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Treatment of lethal Pichinde virus infections in weanling LVG/Lak hamsters with ribavirin, ribamidine, selenazofurin, and ampligen

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Summary

A lethal Pichinde (An 4763 strain) virus infection was produced in 3-week-old random-bred Golden Syrian (LVG/Lak strain) hamsters inoculated intraperitoneally with virus, causing mortality in 6–9 days. High virus titers ($\geq 10^{7.5}$ cell culture infectious doses/g) were present in visceral organs, serum, brain and salivary glands near the time of death. Intraperitoneal treatments with ribavirin (10 and 32 mg/kg) and ribamidine (32, 100, and 320 mg/kg) for 10 days starting 24 h after virus challenge significantly decreased mortality and reduced virus titers by 100- to >10 000-fold in liver, spleen, brain, and serum. Serum alanine aminotransferase (an indicator of liver damage) was also reduced in animals treated with the two compounds (ribavirin at 32 mg/kg; ribamidine at 100 and 320 mg/kg). Intraperitoneal selenazofurin (1–100 mg/kg per day for 10 days) and ampligen (0.5 and 5 mg/kg every other day for 5 injections) treatments provided neither protection from the lethal infection nor increased mean survival times. In fact, selenazofurin was overtly toxic, causing death of uninfected hamsters at 32 and 100 mg/kg. The random-bred LVG/Lak hamster appears to be a viable and cost-effective model for evaluating new therapies for arenavirus infections.

Pichinde virus; Arenavirus; Ribavirin; Interferon inducer

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Introduction

Arenaviruses are a group of rodent-transmitted infectious agents that cause serious life-threatening hemorrhagic fevers in man (Canonico et al., 1984). Some of the more dangerous viruses in the group include Junin, Machupo, and Lassa fever, which are endemic to South America or Africa (Andrewes et al., 1978). Pichinde virus is an arenavirus that is much less pathogenic to humans, and thus has been used in infection studies in guinea pigs (Jahrling et al., 1981) and hamsters (Stephen et al., 1980; Walker and Murphy, 1987). Formerly, only the MHA strain of hamster was thought to develop lethal Pichinde virus infections in adult animals (Buchmeier and Rawls, 1977; Gee et al., 1979, 1980). These animals, when inoculated subcutaneously with virus, die in 10–13 days, whereas random-bred adult LVG/Lak hamsters survive the infection if similarly infected. We have determined that intraperitoneal Pichinde virus challenges are lethal to random-bred 3-week-old LVG/Lak hamsters using the An 4763 strain of virus, and that this animal species is suitable for conducting antiviral chemotherapy experiments. LVG/Lak hamsters are much more readily available than MHA hamsters, have a milder temperament, and are considerably less expensive.

Because of the serious and often life-threatening nature of arenavirus infections, development of new treatments for these diseases is warranted. Ribavirin, first reported to be active against Pichinde and Lassa fever arenaviruses in animals (Jahrling et al., 1980; Stephen et al., 1980), was later shown to be effective against Lassa fever virus in humans (McCormick et al., 1986). In our laboratory we first evaluated ribavirin in the LVG/Lak hamster model of Pichinde virus infection to establish the drug as a positive control for future studies. Three previously untested compounds were also evaluated in the same model. These included ribamidine, a ribavirin derivative with activity similar to that of ribavirin against other viruses (Witkowski et al., 1973; Sidwell et al., 1988a); selenazofurin, a nucleoside analog with demonstrated anti-arenavirus activity in vitro (Huggins et al., 1984); and ampligen, an interferon-inducing mismatched double-stranded RNA molecule that has virus-inhibitory properties (Montefiori and Mitchell, 1987; Sidwell et al., 1992). In the present studies, we found ribavirin and ribamidine to be active against Pichinde virus in hamsters, whereas the other two compounds appeared ineffective against this infection.

Materials and Methods

Compounds

Ribavirin, ribamidine, selenazofurin, and ampligen were provided in dry powder form by the US Army Medical Research Institute of Infectious Diseases (USAMRIID) via Technassociates (Rockville, MD). They were

dissolved in sterile saline for injection into hamsters. Ampligen required heating at 67°C for 16 h then at 37°C for 1 h in order to anneal the strands of the polymer prior to animal treatments.

