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Effects of ribamidine, a 3-carboxamidine derivative of ribavirin, on experimentally induced *Phlebovirus* infections

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Summary

Ribamidine (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine) was inhibitory in rhesus monkey kidney (LLC-MK_2 derivative) cells to Adames and Balliet strains of Punta Toro virus (PTV), a *Phlebovirus* related to Rift Valley fever and sandfly fever viruses. The 50% effective dose was 8 and 12 μg/ml against each respective virus strain; the 50% cytotoxic dose was 320 μg/ml, giving selectivity indices of 40 and 27 against each virus strain. The virus ratings were 1.2 and 1.0, respectively. In radiolabel uptake studies, ribamidine had a moderate effect on [^{3}H]leucine uptake at dosages down to 1 μg/ml, but [^{3}H]thymidine, [^{32}P], and [^{3}H]uridine were inhibited at high (100–1000 μg/ml) doses only. Subcutaneous (s.c.) and oral treatments of Adames PTV-infected mice were equally highly effective, as evidenced particularly by 100% survivors. Reduced hepatic icterus, serum oxalic acid transaminase, serum glutamic pyruvic acid transaminase, and recoverable virus titers from livers and sera of infected mice were also seen as a result of ribamidine treatment. Twice daily treatment for 5 days could be started as late as 72 h post-virus inoculation (p.v.i.) with significant inhibition of PTV infection seen. Single s.c. treatments administered as late as 48 h p.v.i. were similarly effective. Using the chronic therapy schedule, the maximum tolerated dose was 1000 mg/kg/day and the minimum effective dose was 31.3 to 62.5 mg/kg/day. Using single treatment, a maximum tolerated dose was >1000 mg/kg, and the minimum effective dose was 125 mg/kg. Ribamidine s.c. treatment of mice infected intracerebrally with the Balliet strain of PTV resulted in a moderate infection-inhibitory

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effect, seen especially by reduced virus titers in the brains of the infected, treated mice.

Ribamidine; *Phlebovirus*; Punta Toro virus; Ribavirin

Introduction

The synthetic nucleoside 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) is recognized as a broad-spectrum antiviral agent (Sidwell et al., 1972, 1985). The compound is known to be associated with various adverse effects, however. Among the most troubling has been a relatively small therapeutic index, with toxicity seen as development of anemia at doses approaching those desired to be used in antiviral therapy (Hillyard, 1980). A variety of compounds chemically related to ribavirin have been synthesized and evaluated with regard to their antiviral activity as compared to ribavirin (Sidwell and Olsen, 1980; Sidwell et al., 1985). One of the most promising of these derivatives is 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine (ribavirin carboxamidine, ribamidine, Fig. 1). This compound was initially described by Witkowski et al. (1973), with in vitro activity reported against type 1 herpes, type 13 rhino- and type 3 parainfluenza viruses which paralleled that of ribavirin. These investigators also reported ribamidine to be effective against experimentally induced influenza virus infections in mice, but did not define the maximum tolerated dose (MTD) of the compound in mice and, therefore, its subsequent therapeutic index against the influenza infection. No other antiviral studies are known to have been done with this compound.

The present report describes the effect of ribamidine on experimental infections induced in mice by the Punta Toro virus (PTV), a *Phlebovirus* in the Bunyaviridae family. The PTV is closely related to phlebotumus, or sandfly fever (SF) and Rift

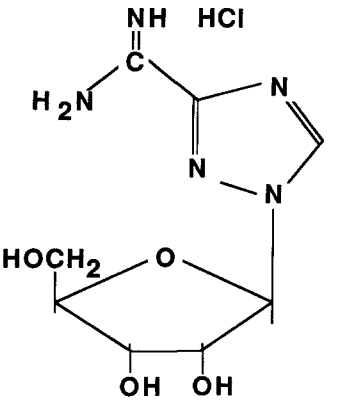

Fig. 1. Ribamidine.

