Antiviral Research, 12 (1989) 279–292
Elsevier

AVR 00391

Arenavirus infection in the guinea pig model: antiviral therapy with recombinant interferon-α, the immunomodulator CL246,738 and ribavirin

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(Received 6 June 1989; revision accepted 10 October 1989)

Summary

Human arenaviral infections have a high mortality, and are dangerous to work with in the laboratory. There is a need for good antiviral agents to treat these infections. Pichinde virus infection of the inbred strain 13 guinea pig is a relatively safe, good animal model for human arenavirus infections. Mortality is consistently 100% between days 12 and 25 (mean 14.8) days after infection. When infected animals were treated with recombinant human interferon αA, or with CL246,783, an immunomodulator known to induce interferon, no beneficial effect was noted. When animals received ribavirin, 25 mg/kg/day for the first 14 days of infection, the course of infection was prolonged, with death occurring a mean of 22.5 days after infection. If ribavirin was administered for 28 days, mortality was reduced to 25%, with those animals dying a mean of 21.0 days after infection. These results confirm the studies that indicate that ribavirin is a useful agent for treating arenaviral infections. However, treatment with this agent must be prolonged. They also demonstrate the potential usefulness of Pichinde virus infection in strain 13 guinea pigs as an animal model of human disease.

Arenaviral infection; Pichinde virus; Strain 13 guinea pig; Interferon; Immunomodulator; Antiviral therapy; CL246,738

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Introduction

Pichinde virus is an arena virus which was originally isolated by Trapido and Sanmartin from *Oryzomys*, a rodent found in Columbia, South America (Trapido and Sanmartin, 1971). Jahrling and co-workers adapted this virus to Strain 13 guinea pigs (Jahrling et al., 1981) in which it causes a severe acute illness, similar to that caused by Lassa Fever in humans. They have described the virology, pathophysiology and pathology of this model infection in detail (Cosgriff et al., 1987; Jahrling et al., 1981; Liu, 1989). Infection with Pichinde virus in humans is not associated with major disease (Buchmeier et al., 1974). Therefore, it serves as a relatively safe first line animal model for the severe human arenavirus hemorrhagic fevers. The human arenaviruses remain a major cause of morbidity and morality in a number of nations (McCormick et al., 1987), and it would be extremely useful to have effective antiviral agents available for therapy.

Ribavirin is a synthetic nucleoside. Its antiviral action is incompletely understood, but appears to be the result of interference with the initiation and elongation of viral messenger RNA by competitive inhibition of viral polymerases, although some antiviral effect may be due to reduction in guanosine triphosphate pools by competitive inhibition of cellular inosine monophosphate dehydrogenase (Rodriguez and Parrott, 1987; Gilbert and Knight, 1986). Ribavirin has been used with success against Lassa Fever in both monkeys and human beings (Canonico et al., 1984; McCormick et al., 1986). and has antiviral activity in the therapy of a second arena virus disease, Argentine Hemorrhagic Fever (Enria et al., 1987). It has been briefly reported to be beneficial in the guinea pig infected with Pichinde virus (Huggins et al., 1984; Stephen et al., 1977).

The interferons are also known to have antiviral action. The extent and usefulness of this action varies with the virus involved. Antiviral activity is especially pronounced against vesicular stomatitis virus (VSV), for example, while it is much less marked against the herpesvirus (Kern and Glasgow, 1982). Interferon (IFN), can be administered directly, or an organism can be stimulated to produce endogenous IFN by treating with an IFN-inducing compound. With the advent of recombinant DNA techniques, large quantities of pure interferon are available commercially. One of these, recombinant human IFN alpha A (rHuIFN-αA), is known to be active in guinea pigs and in guinea pig cell cultures (Overall et al., 1984). Therefore, we used this agent in our initial studies.