Virus and cells

Pichinde virus (PCV) strain An 4763 was provided by Joseph D. Gangemi, University of South Carolina School of Medicine, Columbia, SC. Virus stocks were prepared in Vero 76 cells (obtained from the American Type Culture Collection, Rockville, MD) from twice-plaque-purified PCV, then were stored frozen at −80°C. The titer of the virus was determined by plaque assay in Vero 76 cells following methods described previously (Smee et al., 1992). The cells were grown in Eagle's minimal essential medium containing 10% fetal bovine serum (FBS), 0.1% sodium bicarbonate and 50 μg gentamicin/ml in 5% CO_2 at 37°C.

Animals and virus infection model

Three-week-old specific-pathogen-free female random-bred Golden Syrian (LVG/Lak strain) hamsters, weighing approx. 50 g each, were obtained from SASCO (a division of Charles River Labs), Omaha, NE. PCV, in a volume of 0.2 ml per injection, was inoculated into the animals intraperitoneally (i.p.) both on the right and left sides of the abdomen to insure that an i.p. injection was achieved, since subcutaneous (s.c.) inoculations of the virus are not lethal to weanling animals (Buchmeier and Rawls, 1977). Other hamsters were not infected and served as drug toxicity controls. The animals were quarantined 24–48 h prior to use, housed 5 to a cage, and fed hamster chow and tap water ad libitum.

In vivo chemotherapy studies

Except where indicated, PCV was inoculated i.p. into hamsters at a dose of 1000 plaque forming units (PFU) per animal. Starting 24 h after virus challenge, the nucleoside analogs (ribavirin, ribamidine, and selenazofurin) were administered i.p. twice daily for 10 days. Ampligen was administered i.p. every other day for 5 injections in order to avoid the hyporesponsive phenomenon that accompanies treatment with interferon inducers (Stringfellow, 1977). The animals were weighed daily to insure constant mg/kg dosages. Doses of each compound (see tables and text) were selected based upon our experience with each substance in mice infected with Punta Toro virus (Sidwell et al., 1988a,b, 1992; Smee et al., 1990) or as was reported by others using ribavirin against PCV in MHA hamsters (Stephen et al., 1980). Death was monitored daily for 21 days using 10 animals in each drug-treated group and 20 hamsters in the placebo control. An additional 5 hamsters/group were held for tissue virus titer and serum alanine aminotransferase

determinations. Five uninfected animals/group, maintained in an area remote from the infected hamsters, were used to monitor drug toxicity. Their numbers were recorded daily, and weights were noted before the first and 24 h after the last treatment.

For virus titer determinations, serum was obtained and tissues removed and stored frozen at −80°C until assayed. 10% homogenates of tissues were made using a Stomacher™ (Techmar, Cincinnati, OH) in cell culture medium. Tissues and serum from infected hamsters were each titrated separately. Samples were titrated at 10^{-1} to 10^{-8} dilutions in Vero 76 cells in 96-well microplates by end point dilution method (Reed and Muench, 1938), and the virus titers expressed as $\log_{10}$ cell culture infectious units ($CCID_{50}$) per g of tissue or serum. Because PCV does not readily exhibit a discernible cytopathic effect, an immunofluorescence assay was used to detect the presence or absence of virus in each well. Briefly, cells inoculated with dilutions of virus-containing tissue homogenates were incubated in medium with 2% FBS for 6 days. Plates were inverted and blotted to remove the medium, then were dried 1 week or longer. A fluorescein-labeled monoclonal antibody against PCV described previously (Burns et al., 1988) was used to stain the infected monolayers for 2 h at 37°C. Plates were inverted and blotted to remove the immunoconjugate. When wells were dry, they were checked for virus using an inverted fluorescence microscope.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) determinations were made using colorimetric kits (Sigma, St. Louis, MO) following the manufacturer's instructions. Animals were bled by cardiac puncture to obtain serum samples.

Statistical analyses

Survivor increases in infected and uninfected groups were evaluated using chi-square analysis with Yates' correction. The Mann-Whitney *U* test was used to analyze increases in mean survival times of animals that died before day 21 and reductions in tissue and serum virus titers. Since virus titers in placebo control groups exceeded the dilution endpoint, we assumed an arbitrary standard deviation of 2.0 for statistical analyses. This we considered to be reasonable, since most other standard deviations on the tables were less than this. Significant decreases in ALT levels were determined using the Student's *t*-test. In all cases, values of statistical significance were made comparing drug-treated groups to respective placebo controls. The thresholds of statistical significance were $P < 0.05$ and $P < 0.01$, using two-tailed analyses.

