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Antiviral treatment of Argentine hemorrhagic fever[1]

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Summary

Argentine hemorrhagic fever is a systemic viral disease caused by Junin virus, with a mortality of 15–30% in untreated individuals. Current specific therapy is highly effective in reducing mortality, and consists of the early administration of immune plasma in defined doses of specific neutralizing antibodies per kg of body weight. However, several reasons suggest the need to investigate alternative therapies. Ribavirin, a broad spectrum antiviral agent, is effective in the treatment of other viral hemorrhagic fevers, and the studies done with Junin virus infections to date indicate that this drug may also have a beneficial effect in Argentine hemorrhagic fever

Junin virus; Hemorrhagic fever; Immune plasma; Ribavirin

Introduction

Argentine hemorrhagic fever (AHF) is a systemic viral disease caused by Junin virus (JV), a member of the arenaviruses. Since the disease was first recognized (Arribalzaga, 1955), annual outbreaks have been registered without interruption, with more than 24000 cases notified to 1993. The endemo-epidemic area of the disease is located in the humid pampa, the most fertile farming land of Argentina. An important epidemiologic characteristic of AHF is the progressive extension of the endemic region (Maiztegui, 1975; Maiztegui et al., 1986).

The disease is characterized by renal, vascular, hematological and

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TABLE 1

Controlled therapeutical trial (1974–78): mortality in patients with AHF-treated with immune or normal plasma*

Treatment	Improved	Died	Total	Mortality
Immune plasma	90	1	91	1.1%
Normal plasma	81	16	97	16.5%
Total	171	17	188	

X^2: 11.72; $P = 0.0006$. *Maiztegui et al., 1979.

immunological alterations, with a mortality of 15–30% in untreated individuals (Maiztegui, 1975).

An attenuated live vaccine is available for AHF. This vaccine has shown efficacy in preventing the disease and is being used in the population exposed to the higher risk of infection (Maiztegui et al., 1991). Even with an effective vaccine, sporadic cases and even occasional outbreaks of AHF are expected to occur due to conditions linked to the natural reservoirs of the disease (Rugiero et al., 1959; Parodi et al., 1961; Sabattini and Contigiani, 1982; Weissenbacher et al., 1985; Mills et al., 1991).

Current therapy for AHF

AHF is one of the few viral diseases for which a specific treatment is available: the administration of immune plasma within the first 8 days from onset of symptoms. A controlled therapeutic trial, using 500 ml of either immune plasma or normal plasma in AHF cases within the first 8 days of illness demonstrated the efficacy of immune plasma in decreasing the mortality of AHF. (Table 1) (Maiztegui et al., 1979).

This treatment was then standarized based on the amount of neutralizing antibodies given to each patient (Enria et al., 1984). First, in a retrospective study the amount of neutralizing antibodies received by each patient was calculated on the basis of the following formula: "Therapeutic units by kg of body weight"

$$\text{TU/kg} : \frac{\Sigma(\text{weight} \times \text{titer of neutralizing antibodies of each unit})}{\text{body weight in kg}}$$

As a result of this study, it was demonstrated that the lower doses of neutralizing antibodies were associated with a higher mortality (Table 2). On the basis of these results, a prospective study was initiated, giving all patients more than 1000 TU/kg of neutralizing antibodies against JV (Table 3). A dose of 3000–4000 TU/kg is currently recommended.

Rationale for the evaluation of alternative forms of treatment in AHF

Immune plasma therapy is of no benefit to AHF patients when it is initiated

TABLE 2

Dose of neutralizing antibodies in treatment of AHF with immune plasma* retrospective study (1978–81)

Outcome	TU/kg		
	< 1000	⩾ 1000	Total
Died	4	3	7
Improved	3	27	30
Total	7	30	37

X^2: 5.44; P = 0.020. *Enria et al., 1984.

after 8 days of illness (Table 4). In this case, treatment is limited to supportive and symptomatic measures. In addition to this lack of efficacy in advanced cases of AHF, plasma therapy entails the risk of transfusion-borne diseases and, as well as the presentation of a late neurologic syndrome, that has been demonstrated in 10% of treated survivors (Maiztegui et al., 1979; Enria et al., 1985). These considerations, together with the difficulties with the maintenance of adequate stock of immune plasma, provide the rationale for the evaluation of alternative forms of therapy in AHF.