CL246,738 [3,6-bis(2-piperidinoethoxy) acridine trihydrochloride] is a water-soluble compound capable of inducing both cellular and humoral immune response in rodents (Durr et al., 1983). This acridine derivative stimulates tumoricidal macrophages (Sarzotti et al., 1989; Wang et al., 1985), activates splenic natural killer cells (Wang et al. 1986), prolongs the survival of tumor-implanted mice, and induces serum interferon (Durr et al., 1983; Sarzotti et al., 1989). We have shown that CL246,738 is an extremely potent antiviral agent in vivo. This compound protected outbred weanling mice against the lethal encephalitis produced by Semliki Forest virus, an alphavirus, and Banzi virus, a flavivirus, when administered 24 h before virus challenge (Sarzotti et al., 1989). Earlier studies also showed

in vivo protection against a third RNA virus, encephalomyocarditis virus, as well as herpes virus (Durr et al., 1983). CL246,738 produces high levels of circulating IFN-α in mice and induces peritoneal exudate cells to produce IFN-β. In our studies on the amelioration of togavirus infections with this compounds, the production of IFN-α and β was shown to be directly responsible for the antiviral action of the drug (Sarzotti et al., 1989).

We therefore tested the activity of commercially available rHuIFN-αA and CL246,738 against Pichinde virus infection in the guinea pig, and compared this activity to that seen with ribavirin therapy.

Materials and Methods

Guinea pigs

450–500 g male and female strain 13 guinea pigs were obtained from the University of Texas Cancer Center Veterinary Resource Division, Science Par, Bastrop, Texas. Animals were free of detectable adventitious infections.

Virus strain

Pichinde virus derived from the original guinea pig adapted strain was obtained from Dr David Gangemi, University of South Carolina. He had received the virus from Dr P.B. Jahrling, who had derived the guinea pig virulent isolate. We received virus which was passage 12 as derived by Dr Jahrling (Jahrling et al., 1981). Stock virus preparation was obtained by inoculating this preparation into Strain 13 guinea pigs. The animals were sacrificed on day 6 after infection, and the spleens removed aseptically. The spleens were minced at 1:10 dilution with Hanks Balanced Salt Solution (HBSS), and stock virus was prepared as previously described (Jahrling et al., 1981). This stock virus contained 4.5×10^4 PFU/0.1 ml, with an LD50 of $10^{-4.5}$ ml.

Animal infection

The guinea pigs were inoculated i.p. on day 0 with 0.1 ml of spleen homogenate (passage 14 or 15 counting from Jahrling's original passage). They were weighed daily, and their condition noted.

Virus isolation and quantification

Vero cells were cultured to subconfluence in 24-well panels. Tissues for virus isolated were minced finely, and a 10% (w/v) suspension was prepared in Eagles Minimal Essential Medium (EMEM) containing Earle's salts, with 1% donor calf serum. Serial dilutions at 0.1 ml volumes of either serum or minced tissue were inoculated into each well and covered with 1 ml EMEM with 25 mM HEPES and

0.67% methyl cellulose. The plates were incubated for 6 days at 37°C, in 5% CO_2, 95% air. The panels were fixed with 10% neutral buffered formalin and stained with crystal violet, and the plaques were counted.

Interferon assay

The methods for assaying for guinea pig IFN have been previously described (Chen et al., 1988). In brief, guinea pig embryo cell monolayers, grown to confluence in 24 well panels, were treated with EMEM with Hank's balanced salts with 5% donor calf serum, containing serial dilutions of the test samples at 24 h at 37°C. After the culture fluids were removed, each well was inoculated with 50 PFU of VSV. The virus was allowed to adsorb for 1 h, after which the cells were overlaid with EMEM with 25 mM HEPES with 10% donor calf serum and 5% methylcellulose. After incubation at 37 degrees for 2 days, the overlay was removed, the monolayers were treated for 15 minutes with 10% formalin containing 1.3% crystal violet, rinsed, dried, and the plaques were counted. One unit of interferon activity resulted in 50% plaque inhibition.

Evaluation of the biological effect of CL246,738 in the guinea pig

Normal uninfected guinea pigs were given a high dose (15 mg/kg) of CL246,738 i.p. Serum was obtained 6, 12, 18 and 24 hours after administration of CL246,738. The peritoneal cavity was lavaged with sterile EMEM with Earle's salts with 2% fetal bovine serum (FBS). The fluid was recovered, and centrifuged, and the cells were obtained. The spleen was removed aseptically, weighed, and minced. The fragments were further disrupted by gently grinding them between the frosted ends of two sterile microscope slides. The cells were collected in sterile EMEM with 2% FBS. Both spleen cells and peritoneal cells were centrifuged at 1000 rpm for 10 min in a refrigerated centrifuge. The supernatant was discarded, and the cell pellets were resuspended in cold Roswell Park Medical Institute Medium No. 22 (RPMI 22) with 5% FBS and 0.1% gentamicin. The cells were plated out at 8×10^5 to 9×10^5 per well in 48-well plates, and incubated at 24 hours at 37°C. The panels were then frozen to −120°C and thawed to 37°C, and the contents of the wells were centrifuged at 1000 rpm for 10 min. The supernatant was assayed for interferon activity.