Valley fever (RVF) viruses, inducers of diseases which have had a major impact in Europe, the Middle East and Africa (Sabin, 1948, Meegan et al. 1981), and are as yet uncontrolled by antiviral drugs. PTV induces in inbred strains of peripherally inoculated mice a hepatocellular necrotic disease, leukopenia and lymphopenia which resemble the disease in man induced by SF and RVF viruses (Pifat and Smith, 1987). Our observations with ribamidine indicate the compound to have a significant inhibitory effect in vitro and in vivo against PTV and to have a much less toxic effect in mice when compared with ribavirin.

Materials and Methods

Virus

The Adames and Balliet strains of PTV as described previously (Sidwell et al., 1988) were used. Both were originally isolated from patients presumably infected in Panama. Each was twice plaque purified, pools made, and the viruses titrated in cells and mice.

Cells

A derivative strain of continuously passaged monkey kidney cells (LLC-MK_2), maintained in minimum essential medium (MEM, Grand Island Biological, Grand Island, NY) containing 5% fetal bovine serum (FBS, HyClone Labs, Logan, UT) and 0.1% $NaHCO_3$ without antibiotics were used. The cells were determined to be free of mycoplasma.

Animals

Specific pathogen-free female C57BL/6 mice weighing 10–12 g were obtained from Simonsen Laboratories (Gilroy, CA) for these studies. The animals were quarantined 24–48 h prior to use, housed five or ten to a cage, and fed Wayne Laboratory Chow and tap water ad libitum.

Test compound

Ribamidine and ribavirin were provided in dry powder form by the U.S. Army Medical Research Institute for Infectious Diseases via Technassociates, Inc. (Rockville, MD). Each was prepared in MEM for in vitro studies and in saline or water for animal experiments. Ribamidine was analyzed by high pressure liquid chromatography to determine its purity. It was found to be 99.8% pure, with 0.1% ribavirin as the only major contaminant. The compound is relatively stable, but will hydrolyze to ribavirin in aqueous solution after prolonged storage at room temperature. The material was freshly prepared for these studies. When used in vivo, it was stored between treatments at −20°C.

In vitro antiviral experiments

Antiviral activity against both Adames and Balliet virus strains was determined in LLC-MK_2 cells using inhibition of viral cytopathic effect (CPE) as described

previously (Sidwell et al., 1988). Confirming experiments were run using CPE inhibition and also virus titer reduction (VTR) at the maximum tolerated dose (MTD) of test compound. The MTD was initially determined as the highest dose not causing microscopically discernible cytotoxic effects in concomitantly run toxicity controls. Other cytotoxicity studies were as described below. Antiviral activity was expressed as virus rating (VR) as described previously (Sidwell and Huffman, 1971) and by 50% effective dose (ED_{50}) determined by plotting dosage vs CPE inhibition. Therapeutic indices (TI) were determined as 50% cytotoxic dose (CD_{50}) divided by ED_{50}. Virus yield of multiple 10-fold dilutions of eluates from frozen and thawed cells and cell supernates was assayed in triplicate in LLC-MK_2 cells, using CPE as endpoint.

In vitro cytotoxicity determinations

Initial cytotoxicity determinations used visually discernible morphologic changes in cellular appearance to determine CD_{50}. To evaluate the potential cytotoxic or cytostatic effects of ribamidine further, the effects on cellular DNA, RNA and protein synthesis were determined using uptake of [^{3}H]thymidine, inorganic [^{32}P], [^{3}H]uridine and [^{3}H]leucine in a 24 h monolayer of LLC-MK_2 cells treated with varying 10-fold concentrations of ribamidine. The overall procedure for this experiment was described by Smee et al. (1980). Vital dye (neutral red) uptake was also used as a measure of cytotoxicity. The neutral red uptake method as described by Finter (1969) was adapted for use in 96-well tissue culture plates for these experiments.

