Med Microbiol Immunol
(1986) 175:183–186

Medical
Microbiology and
Immunology

© Springer-Verlag 1986

Diseases of experimental animals

Chairman: W. Klinkert

Effect of Ribavirin and immune serum on Junin virus-infected primates

M.C. Weissenbacher, M.M. Avila, M.A. Calello, M.S. Merani, J.B. McCormick, and M. Rodriguez

Cátedra de Microbiologia, Parasitologia e Inmunologia, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, 1121 Buenos Aires, Argentina

The only specific treatment available for Argentine Hemorrhagic Fever (AHF) is the administration of convalescent plasma within 8 days of onset of symptoms [5]. This therapy reduces mortality from 16% to 1–2%, but it seems to induce in 10% of patients a late neurological syndrome whose pathophysiology is as yet unknown [3].

Ribavirin treatment increases the number of survivors and reduces viremia in monkeys infected with Machupo or Lassa viruses [1]. In a therapeutic trial with Ribavirin in Lassa Fever patients, the lethality among high- and low-risk cases was significantly reduced; when immune serum was also administered, the number of survivors was not greater [4].

Previous reports from our laboratory indicate that the marmoset, *Callithrix jacchus*, experimentally infected with Junin virus (JV) develops a fatal disease which in several aspects mimics the natural infection in man. In this animal the clinical course of the disease, which proves 100% lethal around the 3rd week of infection, is characterized by hemorrhagic or neurological signs, pronounced and longlasting viremia, wide virus spread, leukopenia, anemia, and thrombocytopenia [6]. Recent findings in this animal model show that JV-immune serum treatment significantly reduced lethality, although a late neurologic syndrome was also observed. This work was carried out to assess the effect of Ribavirin on JV infection in vitro, followed by an attempt to determine the effect of Ribavirin alone or together with immune serum on the clinical course of the disease in the JV-infected marmoset.

A sterile solution of Ribavirin (1 β D ribofuranosyl 1,2,4-triazole-3-carboxamide) containing 100 mg/ml was obtained from Viratek, Covina, California, U.S.A., and diluted in Eagle's minimum essential medium. JV primate immune serum was from a pool of sera from 10 *C. jacchus* which had been immunized for a separate experiment with 10^3 $TCID_{50}$ of the attenuated $XJCl_3$ strain, challenged 60 days after infection with 10^3 $TCID_{50}$ of the pathogenic XJ strain, and bled 2 months later. The titer of neutralizing antibody was 1,280. Stocks of the pathogenic XJ strain and the attenuated $XJCl_3$ strain were produced in newborn mouse brain and had titers of 3×10^6 and 1×10^6 plaque-forming units (PFU) per ml, respectively. Vero cells were seeded in test tubes, in 96-well plates or 25 cm^2 glass bottles, and 48 h later, when confluent, virus in blood was assayed by intracerebral inoculation of suckling mice, whereas virus in organs was evaluated by inoculation of Vero cells. Neutralization tests were performed on Vero cell monolayers, using the constant virus-varying serum dilution technique. Antibody titers were expressed as the reciprocal of the highest dilution of serum which entirely inhibited the cytopathic effect of 100 $TCID_{50}$ of JV.

In order to quantify the effect of Ribavirin on the replication of JV in vitro, Vero cells grown in 96-well microplates or 25 cm^2 flasks were infected with 100 or 500 PFU of the attenuated strain. After 1 h adsorption, medium containing 0, 3.12, 6.25, 12.5, 25, 50, or 100 μg/ml of Ribavirin was added.

Three parameters were determined: 1) the cytopathic effect (CPE) of the virus by microscopic examination; 2) viral yield by titrating supernatants at 5–8 days post infection; and 3) the inhibition of PFU semisolid medium containing Ribavirin dilutions. Results are outlined in Table 1. At 25 μg/ml or above, Ribavirin inhibited CPE and reduced both viral replication and PFU formation to levels undetectable by the methods employed. Moreover, 6.2 and 12.5 μg/ml concentrations of the drug significantly depressed the parameters measured.

These findings, together with the reported low toxicity of the drug for mammals and the therapeutic effect observed in clinical trials with other arenaviruses, encouraged us to test Ribavirin in primates infected with JV. In all, 26 *C. jacchus* marmosets weighing between 240 and 340 grams were employed. Of these, 24 were inoculated intramuscularly with 10^3 PFU of XJ, while the remaining two were used for drug con-

Table 1. Effect of Ribavirin treatment on Junin virus-infected Vero cells

	Ribavirin concentration (μg/ml)				
	0	3.1	6.2	12.5	25–50–100
CPE[a] (day 5)	++	–	–	–	–
CPE (day 8)	++++	++	++	+	–
Virus	4.8[b]	3.7	3.1	2.5	0.7
PFU	430[c]	48	30	20	0

[a] Cytopathic effect

[b] Log_{10} plaque-forming units per ml in fluids of cultures infected with 100 plaque-forming units

[c] Plaque-forming units in cultures infected with 500 plaque-forming units

trol. All but six of the infected marmosets received a variety of treatments. The primates were bled at various times after infection to determine viremia and serum antibodies as well as for hematologic studies. Organ virus levels were determined in animals killed or found dead.