Detection of precipitating antibody

Precut gels for radial immunodiffusion were obtained from ImmunoMycologics (Norman, OK). Virus inoculum (passage 14) was placed in the central well, and serum, undiluted and diluted 1:2 was placed in the encircling wells. The gels were incubated at room temperature, and examined at 18 and 36 h for lines of precipitation.

TABLE 1

Treatment with Recombinant Interferon-αA

Treatment[a]	No. animals	% Mortality	Mean day of death (±1 SD)
556 U	8	100	13.7 ± 1.1
1667 U	8	100	13.6 ± 1.1
16,667 U	4	100	13.7 ± 0.96
166,667 U	4	100	14.0 ± 0.81
Virus only	12	100	13.9 ± 0.79
Uninfected Interferon Treated 166,667 U	6	0	–

[a]Interferon treatments are shown as guinea pig equivalent units, as determined against vesicular stomatitis virus on primary guinea pig cells. Actual international units of IFN given in each experiment are 18-fold higher, i.e. 166,667 g.p. units = 3×10^6 I.U. rHuIFN-αA. IFN was administered as a single i.p. injection 1 h prior to virus challenge.

Drug treatment

Interferon

Recombinant leukocyte IFN-αA was obtained from Dr I. Sim, of Hoffmann-LaRoche. This preparation is known to be active in guinea pigs, and has been used in a number of experiments (Kataoka et al., 1986; Overall et al., 1984). The activity of the IFN in guinea pig cells was assayed, and 18 U of labelled human activity were found to equal 1 U of guinea pig activity. The animals were divided into 6 groups (Table 1). Drug-treated animals received 556 units of guinea pig activity, 1667 U, 16557 U, or 166667 U by i.p. injection one hour prior to inoculation of virus. 166667 U was the maximal practical volume that could be injected. Control groups received virus only, or drug only.

CL246,738

This agent was obtained from Dr F. Durr, Lederle Labs. The effects of this agent on normal pigs was determined. The animals were divided into 4 groups (Table 2). Drug treated animals received either a low dose (5 mg/kg) or a high dose (15 mg/kg), injected i.p. 24 h before virus inoculation. Control groups received virus only, or drug only.

TABLE 2

Treatment with CL 246,738

Treatment	No. animals	% Mortality	Mean day of death (±1 SD)
15 mg/kg	8	100	14.3 ± (0.46)
5 mg/kg	8	100	14.6 ± (0.52)
Virus only	8	100	15.7 ± (0.82)
Uninfected 15 mg/kg	6	0	–

TABLE 3

Treatment with ribavirin

Treatment	No. animals	% Mortality	Mean day of death (±1 SD)
25 mg/kg/day 14 days	8	100	22.5 ± 2.8[a]
25 mg/kg/day 28 days	12	25[b]	21.0 ± 2.0[c]
Virus only	7	100	15.6 ± 4.6
Drug only 25 mg/kg/day 28 days	3	0	–

[a]Student's t-test $P = 0.005$ compared to untreated animals.
[b]$\chi^2 = 9.98$ $P = 0.002$ compared to untreated animals.
[c]Student's t-test $P = 0.02$ compared to untreated animals.

Ribavirin

In the first group of experiments, animals received 25 mg/kg/day ribavirin i.p., starting on the day of infection and continuing for 14 days, virus only, or drug only (Table 3). In the second set of experiments, ribavirin treatment (25 mg/kg/day; i.p.) was started on the day of infection and continued for 28 days, with appropriate control groups. In a third set of experiments, the guinea pigs received 25 mg/kg twice a day for 28 days.

Histopathological examination

When the infected animals were moribund, or recently dead, tissue was obtained for histologic examination. Drug control animals were also examined at this time. Liver, lung, spleen, pancreas, kidney, brain, and adrenal tissues were examined. The tissues were removed from the animal and fixed overnight in buffered aqueous formalin solution. Then, the tissues were dehydrated through graded alcohols to xylene, embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin.