In vivo antiviral experiments

The in vivo experiments varied somewhat according to the objectives of the experiment, but were generally run as follows: Twenty mice infected subcutaneously (s.c.) with Adames PTV were treated s.c. or per os (p.o.) with varying 2-fold dilutions of each drug dosage, and 40 infected mice were treated with saline or water as virus controls. The mice were held ten to a cage. Five sham-infected animals were treated with each drug dosage as toxicity controls, with the mice used as normal controls. These uninfected animals were held in an area remote from the infected animal holding rooms. The toxicity and normal controls were weighed prior to initial treatment and 18 h following treatment termination. Three days after virus inoculation, mice from one cage of each treatment group, one cage of normal controls, and two cages of virus controls were killed, bled, and their livers removed. Hepatic icterus, characterized by discoloration of the liver, was assigned a score of 0 (normal) to 4 (maximal discoloration), frozen at −70°C and later assayed for infectious virus. The serum was assayed for level of serum glutamic oxalic acid transaminase (SGOT) and pyruvic acid transaminase (SGPT). Frozen serum samples were also later assayed for infectious virus titer. Titration of SGOT and SGPT was done using colorimetric kits from Sigma. Infectious virus in serum and liver homogenates was quantified as described above in LLC-MK_2 cells. Animals not killed on day 3 were held through 21 days post-virus inoculation (p.v.i.) with deaths noted daily. In some experiments, only death was used as an end-

point, in which case 10 mice were used in each infected, ribamidine-treated group and 20 infected, placebo-treated animals were included as virus controls. Toxicity and normal controls in such experiments were as described above.

Statistical evaluations

Survivor increases were evaluated using chi square analysis with Yates' correction. The *t* test was used to analyze increases in mean survival times of animals that died before day 21 and reductions in SGOT, SGPT and PTV levels in liver and serum. Liver score inhibition was compared using ranked sum analysis. In all cases, values for infected, treated groups were compared to those of the concomitantly run placebo-treated controls.

Results

In vitro anti-PTV effects of ribamidine

The PTV-inhibitory effects of ribamidine in vitro are seen in Fig. 2. Both strains of PTV appeared approximately equal in their sensitivity to the compound. Against the Adames PTV, ribamidine at 100 μg/ml, a partially cytotoxic dose, reduced the virus yield by greater than 5 $\log_{10}$; at 10 μg/ml, a virus titer reduction of 1 $\log_{10}$ was seen. With the Balliet PTV, 32 μg/ml of ribamidine (MTD) reduced the virus yield by 2 $\log_{10}$; a 1.2 $\log_{10}$ virus reduction occurred using 3.2 μg/ml. Vital dye uptake indicated ribamidine partial cytotoxicity only at 1000 μg/ml; ribavirin displayed partial cytotoxicity at 1000 and 320 μg/ml levels using this dye uptake assay.

Biochemical cytotoxicity determinations

The effects of ribamidine on cellular macromolecular synthesis are presented in Table 1. This compound had an inhibitory effect on [^{3}H]leucine incorporation of 36% to 56%, depending on the dosage level, implying a moderate inhibition of protein synthesis. Inhibition of [^{3}H]uridine uptake was seen at 100 and 1000 μg/ml levels only, whereas [^{32}P] was significantly inhibited, and [3H]thymidine to a lesser extent at the 1000 μg/ml dose level only of ribamidine, dose levels which also caused the slight to moderate morphological alterations in toxicity control cells seen in Fig. 2. Ribavirin was run in a parallel experiment (data not shown) and was almost identical to ribamidine in its effects on [^{3}H]leucine, [^{3}H]uridine and [^{3}H]thymidine. Ribavirin inhibited [^{32}P] incorporation in LLC-MK_2 cells from 40 to 60% at all dosage levels from 1 to 1000 μg/ml; in contrast, ribamidine inhibited the uptake by 40% at 1000 μg/ml only. This comparison of ribamidine to ribavirin suggests a possible difference in biochemical effects of these two closely related compounds.