Results

Infection parameters in PCV-infected LVG/Lak hamsters

As part of developing the LVG/Lak hamster model of PCV infection, various disease parameters were determined daily though 8 days of infection in animals inoculated with 1000 PFU of virus (Fig. 1). Virus in kidney, liver, lung,

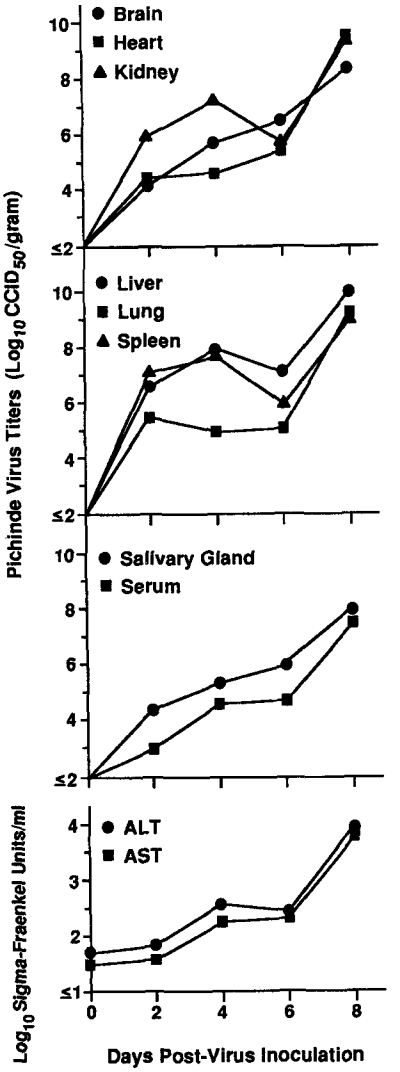

Fig. 1. Development of PCV titers, and effects of infection on serum alanine (AST) and aspartate (AST) aminotransferase activities in LVG/Lak hamsters. The virus challenge dose was 1000 PFU per animal administered by i.p. route.

spleen, brain, heart, serum, and salivary gland tissues rose steadily through the acute infection, and mean virus titers exceeded 10^7 $\log_{10}$ $CCID_{50}$/g in all tissues analyzed. ALT and AST values in serum increased to high levels by day 8, indicating severe liver damage. Yellow discoloration (icterus) of the liver was observed in animals near death. In addition, spleens of PCV-infected hamsters were markedly necrotic relative to uninfected animals. Histopathology of the virus-infected organs and tissues was not performed as part of these evaluations, however. Overt symptoms of the disease included hunching, ruffled fur, squinting and watery eyes, and staggering body movements. The virus, when properly inoculated i.p. into these animals, was uniformly fatal and caused death between 6 and 9 days post-virus inoculation.

Effect of virus dose on ribavirin activity

Since antiviral activity is dependent upon virus dose, an experiment was conducted to determine an appropriate PCV challenge dose for subsequent studies. This was accomplished by evaluating the efficacy of ribavirin in hamsters inoculated with different PFU of virus (Table 1). Ribavirin-treated (40 mg/kg) animals inoculated with 10^4 PFU survived the infection, but only half of the animals survived in the 20 mg/kg group. With two exceptions, virus titers in tissues and serum were only moderately reduced in the ribavirin-treated groups at this virus challenge dose. Serum and spleen virus titers were markedly decreased in the 40 mg/kg group, with inhibition of spleen virus titers being statistically significant.

As the infecting virus dose decreased to 10^3 and 10^2 PFU/animal, the degree of antiviral activity of ribavirin increased (Table 1). All ribavirin-treated animals survived these virus challenge doses. The amounts of virus recovered from tissues and sera of ribavirin-treated hamsters were much less than those seen in the placebo controls. Virus titers were suppressed to a greater extent in ribavirin-treated groups infected with 10^2 PFU than in the groups receiving higher virus challenge inocula. In this experiment, it appeared that low spleen and serum virus titers correlated well with a favorable prognosis for recovery from the lethal infection.