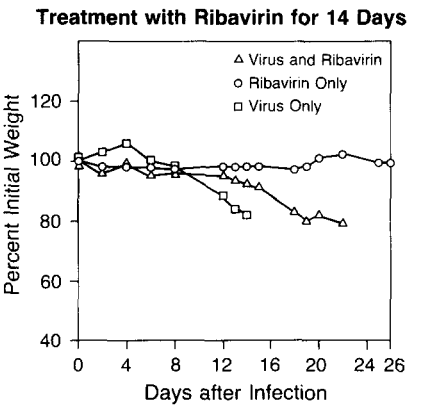

Fig. 1. Weight curve for guinea pigs infected with Pichinde virus and treated with ribavirin for 14 days.

Results

All untreated infected guinea pigs died between days 12 and 25 after virus inoculation (mean day of death = 14.8). Most untreated animals died between days 14 and 19. Prior to death, all animals demonstrated a marked loss of weight, beginning about day 6 post-infection, ultimately losing up to 20% of their initial body weight (Fig. 1).

When serum was evaluated for viremia, virus was first detected on day 4 after infection (Table 4). The quantity of virus rose rapidly, and on days 14–16, at the time of death, levels of 10^4 to 10^5 plaque forming units (pfu)/ml of serum were present. When the various organs were assayed for virus, the spleen consistently contained high levels of virus (Table 5). By day 4 post-infection, 7.7×10^3 pfu/0.1 g. tissue was detected, with the highest levels 2.1×10^5 being reached by day 14. The liver contained less virus than the spleen on day 4 post infection, but by day 7 the levels were similar. These levels were higher than those seen in serum. In contrast, the kidney always contained less virus than liver or spleen, with levels equal to or less than that seen in serum. Viremia, and virus titers in various organs peaked at the time of death. IFN is detectable in the serum in animals on days 7 and 16 post-infection (data not shown).

Histologic evaluation of the organs revealed surprisingly little alteration. The spleen demonstrated bands of necrosis surrounding the periarteriolar lymphoid sheathes on days 14–16 post-infection. The liver contained scattered foci of single cell necrosis on days 11–16. Occasionally a few polymorphonuclear leukocytes would be present in these areas. There was also marked fatty metamorphosis of the hepatocytes beginning on day 11 after infection and continuing until death. The adrenals showed scattered foci of necrosis, 1–2 cells in extent, with a small sprinkle of mixed cellular infiltrate on days 14–16. There was interstitial pneumonia, ranging in extent from minimal to severe by days 14–16. However, many animals

TABLE 4

Virus quantification in serum

Group	Days after infection	No. animals	Geometric mean pfu/ml of serum
Virus only	4	3	2.1×10
	7	3	3.9×10^2
	11	3	2.3×10^4
	14	6	4.8×10^5
	16	4	2.9×10^4
CL246,738 5 mg/kg	15	4	1.0×10^4
15 mg/kg	15	2	1.0×10^4
Interferon 300,00 I.U. ribavirin	13	3	3.0×10^6
25 mg/kg/day for 28 days	14	2	7.1×10^{3a}
25 mg/kg/12 h for 28 days	14	4	2.9×10^{3b}

[a]Student's t-test $P = 0.002$ compared to untreated infected animals.

[b]Student's t-test $p = 0.005$ compared to untreated infected animals.

TABLE 5

Virus quantification in tissue of untreated animals

Days after infection	No. animals	Organ	Geometric mean pfu/g of tissue
4	3	Spleen	7.7×10^3
		Liver	4.6×10^1
		Kidney	NVD
7	3	Spleen	3.5×10^4
		Liver	1.5×10^4
		Kidney	4.6×10^1
11	3	Spleen	9.8×10^4
		Liver	8.7×10^4
		Kidney	3.3×10^3
14	3	Spleen	2.1×10^5
		Liver	1.9×10^5
		Kidney	1.6×10^4
16	3	Spleen	1.4×10^4
		Liver	3.7×10^4
		Kidney	2.1×10^4

NVD, no virus detected.

died without any demonstrable pneumonia. No consistent histologic abnormalities were noted in any other organ system.