Effects of subcutaneous ribamidine treatment

Three experiments were initially run to determine the effect of ribamidine on PTV infections. In these experiments, the compound was administered s.c. twice daily (b.i.d.) for 5 days beginning at varying times relative to virus inoculation

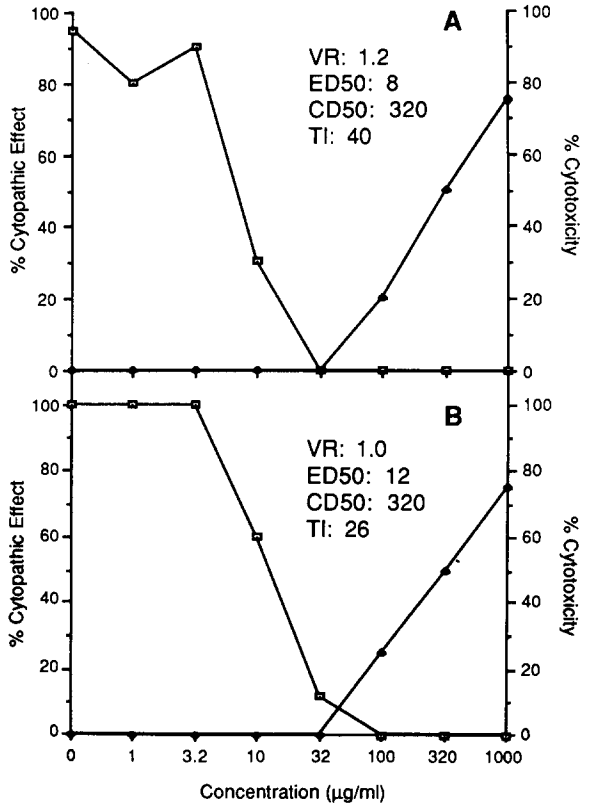

Fig. 2. Effect of ribamidine on PTV infections in LLC-MK2 cells. A, Adames strain of PTV; B, Balliet strain of PTV. □, Effects on viral CPE; ▲, cytotoxic effects.

(Table 2). In Experiment 1, treatments from 31.3 to 250 mg/kg/day were used beginning 4 h pre-virus inoculation. Each dosage was effective against the infection, although the 31.3 mg/kg/day dose level, while preventing death, had little effect on the other disease parameters. The highest dose used was well tolerated in toxicity controls. In Experiment 2, treatments were initiated 24 or 48 h p.v.i.; in this experiment the dosage range was shifted to range from 62.5 to 500 mg/kg/day. All dosages were again well tolerated, and were significantly inhibitory to the PTV infection. The 62.5 mg/kg/day dosage exhibited less efficacy when given in these delayed therapy experiments, especially when begun 48 h p.v.i. Experiment 3 was then run using only 125 to 500 mg/kg/day dosages begun 72 h p.v.i. All three dosages were highly effective against the infection.

Using increased survivors and mean survival time only as endpoint, an experiment was run using the same b.i.d. × 5 s.c. treatment regimen beginning 4 h previrus exposure, with ribamidine dosages ranging from 7.8 to 2000 mg/kg/day (data not shown). This experiment determined the TI of the ribamidine treatment. The 2000 mg/kg/day dosage was toxic, killing 4 of 5 toxicity controls; the 1000 mg/kg/day

TABLE 1

Effect of ribamidine on [^{3}H]leucine, [^{3}H]uridine, [^{3}H]thymidine and inorganic [^{32}P] uptake into LLC-MK$_2$ cells[a]