Comparative antiviral activities of ribavirin and ribamidine

Although ribavirin has been evaluated before in Pichinde virus animal infection models, the antiviral activity of the related nucleoside ribamidine had up to this point not been examined. Thus, a comparative dose-response study was initiated using a virus infecting dose of 10^3 PFU/animal (Table 2). Ribavirin completely protected hamsters from mortality at 32 mg/kg, was weakly active at 10 mg/kg and ineffective at 3.2 and 1 mg/kg. The 10 mg/kg dose prolonged life in those animals that died from the infection. The 32 mg/kg dose of ribavirin caused statistically significant reductions in all of the virological and enzymatic parameters. Based upon previous studies (Sidwell et

TABLE 1

Effect of virus challenge dose on the PCV-disease-inhibitory activity of ribavirin in LVG/Lak hamsters

Virus challenge (Log_{10} PFU/animal)	Ribavirin[a] (mg/kg per day)	Survivors/total	Mean day to death	Virus titer[b] (Log_{10} $CCID_{50}$/g) in			
				brain	liver	spleen	serum
10^2	0	0/20	7.3 ± 0.7[c]	⩾10.5 ± 0.0	⩾10.5 ± 0.0	⩾10.5 ± 0.0	⩾9.5 ± 0.0
10^2	20	10/10**	>21	8.6 ± 1.8	6.5 ± 2.3**	5.9 ± 1.0**	5.4 ± 1.3**
10^2	40	10/10**	>21	7.3 ± 1.6*	6.4 ± 2.1**	5.4 ± 0.6**	4.1 ± 2.7**
10^3	0	0/20	6.9 ± 0.5	⩾10.5 ± 0.0	⩾10.5 ± 0.0	⩾10.5 ± 0.0	⩾9.5 ± 0.0
10^3	20	10/10**	>21	8.3 ± 2.0	7.8 ± 1.8	6.8 ± 2.1**	6.0 ± 2.1**
10^3	40	10/10**	>21	8.8 ± 1.8	8.6 ± 1.7	6.0 ± 0.8**	5.6 ± 1.5**
10^4	0	0/20	6.8 ± 0.4	⩾10.5 ± 0.0	⩾10.5 ± 0.0	⩾10.5 ± 0.0	⩾9.5 ± 0.0
10^4	20	5/10**	10.4 ± 3.5*	9.7 ± 1.4	9.9 ± 1.7	9.2 ± 1.8	9.2 ± 0.9
10^4	40	10/10**	>21	9.0 ± 2.0	9.6 ± 1.4	6.9 ± 2.4*	6.9 ± 2.4

[a] Treatments were twice daily for 10 days starting 24 h after virus inoculation.

[b] Determined 7 days after virus challenge.

[c] Standard deviation.

*$P < 0.05$, **$P < 0.01$.

TABLE 2

Effects of ribavirin and ribamidine on PCV infections in LVG/Lak hamsters

Compound	Dose[a] (mg/kg per day)	Survivors/ total	Mean day to death	Virus titer[b] Log_{10} $CCID_{50}$/g) in				ALT[c]
				brain	liver	spleen	serum	
Placebo	–	0/20	7.3 ± 1.1[d]	⩾10.5 ± 0.0	⩾10.5 ± 0.0	⩾10.5 ± 0.0	⩾9.5 ± 0.0	2973 ± 1714
Ribavirin	1	0/10	7.9 ± 0.6	10.1 ± 0.5	10.1 ± 0.9	10.4 ± 0.3	9.4 ± 0.3	5000 ± 608
Ribavirin	3.2	0/10	8.0 ± 1.2	9.5 ± 0.8	6.4 ± 0.7**	9.3 ± 0.5	6.9 ± 1.1*	5150 ± 2333
Ribavirin	10	3/10*	9.6 ± 1.5*	8.5 ± 1.4	7.0 ± 1.5*	7.3 ± 1.1*	5.3 ± 1.4**	3400 ± 2005
Ribavirin	32	10/10**	>21	5.7 ± 0.2**	5.4 ± 1.0**	4.9 ± 1.0**	4.5 ± 0.5**	264 ± 143*
Ribamidine	10	2/10	7.5 ± 0.5	⩾10.5 ± 0.0	10.0 ± 0.5	10.4 ± 0.4	9.3 ± 0.6	4700 ± 2463
Ribamidine	32	5/10**	11.2 ± 4.5*	9.1 ± 1.3	8.9 ± 1.9	8.3 ± 1.9	6.4 ± 0.7*	1987 ± 2070
Ribamidine	100	8/10**	9.0 ± 0.0*	7.9 ± 1.3*	8.2 ± 1.7	6.8 ± 0.9**	5.3 ± 0.4**	80 ± 94**
Ribamidine	320	10/10**	>21	8.6 ± 0.7*	6.6 ± 0.9**	5.4 ± 0.7**	3.4 ± 0.3**	23 ± 6**

[a] Treatments were twice daily for 10 days starting 24 h after virus inoculation.