Interferon

All IFN-treated infected animals died between days 13 and 14 after virus inoculation. The mean day of death for each of the groups was between 13.8 and 14.0 (Table 3). Virus levels in the serum of these animals were similar to those seen in control infected animals (Table 1). Histologic evaluation demonstrated no difference from control animals. The weight curves for treated animals showed the same severe decline as that of untreated infected animals. No difference was apparent between the different dosages of IFN. Uninfected, drug controls showed no adverse effects, and gained 4% over initial body weight by day 14, as expected in animals of this age.

CL246,738

No interferon was detectable in the serum of uninfected guinea pigs treated with CL246,738 at 6, 12, 18 or 24 h. The spleen cells did not produce interferon after in vitro cultivation. However, the adherent peritoneal macrophages isolated at 6 and 24 h after treatment, produced detectable levels of interferon ranging from 10 to 100 U/10^5 cells when cultivated in vitro, indicating a response to CL246,738 at the doses used.

All infected animals treated with CL246,738 died between days 14 and 16 after infection. Mean day of death for animals receiving 15 mg/kg was 14.3, for those receiving 5 mg/kg 14.6, and for untreated infected animals 15.7 (Table 4). The slight tendency for treated animals to die earlier than virus controls was not significant; times of death were within the range usually seen for infected animals. No difference was seen in levels of virus or in histologic abnormalities between treated and control infected animals. Uninfected animals receiving drug appeared healthy, and gained 1% over initial body weight by day 14.

Ribavirin

When the animals were treated with ribavirin for 14 consecutive days, they remained relatively healthy for those 14 days, although a small loss of weight (8–10% initial weight) can be documented on days 12–14. However, when ribavirin therapy was stopped, the animals became very sick, rapidly lost weight, and died between days 19 and 28 (mean day of death 22.5) (Fig. 1). Serum virus titers obtained from these animals on day 14 were lower than control animals (Table 1). Histologic evaluation at the time of death demonstrated the same abnormalities seen in control-infected animals dying on days 14–16 after infection. As indicated above, virus control animals showed marked weight loss beginning on day 6 post-infection. Since ribavirin therapy was clearly delaying the day of death, we determined to extend the therapeutic treatment. When animals were treated for 28 days, there was only a 25% mortality (mean day of death for dying animals was 21.0). The remaining 75% appeared well and active. Virus evaluation of the serum of these survivors on day 14 post-infection was similar to the ribavirin treated animals in the preceding experiment. These survivors experienced some loss of weight on days 12–14 after infection (Fig. 2). This weight loss occurred at the same point as in those animals for which ribavirin therapy was determined on day 14 post-infection. Thereafter, they regained the lost weight, and then continued to gain weight. Although they were observed for another 40 days, they showed no illness, and

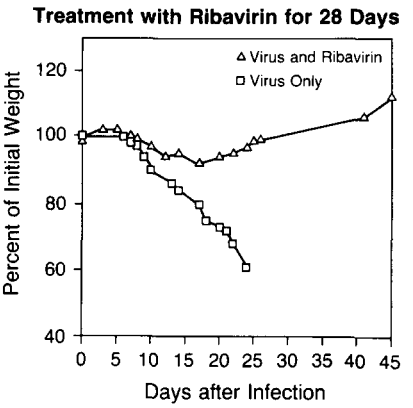

Fig. 2. Weight curve for guinea pigs infected with Pichinde virus and treated with ribavirin for 28 days.

gained weight steadily. In particular, there were no neurologic symptoms. These animals were sacrificed at days 50–300 after infection, and no virus was detected, and no abnormalities were noted. These animals developed precipitating antibody to Pichinde virus as measured by the radial immunodiffusion technique. All uninfected, drug-treated controls gained weight as usual, and showed no adverse effects (Table 5).

In an effort to improve survival, we increased the dose of ribavirin to 50 mg/kg/day. When the animals received 25 mg/kg twice a day, there was no improvement in mortality (67% mortality, days 14–23 after infection). In addition, 2/2 uninfected, drug treated control animals died on days 16 and 27. Histologic examination of the drug treated animals including the controls, revealed extensive acute hepatocellular necrosis. No virus was recovered from the control animals and no other histologic abnormalities were noted in them. All virus infected animals had lesions similar to those described above in other virus infected animals.