Concentration (μg/ml)	[^{3}H]leucine		[^{3}H]uridine		[^{3}H]thymidine		[^{32}P]	
	CPM[b]	%Control ± S.E.	CPM[b]	Control ± S.E.	%CPM[b]	%Control ± S.E.	CPM[b]	%Control ± S.E.
1000	77394	44 ± 4	49489	44 ± 8	22535	90 ± 18	3677	62 ± 10
100	113340	65 ± 10	46552	42 ± 5	24522	102 ± 10	6486	110 ± 3
10	107411	61 ± 12	130636	118 ± 2	32617	137 ± 5	6199	104 ± 3
1	112634	64 ± 12	121897	110 ± 7	31056	130 ± 10	7317	124 ± 2
0	175252	100 ± 7	110617	100 ± 7	23887	100 ± 5	5924	100 ± 6

[a] 24 h monolayer incubated with ribamidine 23 h, followed by 1 h radiolabeled pulse.
[b] Counts per minute, mean of four replicates.

TABLE 2

Effect of chronic s.c. administration of ribamidine on PTV infections in mice[a]

Expt. No.	Start of treatment[b]	Dose (mg/kg/day)	No. Survivors/ Total	Mean survival time[c] (days)	Mean[d] liver score	Mean SGOT[e]	Mean SGPT[e]	Mean liver virus titer[f]	Mean serum virus titer[f]
1	4 h pre	250	9/10[h]	2.0	0.7[h]	204[h]	37[h]	3.4	0.9[h]
		125	10/10[h]	>21.0[h]	0.7[h]	297[h]	80[h]	3.2	4.1[g]
		62.5	10/10[h]	>21.0[h]	0.6[h]	552[h]	274[h]	3.1	4.8
		31.3	9/10[h]	6.0	1.5[h]	2397	2028	2.9	4.9
		0	7/20	4.8	2.5	4704	3757	3.2	6.4
2	24 h post	500	10/10[h]	>21.0[h]	0.8	149[h]	31[h]	2.3	0.0[h]
		250	10/10[h]	>21.0[h]	0.6[h]	134[h]	37[h]	1.0[h]	1.1[h]
		125	10/10[h]	>21.0[h]	0.5[h]	238[h]	254[h]	0.0[h]	3.5[h]
		62.5	10/10[h]	>21.0[h]	0.7[g]	493[h]	391[h]	1.0[h]	4.9
	48 h post	500	10/10[h]	>21.0[h]	0.5[h]	150[h]	48[h]	0.3[h]	0.5[h]
		250	10/10[h]	>21.0[h]	0.4[h]	197[h]	87[h]	0.3[h]	3.4[h]
		125	10/10[h]	>21.0[h]	0.2[h]	306[h]	198[h]	0.0[h]	5.3
		62.5	5/10	6.6[h]	0.8	3129	2857	2.9	6.0
		0	8/20	4.9	1.8	1189	1952	3.1	5.6
3	72 h post	500	10/10[h]	>21.0[h]	2.2	2568	2093	1.3[h]	4.5[h]
		250	10/10[h]	>21.0[h]	1.1[h]	392[h]	275[h]	1.8[h]	4.9[h]
		125	10/10[h]	>21.0[h]	1.8	2641	2695	0.5[h]	5.4[g]
		0	5/20	4.9	2.3	3917	3687	5.0	6.0

Toxicity controls: All survived at all dose levels, and gained 2.5 to 4.7 g in weight during the treatment period compared to similar weight gains in normal controls.

[a] Treatment twice daily for 5 days beginning at times indicated.

[b] Relative to virus inoculation.

[c] Mice that died before day 21.

[d] All means are of 10 animals treated, infected groups and 20 animals in saline-treated virus controls. Mice killed on day 4 post-virus inoculation.

[e] Sigma-Fraenkel units per ml on serum taken on day 4.

[f] Titers expressed as $\log_{10}$.

[g] $P<0.05$.