[b] Determined 7 days after virus challenge.

[c] Serum alanine aminotransferase activity expressed in Sigma-Fraenkel units/ml. Normal values for ALT in uninfected, untreated hamsters are <100 units/ml.

[d] Standard deviation. * $P < 0.05$, ** $P < 0.01$.

al., 1988a) it was anticipated that ribamidine would be active but less potent than ribavirin against PCV in vivo, thus higher doses of this agent were chosen for evaluation. Only the 320-mg/kg dose of ribamidine completely prevented death, but even the 100- and 32-mg/kg doses protected a significant number of animals. The two highest doses of ribamidine significantly reduced virus titers (in most tissues) and ALT values relative to the placebo group.

In comparing the antiviral activities of the two compounds, ribavirin (32 mg/kg) was more effective than ribamidine (100 and 320 mg/kg) to reduce brain ($P < 0.01$) and liver ($P < 0.05$) virus titers. The 32 mg/kg dose of ribavirin also reduced spleen virus titer significantly ($P < 0.01$) compared to the 100 mg/kg dose of ribamidine. The brain virus titer results suggest that ribavirin may be more effective than ribamidine to enter the central nervous system. Ribamidine suppressed virus-induced rises in ALT values at 100 and 32 mg/kg whereas ribavirin did so only at 32 mg/kg. In spite of the virus titer and ALT level differences, there were no statistically significant differences in mortality between the high doses of ribamidine vs. the highest dose of ribavirin. Overall, ribavirin activity at 32 mg/kg was similar to ribamidine activity at 100 and 320 mg/kg, indicating that the two compounds were approximately equally inhibitory to the infection but that ribavirin was 3–10-times more potent.

Toxicity evaluations of ribavirin and ribamidine were performed in uninfected hamsters (5 animals/group) in parallel with the above experiments. 10-day treatments with these compounds were not acutely toxic, since no animals died or lost weight. There were moderate degrees of suppression of weight gain at certain doses, however. The placebo controls gained a mean of 21.6 over 10 days compared to 14.7 g, 11.2 g, and 15.5 g for ribamidine groups treated with 320, 100, and 32 mg/kg per day, respectively. By comparison, the 32 mg ribavirin/kg per day group gained 16.7 g, which is also less than the placebo control. Lower doses of either compound did not suppress weight gain. The above results were not evaluated statistically, since animals were weighed in groups instead of individually. However, it is our experience that animal-to-animal weight variation within groups is low for these types of studies, suggesting that weight differences between the placebo and the above mentioned drug treatment groups were statistically significant. ALT values in uninfected hamsters were not elevated by treatments with ribavirin and ribamidine at the indicated doses. Other types of assays indicative of toxicity, such as hematocrit, creatinine and blood chemistry were not performed.

Antiviral activities of selenazofurin and ampligen

In experiments performed similarly to those described above, two other previously untested agents, selenazofurin and ampligen, were evaluated in this PCV infection model using a virus challenge dose of 1000 PFU/hamster. Doses of selenazofurin, ranging by half-$\log_{10}$ increments from 1 to 100 mg/kg per day, protected no animals from death nor extended mean survival times relative to placebo controls. Similarly, ampligen at 0.5 or 5 mg/kg given every other day

for 5 treatments provided no protection to the animals. In this experiment, the drug-treated animals died between 6.3 and 8.4 days. All placebo-treated animals died, with a mean day to death of 7.6 days. Ribavirin (32 mg/kg per day), included as a positive control, protected all of the hamsters from the lethal PCV infection.

In addition to the lack of antiviral activity of selenazofurin, the compound was overtly toxic to uninfected animals at two doses. The 100-mg/kg per day dose killed all hamsters, with a mean day to death of 6.5 days. At 32 mg/kg per day the animals all died, with a mean day to death of 13.3 days. Doses $\leqslant 10$ mg/kg were not overtly toxic, nor did they suppress weight gain relative to the placebo control. Ampligen was not lethally toxic nor suppressed weight gain at the two doses tested.

