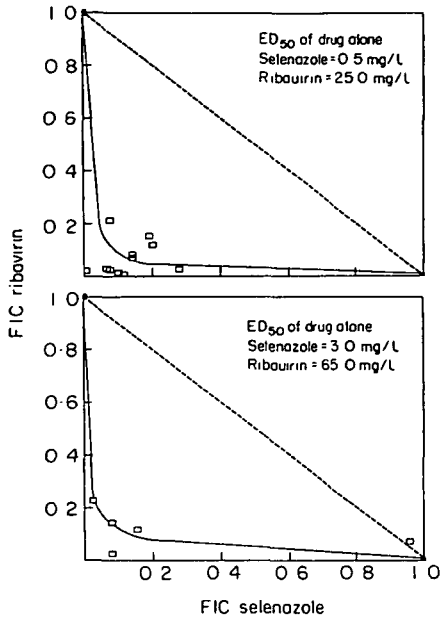


Figure 9. Isobologram for the combination of ribavirin and selenazole against Venezuelan equine encephalitis (top) and Japanese encephalitis (bottom). The combined fractional inhibitory concentration are 0·1 and 0·2, respectively.

against Japanese encephalitis virus infection of mice (Stephen *et al.*, 1977). Poly(ICLC) was also effective in increasing the percentage of survivors and the time to death of Japanese encephalitis virus-infected rhesus monkeys.

Ebola, an unclassified virus, is quite insensitive to alpha-fibroblast interferon *in vitro* and treatment with leucocyte interferon did not protect monkeys against Ebola infection (Bowen *et al.*, 1978). Arenaviruses, as well, are uniformly insensitive to beta-fibroblast interferon *in vitro*, an observation which extends to poly(ICLC) treated rhesus monkeys infected with Machupo virus. In these studies, monkeys developed greater viraemia than untreated controls in spite of the appearance of interferon in the serum. The mean time death of these poly (ICLC)-treated monkeys was similar to that for infected control monkeys (Stephen *et al.*, 1977).

Of the Bunyaviridae, Rift Valley fever is relatively sensitive to both alpha- and beta-fibroblast interferon *in vitro*. *In-vivo* studies indicate that RVF infected mice are protected by treatment with the interferon inducer and macrophage activator poly(ICLC) even when given as late as 3 days postinfection (C. J. Peters, personal communication). More recently, the value of interferon prophylaxis in Rift Valley fever virus infection was evaluated in the rhesus monkey (Jennings & Peters, personal communication). In these studies monkeys received 5×10^{-5} units/kg of either gene-cloned alpha-interferon or Sendai-induced human leukocyte interferon, predominantly alpha-interferon, for 5 consecutive days beginning 1 day prior to virus inoculation. The ensuing viraemia in all 6 control monkeys reached a peak of about 10^{-6} pfu/ml within 30 to 48 h, resolving by day 4 to 5 in the four monkeys which survived. All five monkeys receiving cloned interferon survived and only three of the monkeys developed a mild viraemia of brief duration. With the exception of a single measurement, none of the five monkeys receiving human leukocyte interferon became viraemic and all survived.

Table IV. Relative interferon sensitivity of RNA viruses

Virus	Assay cell line	Interferon sensitivity*		
		Alpha	Beta	Gamma
Vesicular stomatitis	MRC-5	1	1	1
Togaviridae				
Alphavirus				
Eastern equine encephalitis	MRC-5		2·5	0·2
Western equine encephalitis	MRC-5		2·5	
Sindbis	MRC-5		3·2	
Venezuelan equine encephalitis	MRC-5	0·2	0·5	
O'nyong-nyong	MRC-5		1	
Mayaro			2·5	
Chikungunya			2·5	
Flavivirus				
West Nile	LLC-MK2		0·1	
Yellow fever	LLC-MK2		0·004	
Japanese encephalitis	LLC-MK2		10	
St Louis encephalitis	VERO		2·5	
Langat	LLC-MK2		0·6	
Dengue type 1	LLC-MK2		0·03	
Dengue type 2	LLC-MK2		0·01	
Unclassified				
Ebola	VERO	0·001		
Bunyaviridae				
Oropouche	MRC-5		10	
California encephalitis (LaCross)	MRC-5		0·5	
Rift Valley fever	MRC-5	3	4	
Korean haemorrhagic fever	VERO-E6	0·1	0·3	0·001
Congo-Crimean haemorrhagic fever	MRC-5		1·3	
Arenaviridae				
Lymphocytic choriomeningitis	BSC-1		0·03	
Lassa	VERO		0·0003	
Pichinde	BSC-1	0·003	0·001	
Machupo (BHF)	BSC-1		0·02	
Junin (AHF)	BSC-1		0·13	

*Concentration of interferon causing a 50% plaque reduction with vesicular stomatitis virus.

Concentration of interferon causing a 50% plaque reduction with virus.

Summary

In summary, effective treatments for exotic RNA virus infections are being sought on various fronts. It is unlikely that a single remedy will be effective against these various diseases. Progress has been made in the development of drugs, biological response modifiers including interferons, combination chemotherapy and drug

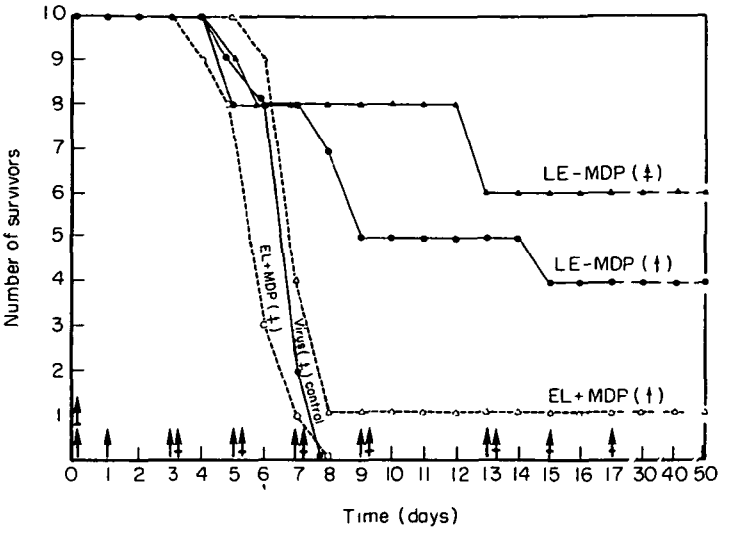


Figure 10. Efficacy of prophylactic (−2 h) and therapeutic (day 3) regimens of liposome encapsulated MDP (LE-MDP) or liposome-MDP mixture (EL + MDP) against Rift Valley fever infection in mice. Treatments were intravenous with LE-MDP or EL + MDP (MDP = 50 μg) on days 0 (−2 h) to 13 (plain arrows) or on days 3 to 17 (barred arrows). A challenge dose of 25 PFU of RVF virus was injected subcutaneously on day 0. MDP = muramyl dipeptide-phosphatidylethanolamine.

delivery which give great promise that effective treatments will be forthcoming. It is clear that the earlier treatments are initiated the greater the potential for efficacy. Hence, a key to effective antiviral therapy for these life threatening viral diseases may rest with the concurrent development of effective, low cost laboratory procedures for the rapid diagnosis of exotic viruses particularly in less developed areas of the world where they are endemic. On the other hand, the high lethality associated with many of these infections would permit treatment modalities carrying greater risk which otherwise would not be acceptable for less severe, self limiting viral diseases.

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