[h] $P<0.01$.

dosage was reasonably well tolerated, with all toxicity controls surviving and a 1.4 g increase in weight during the period of treatment compared to 4.1 g weight increase in concomitantly held normal controls. All other dosages were well tolerated with weight gains approaching that of the normal controls. Significant increases in survivors occurred down to 62.5 mg/kg/day. At this dose level, 8 of 10 mice survived, compared to 0 of 20 virus control mice. Lower dosages were ineffective. The TI was determined to be 16; this was calculated as the maximum tolerated dose (1000 mg/kg/day) divided by the minimum effective dose (62.5 mg/kg/day).

Another series of experiments were run to determine the effect of single ribamidine s.c. treatments administered at varying times relative to virus inoculation (Table 3). Dosages ranged from 125 to 1000 mg/kg. Maximum activity was seen when ribamidine was given 48 h p.v.i. with treatment at all doses resulting in significant increases in survivors. No activity was seen when treatments were given at 72 h post-virus inoculation.

Effects of p.o. ribamidine treatment

To determine if p.o. ribamidine therapy was effective against PTV infections, mice were treated by gavage b.i.d. × 5 beginning 4 h pre-virus inoculation. Treatments ranged from 7.8 to 2000 mg/kg/day. As seen in Table 4, the 2000 dosage was somewhat toxic, killing 1 of 5 control mice. All other dosages appeared well tolerated, although none of the mice gained as much weight as the normal controls. The p.o. treatment resulted in a marked antiviral effect which was almost identical to that seen when the compound was administered s.c. by the same treatment schedule, although the infected mice treated with 1000 mg/kg/day, while exhibiting a significantly increased mean survival time, did not survive the infection. In addition, a significant increase (35%) in survivors was also seen in the lowest dosage group. Assuming 1000 mg/kg/day as the maximum tolerated dose, and not considering the out-of place effect at 7.8 mg/kg/day, the TI using ribamidine orally was 16.

A second experiment was also run using this same treatment schedule, with dosages ranging from 7.8 to 1000 mg/kg/day (data not shown). In this experiment, a lower viral inoculum was used, resulting in a 50% death of saline-treated virus controls. The infected, ribamidine-treated mice in the 125 through 1000 mg/kg/day dosages survived the infection ($P<0.01$). At 62.5 mg/kg/day, nine of ten infected mice survived ($P<0.05$). At 31.3 mg/kg/day, an 80% survival rate occurred ($P<0.05$ due to 50% survivors in virus controls), and a mean survival time of 7.5 days was seen compared to 6.5 days in the virus controls ($P<0.01$). No activity was seen at the lower dosages. In this experiment, using 31.3 mg/kg/day as the minimum effective dose, the TI was determined to be 32.

Effects of subcutaneous treatment on an intracerebral (i.c.) PTV infection

An experiment was run to determine if s.c. ribamidine treatment administered b.i.d. × 5 beginning 24 h pre-virus inoculation would influence an infection induced by the neurotropic Balliet strain of PTV injected i.c. into four-week-old mice.

TABLE 3

Effect of single s.c. administrations of ribamidine on PTV infections in mice

Time of treatment[a]	Dose (mg/kg)	Toxicity controls		Infected, treated			
		Surv/ total	Host wt. change[b] (g)	Surv/ total	P[c]	Mean survival time[d] (days)	P
4 h post	1000	5/5	0.3	8/10	<0.01	9.0	>0.05
	500	5/5	0.3	10/10	<0.01	>21.0	<0.01
	250	5/5	0.4	7/10	<0.01	5.7	>0.05
	125	5/5	1.1	1/10	>0.05	5.6	>0.05
24 h post	1000	5/5	0.3	10/10	<0.01	>21.0	<0.01
	500	5/5	0.3	10/10	<0.01	>21.0	<0.01
	250	5/5	0.4	5/10	<0.05	6.6	>0.05
	125	5/5	1.1	3/10	<0.05	6.4	>0.05
48 h post	1000	5/5	0.3	10/10	<0.01	>21.0	<0.01
	500	5/5	0.3	8/10	<0.01	8.0	>0.05
	250	5/5	0.4	9/10	<0.01	4.0	>0.05
	125	5/5	1.1	6/10	<0.01	5.8	>0.05
72 h post	1000	5/5	0.3	2/10	>0.05	4.3	>0.05
	500	5/5	0.3	2/10	>0.05	4.3	>0.05
	250	5/5	0.4	0/10	>0.05	4.6	>0.05
	125	5/5	1.1	0/10	>0.05	4.6	>0.05
	0	–	–	0/10		4.2	

[a] Relative to virus inoculation.