Preclinical studies of ribavirin in AHF

Ribavirin (1-β-D-ribofuranosyl-1–2–4-triazole-3-carboxamide) is a broad spectrum antiviral agent, that has been shown to have activity against RNA and DNA containing viruses, in vitro and in vivo (Huggins et al., 1984; Sidwell, 1984). In vitro, ribavirin was shown to be active against JV (Huggins et al., 1984; Rodríguez et al., 1986). Ribavirin therapy for experimental AHF includes studies performed in guinea pigs (Kenyon et al., 1986), the marmoset *Callithrix jacchus* (Weissenbacher et al., 1986), and in rhesus macaques (McKee et al., 1988). In guinea pigs, mortality was not affected by ribavirin or tributylribavirin, although viral replication was delayed and mean time of death was prolonged. In *Callithrix jacchus*, ribavirin modified the pattern of disease,

TABLE 3

Dose of neutralizing antibodies in treatment of AHF with immune plasma prospective study (1982–92)

Outcome	TU/kg		
	1000–1999	2000–2999	3000–3999
Died	2	3	5
Improved	24	46	908
Total	26	49	913
Mortality	7.69%	6.12%	0.55%

X^2: 26.32; P = 0.0002.

TABLE 4
Mortality in AHF patients-treated with immune plasma after 8 days of illness

Outcome	Immune plasma	
	yes	no
Improved	40	74
Died	21	31
Total	61	105
Mortality	34%	30%

X^2: 0.23; $P = 0.63$.

lowered viremia and increased survival, although late neurologic alterations appeared in the JV-infected animals. Rhesus macaques treated with ribavirin at the time of infection were protected from clinical disease. A delay in the initiation of therapy until after onset of illness resulted in an improvement and resolution of the systemic signs of disease; however, survivors subsequently developed a late onset central nervous system infection. The results of these preclinical studies provided evidences for therapeutic and antiviral activity of ribavirin in Junin virus infection.

Clinical studies of ribavirin in AHF

Clinical evaluation of ribavirin in AHF was done in cases with more than 8 days of evolution. These studies were done in 2 phases: first, an open study in 7 patients that received ribavirin (Enria et al., 1987), and second, a double blind trial in 18 cases that received either ribavirin or placebo. In both phases, criteria for selection were: (a) male or non-pregnant female, (b) age 15 or older, (c) clinical diagnosis of AHF of more than 8 days of evolution from onset of symptoms, (d) currently not participating in the active phase of any other clinical protocol, (e) signed informed consent prior to entry in the study. Ribavirin (Viratek, Costa Mesa, California) was given intravenously according to the following schedule: 34 mg/kg as a loading dose, followed by 17 mg/kg every 6 h for 4 days, and by 8 mg/kg every 8 h for the following 6 days. The rest of the treatment consisted of symptomatic and supportive measures, such as appropiate hydration and antipyretics. Superimposed bacterial infections were treated with antibiotics according to the organisms involved.

In all cases, the etiologic diagnosis of AHF was attempted by virus isolation and/or detection of specific antibodies. The following clinical laboratory parameters were measured daily: white blood cell count and differential, reticulocyte, red cell and platelet counts, hematocrit, hemoglobin, urine analysis, SGOT, SGPT, bilirrubin, creatinine, glucose and electrolytes. These tests were also repeated during the period of convalescence (at 20, 25, 30, 45, and 60 days after the onset of illness). Daily samples of blood were obtained for virus isolations as well as for specific antibody and endogenous IFN titrations. JV isolation and titrations were performed by intracerebral inoculation of

TABLE 5

Ribavirin study in AHF patients double blind trial comparability at baseline of the groups

Variable	Ribavirin (*n*:8)	Placebo (*n*:10)
Mean age ± S.D., years (range)	44.63 ± 14.56 (22–63)	38.30 ± 15.13 (27–67)
Distribution by sex, male/female (%)	7/1 (87.5%)	8/2 (80%)
Mean day of evolution ± S.D., days (range)	10.25 ± 0.71 (9–11)	9.80 ± 0.63 (9–11)
Presence of excitability and/or confusion (%)	3 (37.5%)	4 (40%)
Presence of seizures (%)	0 (0%)	1 (10%)
IFN levels ± S.D., log (range)	3.21 ± 0.82 (1.81–4.38)	1.65 ± 1.22 (0.4–3.03)
Presence of superimposed bacterial infection (%)	4 (50%)	4 (40%)

suckling white outbred mice (Ambrosio et al., 1986). Antibodies specific for JV were assayed by means of indirect immunofluorescence (Peters et al., 1973), ELISA (Meegan et al., 1986) and by plaque reduction neutralization tests (Webb et al., 1969). The levels of endogenous interferon were determined in Wish cells infected with VSV (Stewart, 1979).