Discussion

From these studies, we have determined that therapy with interferon or the interferon inducing agent, CL246,738 does not have any beneficial effect on the course of Pichinde virus infection in strain 13 guinea pigs at the dosages and treatment schedule used in this study. Ribavirin, at the level of 25 mg/kg/day does have therapeutic value but treatment must be prolonged. Increasing the dose of ribavirin above this level did not increase efficacy, but led to hepatotoxicity.

Pichinde virus infection in strain 13 guinea pigs was developed and carefully studied by Jahrling and co-workers (Cosgriff et al., 1987; Jahrling et al., 1981; Liu, 1989). It serves as a relatively safe serogate for the more dangerous human arenavirus infections. Our work confirms all the points that they have noted. In particular we confirm the severe weight loss that occurs in the animals in the absence of any marked behavioral change (Liu, 1989). There appears to be loss of both fat and muscle tissue. Especially noteworthy is the absence of severe histopathology. There is little tissue damage in the face of extremely high viremia. The macrophage, particularly the splenic macrophage at the junction of the periarteriolar lymphoid sheathes and the red pulp seems to be the cell supporting extensive viral replication. However, small numbers of hepatocytes and adrenal cortical cells also become damaged. The reason for the weight loss is unclear. Possibly the infected macrophages are releasing mediators of inflammation, such as tumor necrosis factor. The precise cause of death is also unclear. This situation is not unique for Pichinde virus. Walker et al. (1982) came to a similar obstacle in explaining the mechanism of death in Lassa Fever patients. Further studies are needed to answer these questions.

The commercial availability of human recombinant IFN has made possible the therapy of human diseases with this agent. In addition, agents which reliably induce endogenous IFN without significant adverse affects are the subject of clinical trials. Thus, it is now possible to examine the efficacy of IFN in the treatment of many serious viral diseases.

Many viruses are potent inducers of IFN. Thus, it is hardly surprising that serum levels of IFN are elevated during many viral infections. In the case of Argentine hemorrhagic fever, those infected animals and patients with the highest levels of IFN are more likely to die than those with relatively low levels (Levis et al., 1984; Levis et al., 1985). We detected IFN in the serum of Pichinde infected guinea pigs. Because of this observation, it could be argued that administering additional IFN would not be clinically useful. An alternate interpretation, however, is implied by the fact that these levels of IFN appear after the virus has infected, and spread widely. The association with lethal infection might simply reflect the fact that severe disease calls forth more response (IFN), but that this response is too late to be useful. In fact, early treatment with IFN is protective in interferon-susceptible viral infection (Stephen et al., 1977). In the present study, Pichinde virus infection in the guinea pig did not respond to therapy with IFN or a known IFN inducer, CL246,738, even though they were administered before disease was established. This is consistent with previous reports of high resistance of other arenaviruses (Tacaribe, Machupo, and lymphocytic choreomeningitis virus) to IFN (Stephen et al., 1977). Thus, the human arenaviruses may not respond either to exogenous administration or endogenous induction of IFN.

There is a paucity of studies on guinea pig interferon and its induction. It has not been subtyped, or immunologically characterized. All determinations of IFN activity must be performed by bio-assay. However, it is known to exist in two forms. One component is exquisitely labile, and difficult to preserve or measure, while a second form is stabile to temperature and oxygen exposure (Winship et al., 1984a,b). It is not known how these two types of guinea pig interferons relate to the α, β and γ subtyping of mice and humans. Our studies on normal guinea pigs indicate that CL246,738 in doses of 5 and 15 mg/kg, will induce peritoneal macrophages of produce IFN 6 and 24 h after the drug is administered.

CL246,738 is known to act upon macrophages. When it is administered to mice at doses of 5 to 150 mg/kg, it induces spleen cells to produce IFN-α with high circulating levels of IFN-α. However, the peritoneal macrophages were induced to produce IFN-β. In contrast to the experience in mice, we could not detect circulating IFN in guinea pigs and the spleen cells did not produce detectable IFN. These results may mean that the spleen and circulating IFN of guinea pigs is in the labile form, and too easily destroyed for us to be able to assay. Alternately, CL246738, at these dosages by the intraperitoneal route, may act only on peritoneal macrophages. Further work would be necessary to evaluate these two possibilities. However, the peritoneal macrophages were clearly making IFN. Since we inject the virus inoculum into the peritoneum, the virus is encountering this immediately. Lack of an effect on the virus infection cannot be blamed on lack of drug effect in the animal.