[b] Difference between initial weight prior to treatment and weight 18 h following final treatment. Normal controls gained 0.7 g.

[c] Probability compared to placebo-treated virus controls.

[d] Mice that died before day 21.

TABLE 4

Effect of chronic p.o. administration of ribamidine on PTV infections in mice[a]

Dosage (mg/kg/day)	Toxicity controls		Infected, treated			
	Surv./total	Host wt. change[b] (g)	Surv./total	P^c	Mean surv. time[d] (days)	P
2000	4/5	0.7	0/10	>0.05	5.6	<0.01
1000	5/5	2.4	1/10	>0.05	6.7	<0.01
500	5/5	2.1	9/10	<0.01	12.0	>0.05
250	5/5	1.6	10/10	<0.01	>21.0	<0.01
125	5/5	2.8	10/10	<0.01	>21.0	<0.01
62.5	5/5	2.6	9/10	<0.01	6.0	>0.05
31.3	5/5	3.2	2/10	>0.05	4.8	>0.05
15.7	5/5	1.8	3/10	>0.05	5.1	>0.05
7.8	5/5	2.4	5/10	<0.05	4.6	>0.05
0	–	–	3/20		4.0	

[a] Treatment twice daily for 5 days beginning 4 h pre-virus inoculation.

[b] Difference between initial weight prior to treatment and weight 18 h following final treatment. Normals gained 4.1 g.

[c] Probability compared to placebo-treated virus controls.

[d] Mice that died before day 21.

In this experiment, 20 mice were used in each infected, treated group and 40 were designated as virus controls which were injected and treated with saline only. The ribamidine doses were 125, 250, and 500 mg/kg/day. Six days p.v.i., one half of each group of mice was killed, their brains removed and infectious virus in the brains quantified by serial log dilution of brain homogenates in LLC-MK_2 cells, using viral CPE as endpoint. Treatment with ribamidine caused moderate effects on the infection, as summarized in Table 5.

Discussion

This carboxamidine derivative of ribavirin appears to have a consistent and potent inhibitory effect against PTV, both in vitro and in an hepatotropic animal model. This activity was seen using a variety of parameters. The compound was also inhibitory, but to a lesser extent, to encephalitis induced by i.c. injection of a neurotropic strain of PTV. Ribamidine appeared to be well tolerated in mice at antiviral dosages; slight inhibitions of weight gain in p.o.-treated mice compared to normal controls was considered a result of the physical trauma of the gavage treatment, and not the ribamidine itself, which may have slightly lessened the animals' ability or desire to eat.

We have previously shown that PTV infections in mice develop rapidly, with serum and liver virus titers reaching peak levels by 2–3 days p.v.i. (Sidwell et al., 1988). Hepatic icterus maximizes by day 4 p.v.i. The mice begin to die also by day 4. Pifat and Smith (1987) have reported marked PTV-induced hematological changes, seen especially as decreased white blood cells, lymphocytes, and platelet counts, occurring by day 2–3 p.v.i. Thus treatment with ribamidine starting as late as 48 h after initiation of the infection, when the disease was well established, was considered most significant and implies the compounds may be useful as a therapeutic antiviral agent.