From the 7 cases included in the open trial, 4 were severely ill when admitted. In one of these cases, the etiologic diagnosis of AHF could not be established. Five virus isolation attempts performed on successive days were negative, and antibodies against JV could not be detected. This patient was excluded from further analysis. Of the remaining 6 patients, 3 died and 3 survived (mortality rate: 50%)

In the double blind trial, 18 patients were enrolled. In all of them, the etiologic diagnosis of AHF was established. Eight of these cases received ribavirin and the other 10 placebo. Comparison at baseline of the two groups is shown in Table 5. Both groups had a similar distribution by age, sex and days of evolution at entry. A non-statistically significant tendency for greater values of endogenous IFN levels was observed in the ribavirin group. One out of the 10 patients from the placebo group (case 2) had seizures at entry. This patient died 48 h later, and could modify the comparability of the groups.

Mortality in the ribavirin group was 12.50% (1/8). Mortality in the whole placebo group was 40% (4/10), and excluding case 2, 33.33% (3/9) (Table 6). Overall mortality in AHF cases treated with ribavirin (6 from the open trial, and 8 from the double blind) was 28.57% (4/14).

TABLE 6

Mortality in double blind trial with ribavirin in AHF patients

Outcome	Ribavirin *n*:8	Placebo *n*:10	*n*:9*
Improved	7	6	6
Died	1	4	3
Mortality %	12.5%	40%	33.3%

X^2: 0.59; $P = 0.44$. *Excluding case number 2.

In all of the 14 AHF patients treated with ribavirin, clearance of viremia and decrease in IFN titers was observed. 4 Days after beginning treatment, all virus isolations were negative. In the 4 fatal cases, JV could not be isolated from either blood or liver at the time of death. In this respect, JV can be isolated from blood in almost 80% of terminal AHF cases at the time of death (Maiztegui et al., 1982). In all patients, a drop in endogenous interferon titer was observed 2 days after initiation of treatment with ribavirin. In the 4 fatal cases treated with ribavirin, the time of death appeared to be prolonged. One of the cases died on day 17 of the disease, while the other 3 died at day 14. Mean time of death in AHF is 12 days, with only 13% of cases dying after day 13.

The only adverse effect observed in the ribavirin treated AHF patients was the development of anemia, which has been documented previously among the secondary reactions of this drug (Canonico et al., 1984; Shulman, 1984). AHF is characterized by an acute and transient inhibition of global hematopoiesis of varying degree. This manifests itself as a decreased cellularity in the bone marrow, without persistent or permanent hematological abnormalities (Ponzinibio et al., 1979). In the cases treated with ribavirin, anemia was easily managed and the recuperation period was within the expected time, suggesting that the hematological alterations present in AHF are not a contradication for the use of ribavirin.

One of the ten survivors treated with ribavirin developed a febrile syndrome during early convalescence without any other clinical manifestations. This patient had alterations in the CSF consisting of an increased number of cells per mm^3, the presence of Ab against JV, and abnormalities in the evoked responses consisting of a prolonged time of central conduction and modifications in the morphology of the components. Although this patient had no neurological alterations and, hence, does not fullfill the criteria for being considered as a late neurologic syndrome (LNS), it is noteworthy that the combination of the CSF alterations and the abnormalities in the evoked responses detected in this case have been described in AHF patients with LNS (Enria et al., 1985; Cristiano et al., 1985).

The results described here demonstrate that ribavirin has an antiviral effect in advanced cases of AHF. In spite of the activity of this drug against JV, we were not able to show efficacy in reducing mortality in severe advanced cases of AHF. Available evidence suggests that the hematological, vascular and neurologic alterations seen in terminal cases of AHF may be mediated by vasoactive mediators, lymphokines and/or proteolytic enzymes released by damaged cells, and do not result from the action of virus itself (Bracco et al., 1978; Molinas and Maiztegui, 1981; Levis et al., 1984; Enria et al., 1986; Vallejos et al., 1989). However, the results of the small number of patients studied in a double blind fashion, suggest that ribavirin might still have a beneficial action in some patients with advanced disease. These findings, and those from the efficacy of ribavirin in the treatment of patients with Lassa fever and Hemorrhagic Fever with Renal Syndrome (McCormick et al., 1986; Huggins et al., 1991), suggest that ribavirin may also be effective in the

treatment of AHF if given during the first days of illness.

Conclusions

AHF is a disease with a high mortality in untreated individuals. Eradication of the disease is not possible, and even with the availability of an effective vaccine, sporadic cases and occasional outbreaks of the disease are expected to occur. For these reasons, it is very important to have a safe and effective treatment to offer to patients. Currently, specific treatment of AHF consists of the administration of immune plasma in defined doses of neutralizing antibodies against JV. This treatment is highly effective in reducing mortality if given early after the beginning of symptoms. Several factors indicate the need of alternative treatments. Among antiviral agents, ribavirin may prove useful in the treatment of AHF patients, and the continuity of the evaluation of this drug should be encouraged.

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