Discussion

These studies demonstrated that the LVG/Lak strain of Golden Syrian hamster could be used as a viable model for evaluating antiviral agents against PCV. In each antiviral experiment we performed, the mortality rate in the placebo group was 100%. This was achieved by i.p. injection of a suitable virus challenge dose, as opposed to s.c inoculation which causes non-fatal infections in these animals (Buchmeier and Rawls, 1977; and as confirmed by us in unpublished experiments). Formerly, antiviral chemotherapy studies of PCV infections in hamsters utilized the s.c.-infected MHA animal strain (Stephen et al., 1980). It may have been assumed that lethal infections could not be achieved in weanling LVG/Lak hamsters, based upon the published literature (Buchmeier and Rawls, 1977; Gee et al., 1979, 1980). After reading these reports, it is unclear to us whether these investigators ever attempted i.p. inoculation of weanling (3-week-old) LVG/Lak hamsters using the An 4763 strain of PCV. For example, the studies of Buchmeier and Rawls (1977) describes only s.c. inoculation using the An 3739 virus strain. By this method, animals less than 8-day-old or older animals immunosuppressed using cyclophosphamide died from the infection. Infection of animals by i.p. route was not mentioned in the article. Gee et al. (1980) inoculated PCV (An 3739 strain) i.p. into two inbred hamster strains (MHA and LSH), and found only the MHA strain to be lethally infected. In the same article they reported non-lethal infection experiments in random-bred LVG/Lak hamsters, but did not mention using the i.p. infection route for this particular animal strain. Jahrling et al. (1981) indicated that PCV (An 4763 strain) adapted to kill guinea pigs (a variant of the virus we used) was not lethal to Syrian hamsters. The strain of hamster and route of virus challenge were not described in that report, however.

Whether the strain of virus or particular source of LVG/Lak hamster we used was critical to establishing this new animal model remains to be determined. The An 4763 strain of PCV was the only virus we had in our

collection, thus the reason for its use in the present studies. The SASCO brand of LVG/Lak hamster is specific pathogen-free, whereas the same type of hamster obtained from other sources may not be. Whether the use of these animals contributed to the present results will require analyses in hamsters obtained from other venders. One titration of PCV was conducted using 3-week-old animals obtained from Simonsen Labs (Gilroy, CA), and most of those animals died from the infection (unpublished results), suggesting that the source of the animal may not be critical. Another unanswered question is how old an animal can be lethally infected with this virus. For our purposes, 3-week-old hamsters were quite suitable for antiviral drug evaluations. The oldest age of hamster we used that was susceptible to lethal infection was 5 weeks old; animals older than that were not studied (older animals weigh considerably more and thus require more drug to treat them). What appears to be essential to achieve lethal infections in weanling animals is correctly-delivered i.p. virus inoculations. For this reason we developed the method of delivering the virus in two injections (one on each side of the abdomen), using the full length of a 1-inch needle.

The mean day to death in i.p.-infected LVG/Lak hamsters is shorter (7–9 days) than for s.c.-infected MHA hamsters (10–13 days). Development of high virus titers in both strains of hamster appears to be similar (Stephen et al., 1980; and this report). The main advantages to using LVG/Lak hamsters over MHA hamsters are reduced cost and greater availability. For example, over a 6-month period we were unable to obtain any MHA hamsters from Charles River Laboratories, the sole supplier of this strain. Subsequent to this project we were informed that they have entirely eliminated production of these animals. Although the guinea-pig model of PCV infection has also been employed for antiviral studies and may be more akin to the human infection (Jahrling et al., 1981; Lucia et al., 1989), guinea pigs are very costly and require substantially more drug for treatments than do hamsters.

The effects of ribavirin to inhibit PCV disease in LVG/Lak hamsters was similar to those reported using MHA hamsters (Stephen et al., 1980). Ribavirin appears to be less effective in guinea pigs than in hamsters infected with PCV, however (Lucia et al., 1989). Although personnel affiliated with USAMRIID have evaluated other nucleoside analogs against PCV in animal models, the results have not been published, probably because the compounds have failed to exhibit antiviral activity. Here we report that the ribavirin derivative, ribamidine, exhibited anti-PCV activity in vivo. The potency of ribamidine against this virus infection was between one-third and one-tenth that of ribavirin, as was observed in studies against Punta Toro virus (Sidwell et al., 1988a,b). Since ribamidine was also well tolerated, the results suggest that the therapeutic indices (maximum tolerated dose divided by minimum effective dose) of both compounds are similar.