Because of the close similarity of ribamidine to ribavirin, it is appropriate to compare the two compounds. A potential advantage with ribamidine is seen in that the compound is approximately ten times better tolerated in mice than ribavirin, with an MTD of approximately 1000 mg/kg/day for ribamidine vs 100 mg/kg/day for ribavirin, although the TI of both compounds was approximately 16. Pifat et al. (1988) have reported that ribamidine treatment of rhesus monkeys did not appear to affect the red blood cell parameters usually decreased by similar ribavirin treatment. The biochemical cytotoxicity studies described in this report also suggest ribavirin to have a more pronounced effect on DNA synthesis, as measured by [^{32}P] uptake, than ribamidine, suggesting a possible difference in biochemical effects of the two compounds. The inhibition of [^{32}P] by ribavirin is in direct contrast with those of Drach et al. (1981), who demonstrated that incorporation of [^{3}H]thymidine was significantly inhibited by ribavirin, while inhibition of [^{32}P] was 100-fold less. The work by the latter investigators was done in human carcinoma of the nasopharynx (KB) cells and in human lymphocytes, whereas our studies were performed in monkey kidney cells (LLC-MK_2). We attribute the differences in ef-

TABLE 5

Effect of chronic s.c. administration of ribamidine on neurotropic infections in mice[a]

Dosage (mg/kg/day)	Toxicity controls		Infected, treated					
	Surv/ total	Host wt. change[b] (g)	Surv/ total	*P*[c]	Mean surv time[d] (days)	*P*	Brain virus titers[e]	*P*
500	5/5	2.0	8/10	>0.05	11.0	>0.05	1.5	<0.05
250	5/5	2.1	3/8	>0.05	12.6	>0.05	2.5	>0.05
125	5/5	2.8	5/8	>0.05	10.0	>0.05	0.0	<0.01
Saline	–	–	10/20		10.7		3.2	

[a] Treatment twice daily for 5 days beginning 24 h pre-virus inoculation.

[b] Difference between initial weight prior to treatment and weight 18 h following final treatment. Normal controls gained 3.2 g in each experiment.

[c] Probability compared to placebo-treated virus controls.

[d] Mice that died before day 21.

[e] Log_{10} infectious virus recovered from brains removed on infection day 6.

fect to the different cell lines used in the two studies. It is significant in this regard that Huffman et al. (1973) showed ribavirin to have striking antiviral activity in KB cells, but effects against the same viruses were markedly reduced in monkey kidney cells, implying definite differences in the effects of the compound in the two cell lines. The in vitro and in vivo activity of both compounds against PTV are somewhat similar, although in a previous study we found ribavirin to have no activity against the encephalitis induced by the Balliet strain of PTV (Sidwell et al., 1988), whereas ribamidine used by the identical treatment regimen was marginally effective.

Studies by Willis et al. (1980) have shown ribamidine to be a strong competitive inhibitor of purine nucleoside phosphorylase. Their studies also indicated that ribamidine inhibits IMP dehydrogenase, presumably after being metabolized to a nucleotide via an adenosine kinase-dependent reaction. The latter inhibition has been seen with the 5′-monophosphate of ribavirin (Streeter et al., 1973), although Smith (1980) found ribamidine to be less inhibitory to IMP dehydrogenase than ribavirin. It has generally been concluded that the antiviral effects of ribavirin are not dependent on this IMP dehydrogenase inhibition, since the antiviral activity of some ribavirin derivatives does not correlate with their varying effects on this enzyme (Smith, 1980). It may be speculated that ribamidine may be acting as an analog of adenosine, since the two materials are quite related structurally. It is possible that ribamidine is deaminated and converted to ribavirin, making it a prodrug or a depot form of ribavirin in vivo. Work is continuing in our laboratories to elucidate more clearly the mechanism of action and in vivo metabolism of this compound.

The strong anti-PTV effects of ribamidine, which are equal to or greater than those of ribavirin, and which can be seen when ribamidine treatment is begun after the infection has been established, suggest the possible use of this compound in clinical studies against diseases such as sandfly fever or Rift Valley fever.

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