In a first step, a group of seven marmosets was treated with Ribavirin, 15 mg/kg twice daily, starting on day 6 up to day 19 after infection. Treatment was thus scheduled since most monkeys exhibit viremia at day 6 at a time presumably corresponding to the time when most AHF human patients also have viremia and immune plasma treatment is initiated. As shown in Table 2, none of the infected controls survived: mean time of death (MTD) was 19 days. Two out of the seven treated marmosets survived, while MTD for the rest was 36 days, most of which exhibited late involvement of the central nervous system.

Given these findings with a 15 mg dose, in a second step treatment was raised to 25 mg/kg twice daily, preceded by a 75 mg/kg loading dose. Two groups of infected marmosets received this latter dose from days 6 to 19 after infection, one of which was also given 2 ml immune serum by intraperitoneal route on day 6. A third uninfected drug control group was treated with the same Ribavirin schedule. This highest survival rate was recorded for the monkeys treated with both Ribavirin and immune serum (50%), whereas it was only 20% in those receiving the drug alone. For both groups the MTD was delayed significantly, and many survivors developed late neurological symptoms. Comparing the 15 and 25 mg treatment dose, it was noteworthy that the higher dose actually failed to increase the survival rate. Previous experiments in our laboratory showed that treatment with immune serum alone had led to 75% survival in infected primates (nine out of 12 marmosets). In this experiment, in spite of administering 25 mg/kg of Ribavirin twice a day in addition to the same serum treatment, the survival rate proved unexpectedly to be lower.

Table 2. Effects of Ribavirin and immune serum treatment on Junin virus-infected primates[a]

Treatment	Dead/Total	MTD[b] (days)
Ribavirin 15 mg/kg twice daily on days 6–19	5/7	36 (± 5.4)
Ribavirin, loading dose 75 mg/kg followed by 25 mg/kg twice daily on days 6–19	4/5	36 (± 12.6)
Immune serum, 2 ml on day 6 Ribavirin, loading dose 75 mg/kg followed by 25 mg/kg twice daily on days 6–19	3/6	28 (± 3.0)
Virus control	6/6	19 (± 2.3)
Ribavirin control: loading dose 75 mg/kg followed by 25 mg/kg twice daily on days 6–19	0/2	

[a] 10^3 plaque-forming units of XJ pathogenic strain
[b] Mean time to death

In the drug controls, hematological studies showed severe anemia and leukopenia, also present in infected Ribavirin-treated monkeys, even more pronounced than that induced by virus alone, which would explain the lower protection found even with the higher dose of Ribavirin, alone or associated with immune serum. Viremia levels were higher in untreated controls than in the treated marmosets of either group that survived, but differences were negligible for the treated animals that died. No serum antibodies or very low amounts were detected in the latter, but titers were high in survivors. This suggests that, at least in survivors, the therapy employed had no inhibitory effect on humoral immune response, but its role is unclear in treated marmosets found dead. Differences in organ virus levels between Ribavirin-treated *C. jacchus* which died and untreated controls were negligible. However, levels were slightly lower in Ribavirin- plus serum-treated primates. No viremia or virus were detected in the organs of survivors 2 months after treatment.

The in vitro results indicate that Ribavirin is highly effective in reducing JV replication in Vero cells. Other chemotherapeutic drugs such as Amantadine, glucosamine, 2-deoxi-D-glucose and Bis-benzimidazol have also proven effective against JV infection in vitro, but as a rule their in vivo action has been discouraging [2]. Up to now, Ribavirin is the only drug shown to modify the pattern of the disease, lower viremia, and increase survival in JV-infected animals. The absence of more significant protection and the later appearance of neurological involvement may be attributed to inadequate immune serum or drug concentration in the central nervous system, potential drug toxicity, or greater and early brain virus replication in this animal model, as compared to the disease in humans.

References

1. Huggins JW, Jahrling P, Kende M, Canonico PG (1984) Efficacy of Ribavirin against virulent RNA virus infections. In: Smith RA, Knight V, Smith JAD (ed). Clinical applications of Ribavirin. Academic Press (Harcourt Brace Jovanovich, Publishers) Orlando San Diego New York London Toronto Montreal Sydney Tokyo, pp 49–63
2. León ME, Coto CE, Weissenbacher MC (1977) Drogas inhibidoras del virus Junin. Medicina (Bs. Aires) 37:225–231
3. Maiztegui JI, Fernández NJ, Damilano AJ de (1979) Efficacy of immune plasma in treatment of Argentine Hemorrhagic Fever and association between treatment and a late neurological syndrome. Lancet II:1216–1217
4. McCormick JB, Webb PA, Johnson KM, Scribner CS (1984) Chemotherapy of acute Lassa Fever with Ribavirin. In: Smith RA, Knight V, Smith JAD (ed) Clinical applications of Ribavirin. Academic Press (Harcourt Brace Jovanovich, Publishers) Orlando San Diego New York London Toronto Montreal Sydney Tokyo, pp 187–192
5. Rugiero H, Astarloa L, Cintora A, Gonzalez Cambaceres C, Maglio F, Magnoni C, Milani H, Perez Izquierdo F (1977) Tratamiento de la Fiebre Hemorrágica Argentina. Medicina (Bs. Aires) 37:210–215
6. Weissenbacher MC, Calello MA, Colillas OJ, Rondinone SN, Frigerio MJ (1979) Argentine Hemorrhagic Fever: a primate model. Intervirology 11:363–365