Both ribavirin and ribamidine showed moderate degrees of toxicity in this hamster strain at the antiviral doses used, as manifest by slower weight-gain relative to the placebo control. It should be pointed out that hamsters at this

age are in a rapid weight-gaining mode; older animals which are not growing much may be less sensitive to these effects. Because of the severity of arenavirus infections, their short duration, and the need for life-saving therapeutic agents, the toxicities of these agents appear to be reasonable. It should also be pointed out that in the Lassa fever trials conducted in man, ribavirin was not considered toxic at the doses that provided benefit to the patients (McCormick et al., 1986).

As was anticipated, tissue virus titers in drug-treated animals were reduced relative to those from placebo controls. These determinations were only made through 7 days of the infection in chemotherapy experiments, and the end point was based upon deaths in placebo-treated animals. It would be interesting to follow drug-treated animals for longer periods of time to determine virus clearance rates and improvement in other parameters of the infection.

These studies illustrate the importance of evaluating compounds in animal models to confirm antiviral activity initially established in vitro, as evidenced by the behavior of selenazofurin in both types of assays. Although selenazofurin showed potent anti-PCV activity in cell culture (Huggins et al., 1984), the agent proved to be inactive (and toxic) in infected hamsters. These results would not be predicted, especially knowing that selenazofurin inhibits other RNA viruses in mice (Sidwell et al., 1986; Smee et al., 1990). It may be that the pharmacology or toxicology of selenazofurin in hamsters is unfavorable relative to mice for providing antiviral protection to the animals.

Regarding the lack of efficacy of ampligen in the hamster model, PCV infections were previously found not to be inhibited by interferon or an inducer of interferon (Lucia et al., 1989), suggesting that a similarly acting agent such as ampligen would also be inactive. Arenaviruses are known to be relatively insensitive to the action of interferons (Canonico et al., 1984); apparently, the immunological events accompanying interferon induction in the host do not play a major role in combating PCV infections, either.

Up to the present time, the most potent and useful anti-arenavirus agent known continues to be ribavirin. Ribamidine represents a second compound that may hold clinical promise. A number of other potent anti-arenavirus agents (Andrei and De Clercq, 1990) and ribavirin-like agents (De Clercq et al., 1991) have been identified as active in cell culture screens. These await experimentation in animals to establish their potential utility in the treatment of human arenavirus infections.

Acknowledgments

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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).

References

Andrei, G. and De Clercq, E. (1990) Inhibitory effect of selected antiviral compounds on arenavirus replication in vitro. Antiviral Res. 14, 287–300.

Andrewes, C., Pereira, H.G. and Wildy, P. (1978) Arenaviridae. In: Viruses of Vertebrates, Fourth Edition, pp. 166–173. Cassell and Co., London.

Buchmeier, M.J. and Rawls, W.E. (1977) Variation between strains of hamsters in the lethality of Pichinde virus infections. Infect. Immun. 16, 413–421.

Burns, N.J., III, Barnett, B.B., Huffman, J.H., Dawson, M.I., Sidwell, R.W., De Clercq, E. and Kende, M. (1988) A newly developed immunofluorescent assay for determining the Pichinde virus-inhibitory effects of selected nucleoside analogues. Antiviral Res. 10, 89–98.

Canonico, P.G., Kende, M., Luscri, B.J. and Huggins, J.W. (1984) In-vivo activity of antivirals against exotic RNA viral infections. J. Antimicrob. Chemother. 14, Suppl A, 27–41.

De Clercq, E., Cools, M., Balzarini. J., Snoeck, R., Andrei, G., Hosoya, M., Shigeta, S., Ueda, T., Minakawa, N. and Matsuda, A. (1991) Antiviral activity of 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide and related compounds. Antimicrob. Agents Chemother. 35, 679–684.

Gee, S.R., Chan, M.A., Clark, D.A. and Rawls, W.E. (1980) Susceptibility to fatal Pichinde virus infection in the Syrian hamster. In: Hamster Immune Responses in Infections and Oncolytic Diseases, pp. 327–338. Eds: Streilein, J.W., Hart, D.A., Stein-Streilein, J., Duncan, W.R. and Billingham, R.E. Plenum Press, New York.

Gee, S.R., Clark, D.A. and Rawls, W.E. (1979) Differences between Syrian hamster strains in natural killer cell activity induced by infection with Pichinde virus. J. Immunol. 123, 2618–2626.

Huggins, J.W., Robins, R.K. and Canonico, P.G. (1984) Synergistic antiviral effects of ribavirin and the c-nucleoside analogs tiazofurin and selenazofurin against togaviruses, bunyaviruses, and arenaviruses. Antimicrob. Agents Chemother. 26, 476–480.