Although it has been reported that ribavirin is beneficial in guinea pigs infected with Pichinde, there is no specific data on the course of infection in the treated animals (Huggins et al., 1984; Stephen et al., 1980). Treatment of rhesus monkeys infected with Lassa fever arenavirus with ribavirin for 17 days, gives complete survival if treatment is begun between days 0 to 4. In contrast, untreated animals die

by day 15 post-infection (Canonico et al., 1984). If treatment was delayed past 7 days, only 4/8 monkeys survived, however. In these experiments, ribavirin was given as a loading dose of 75 mg/kg followed by treatment with 15 mg/kg for 17 days post-infection.

In people with Lassa fever, a 2 gm loading dose was followed by 1 g every 6 h for 4 days, reduced to 0.5 g, every 8 h for another 6 days. Assuming a 70 kg patient, these doses are equal to a loading dose of 28 mg/kg, followed by 57 and 21 mg/kg/day. If treatment was begun within 6 days of onset of illness, 18/19 patients survived, while treatment begun later in the course of illness had a survival of 10/19 (McCormick et al., 1986). It has been noted that in a woman with Lassa fever, treated with ribavirin, virus was still detectable after more than 26 days of hospitalization and 3 weeks of ribavirin therapy (Fisher-Hoch et al., 1985).

In our study, treatment of Pichinde infected guinea pigs with 25 mg/kg ribavirin for 14 days delayed virus proliferation. The levels of virus in the blood on day 14 were lower in ribavirin treated animals compared to untreated animals. However, this reduction in virus titer was not sufficient to prevent the ultimate appearance of lethal disease. In contrast, when the animals were treated with the same ribavirin dose for 28 days, 75% of infected animals survived. Increasing the dose of ribavirin did not improve therapy. Not only did the mortality figures not improve, the drug treated, uninfected control animals displayed significant hepatotoxicity. Ribavirin does not totally eliminate virus. We must rely upon the host response for the ultimate elimination of the virus. It is unclear at this point what host response agent is responsible for the ultimate elimination of the infection. The development of precipitating antibodies in the surviving animals leads one to speculate that extended ribavirin treatment allows time for the development of an effective humoral immune response, which is ultimately able to control the virus infection.

In neither monkeys nor humans with Lassa fever is there any mention of recurrent illness when therapy is stopped. However, when strain 13 guinea pigs are infected with Junin virus, the causative agent of Argentine hemorrhagic fever, and treated with ribavirin (45 mg/kg), the hemorrhagic phase of the disease is blocked, but all the animals eventually die of neurologic complications with high virus titers present in the brain (Canonico et al., 1984). Rhesus monkeys also experienced similar problems (McCormick et al., 1987). When 6 patients with Argentine hemorrhagic fever were treated with ribavirin, 3 survived. One of the survivors developed transient fever in convalescence, with 28 white blood cells/mm^3 in the cerebrospinal fluid. Auditory evoked responses of the brain stem showed a prolonged time of central conduction. The patient was otherwise asymptomatic (Enrio et al., 1987). None of the guinea pigs which we infected with Pichinde virus and treated with ribavirin developed any secondary illness, and none displayed any abnormal neurologic findings, although they were observed for up to 10 months.

From these studies we conclude that Pichinde virus infection in strain 13 guinea pigs is a useful animal model for human arenavirus infection. We have found that interferon and an interferon inducing agent, CL246,738 did not have any beneficial effect on the disease. We confirm that ribavirin has a beneficial effect, but note

that therapy must be prolonged, probably at least until viremia resolves. This model infection can be used to evaluate future antiviral agents which are proposed for therapy of human arenavirus infection.

Acknowledgements

This work was supported by the U.S. Army Medical Research and Development Command under Contract No. DAMD17-86-C-6119. Opinions, interpretations, conclusions and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. In conducting research using animals, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals', prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86–23, Revised 1985). The authors thank Ms Tracy Stevens for her technical assistance, and Ms Shirley Wright for her secretarial assistance. A preliminary report of this work was presented at the Second International Congress on Antiviral Research, Williamsburg, U.S.A., April 10–14, 1988.

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