Jahrling, P.B., Hesse, R.A., Eddy, G.A., Johnson, K.M., Callis, R.T. and Stephen, E.L. (1980) Lassa fever infection of rhesus monkeys: pathogenesis and treatment with ribavirin. J. Infect. Dis. 141, 580–589.

Jahrling, P.B., Hesse, R.A., Rhoderick, J.B., Elwell, M.A. and Moe, J.B. (1981) Pathogenesis of a Pichinde virus strain adapted to produce lethal infections in guinea pigs. Infect. Immun. 32, 872–880.

Lucia, H.L., Coppenhaver, D.H. and Baron, S. (1989) Arenavirus infection in the guinea pig model: antiviral therapy with recombinant interferon-α, the immunomodulator CL246,738 and ribavirin. Antiviral Res. 12, 279–292.

McCormick, J.B., King, I.J., Webb, P.A., Scribner, C.L., Craven, R.B., Johnson, K.M., Elliot, L.H. and Belmont-Williams, R. (1986) Lassa fever: effective therapy with ribavirin. N. Engl. J. Med. 314, 20–26.

Montefiori, D.C. and Mitchell, W.M. (1987) Antiviral activity of mismatched double-stranded RNA against human immunodeficiency virus in vitro. Proc. Natl. Acad. Sci. USA 84, 2985–2989.

Reed, L.J. and Muench, M. (1938) A simple method of estimating fifty percent end points. Am. J. Hyg. 27, 493–498.

Sidwell, R.W., Huffman, J.H., Barnard, D.L. and Pifat, D.Y. (1988a) Effects of ribamidine, a 3-carboxamidine derivative of ribavirin, on experimentally induced *Phlebovirus* infections. Antiviral Res. 10, 193–208.

Sidwell, R.W., Huffman, J.H., Barnett, B.B. and Pifat, D.Y. (1988b) In vitro and in vivo *Phlebovirus* inhibition by ribavirin. Antimicrob. Agents Chemother. 32, 331–336.

Sidwell, R.W., Huffman, J.H., Call, E.W., Alaghamandan, H., Cook, P.D. and Robins, R.K. (1986) Effect of selenazofurin on influenza A and B virus infections in mice. Antiviral Res. 6, 343–353.

Sidwell, R.W., Huffman, J.H., Smee, D.F., Gilbert, J., Gessaman, A., Pease, A., Warren, R.P. and Kende, M. (1992) Potential role of immunomodulators for treatment of phlebovirus infections of animals. Ann. NY Acad. Sci. 653, 344–355.

Smee, D.F., Morris, J.L.B., Barnard, D.L. and Van Aerschot, A. (1992) Selective inhibition of arthropod-borne and arenaviruses in vitro by 3′-fluoro-3′-deoxyadenosine. Antiviral Res. 18, 151–162.

Smee, D.F., Huffman, J.H., Hall, L.L., Huggins, J.W. and Sidwell, R.W. (1990) Inhibition of *Phlebovirus* infections in vivo by tiazofurin and selenazofurin. Antiviral Chem. Chemother. 1, 211–216.

Stephen, E.L., Jones, D.E., Peters, C.J., Eddy, G.A., Loizeaux, P.S. and Jahrling, P.B. (1980) Ribavirin treatment of toga-, arena-, and bunyavirus infections in subhuman primates and other laboratory species. In: Ribavirin: A Broad Spectrum Antiviral Agent, pp. 169–183. Eds: Smith, R.A. and Kilpatrick, W. Academic Press, New York.

Stringfellow, D.A. (1977) Comparative interferon-inducing and antiviral properties of 2-amino-5-bromo-6-methyl-4-pyrimidinol (U025,166), tilorone hydrochloride, and polyinosinic-polycytidylic acid. Antimicrob. Agents Chemother. 11, 984–992.

Walker, D.H. and Murphy, F.A. (1987) Pathology and Pathogenesis of Arenavirus Infections. In: Current Topics in Microbiology and Immunology 133, 89–113. Ed: Oldstone, M.B.A. Springer-Verlag, Berlin.

Witkowski, J.T., Robins, R.K., Khare, G.P. and Sidwell, R.W. (1973) Synthesis and antiviral activity of 1,2,4-triazole-3-thiocarboxamide and 1,2,4-triazole-3-carboxamidine ribonucleosides. J. Med. Chem. 16, 935–937.