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Preliminary Report
Tolerance and antiviral effect of ribavirin in patients with Argentine hemorrhagic fever

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Summary

Tolerance and antiviral effect of ribavirin was studied in 6 patients with Argentine hemorrhagic fever (AHF) of more than 8 days of evolution. Administration of ribavirin resulted in a neutralization of viremia and a drop of endogenous interferon titers. The average time of death was delayed. A reversible anemia was the only adverse effect observed. From these results, we conclude that ribavirin has an antiviral effect in advanced cases of AHF, and that anemia, the only secondary reaction observed, can be easily managed. The possible beneficial effect of ribavirin during the initial days of AHF is discussed.

Ribavirin; Argentine hemorrhagic fever; Junin virus

Introduction

Argentine hemorrhagic fever (AHF) is a severe disease caused by Junin virus (JV), one of the four arenaviruses pathogenic for humans. The endemoepidemic area of the disease is located in the humid pampa, the most fertile farming land of Argentina. AHF is characterized by renal, hematological, inmunologic, neurological and cardiovascular changes, with a mortality rate of 15–30% in untreated

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individuals [12]. A specific treatment is available for AHF, which consists of the administration of immune plasma with defined neutralizing antibody titers [5,13]. However, this treatment is only effective when given within the first 8 days after onset of symptoms. Immune plasma therapy is of no benefit to AHF patients when it is initiated after 8 days of illness; in this case treatment is restricted to supportive measures [13]. In addition to this lack of efficacy in advanced cases of AHF, plasma therapy entails the risk of transfusion-borne diseases and the presentation of a late neurologic syndrome (LNS) that has been demonstrated in 10% of treated survivors [6,13]. These considerations have led us to evaluate alternative forms of treatment in AHF.

Ribavirin (1-β-D-ribofuranosyl-1-2-4-triazole-3-carboxamide) is a synthetic nucleoside that has been shown to have a broad spectrum of activity against RNA and DNA containing viruses in vitro and in vivo [8,9,11,19,22].

The present study was designed to evaluate the tolerance and the activiral activity of ribavirin in patients with AHF when treatment was begun 8 days after the onset of symptoms.

Materials and Methods

A prospective study was conducted in 7 cases with a clinical diagnosis of AHF of more than 8 days of evolution. Four of the 7 cases were very ill when admitted. Each individual (or responsible family member when the patient was incapacitated) signed an informed consent prior to his/her entry in the study. Criteria for patient selection included: (1) male or non-pregnant females; (2) age 15 or older; (3) currently not participating in the active phase of any other clinical protocol; and (4) clinical and laboratory findings strongly suggestive of AHF. On admission, a detailed medical history of each patient was obtained and a complete examination was performed daily by 2 experienced physicians. Vital signs were recorded 4 times a day or more frequently if necessary. Ribavirin (Viratek, Inc., 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. Other treatment consisted of symptomatic and supportive measures, such as appropriate 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 antibody [12]. 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, bilirubin, creatinine, glucose and electrolytes. These tests were also repeated during the period of convalescence (20, 25, 30, 45 and 60 days after the onset of illness) in all survivors. Daily samples of blood were obtained for virus isolation as well as for specific antibody and IFN determinations. JV isolation and titrations were performed by intracerebral inoculation of white outbred mice, as described

TABLE 1

Virological findings in AHF cases treated with ribavirin.

Case	Study	Day of evolution of AHF															
		9	10	11	12	13	14	15	16	17	18	19	20	25	30	45	60
1	a	2.2 ↓	2	Pos**	Neg	Neg	Neg	Neg	Neg	Neg							
	b	128	32	8	4	4	4	4	4	4	6	6	4				
	c	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	10	10	2560	2560	2560	2560
	d	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	8	⩾32	⩾32	⩾32
2	a			2.1 ↓	Pos	2.3	Neg	<1.7	Neg	Neg	Neg	Neg	Neg				
	b			8000	2000	256	64	32	4	4	4	4	4				
	c			Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	640	2560	2560
	d			Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	8	⩾ 32	⩾32
3	a		Neg ↓	Neg	Neg	Neg	Neg	Neg	Neg	Neg							
	b		8000	4000	1500	750	48	12	8	4	4	6	4				
	c		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	40	2560	2560	2560
	d		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	16	⩾32	⩾32	⩾32
4*	a		2.2 ↓	Neg	Neg	Neg	Neg	Neg	Neg	Neg							
	b		8000	4000	128	32	4	4	4								
	c		Neg	Neg	Neg	Neg	40	40	40								
	d		Neg	Neg	Neg	Neg	Neg	Neg	Neg								
5*	a		1.7 ↓	2.4	Neg	Neg	Neg										
	b		⩾64000	2000	256	64											
	c		Neg	Neg	Neg	20											
	d		Neg	Neg	Neg	Neg											
6*	a			2.8 ↓	Neg	<1.7	Neg										
	b			48	64	12	24										
	c			Neg	Neg	Neg	Neg										
	d			Neg	Neg	Neg	Neg										

* Died. a = Viral isolation from blood log LD50/ml; b = IFN titer IU/ml; c = PRNT Ab; d = IIF Ab.

↓ Treatment with ribavirin.

** Junin virus isolated in first blind passage of mice.

elsewhere [1]. Antibodies (Ab) specific for JV were assayed by means of indirect immunofluorescence (IIF) and by plaque reduction neutralization tests (PRNT) [12,16]. The levels of endogenous interferon were determined in Wish cells infected with VSV [10].

Results

In one of the 7 cases initially included in this study the etiologic diagnosis of AHF could not be established. This patient died 15 days after the onset of symptomatology and 5 days after the start of ribavirin treatment. Five virus isolation attempts performed on successive days were negative. Antibodies specific for JV could not be detected by either IIF or PRNT; hence, this patient was excluded from further analysis.

Of the remaining 6 patients, 3 died and 3 survived. The results of virus isolations, IFN titers and Ab quantitation are shown in Table 1. Three days after beginning treatment, all virus isolations were negative. Furthermore, in the 3 fatal cases, JV could not be isolated from either blood or liver at the time of death. One of these patients died on day 17 of the disease while the other two patients died 14 days after the onset of symptoms. In all patients, a drop in IFN titer was observed 2 days after initiation of treatment with ribavirin.

The hematologic results showed a drop in hematocrit and hemoglobin beginning on days 5–6 of treatment. The lowest hematocrit recorded was 29%. Anemia was easily managed in all patients. Only patient 1 received a blood transfusion (500 ml packed red cells) when the hematocrit dropped to 29%. In the remaining cases, the anemia accompanying ribavirin treatment required no specific treatment. Peak reticulocyte counts were documented approximately 30 days after the onset of the disease in the surviving patients. This was followed by a rising hematocrit, which returned to normal by 60 days post disease onset.

One of the 3 survivors (case No. 2) developed a febrile syndrome during early convalescence (day 31) without any other clinical manifestations. The patient was readmitted and remained under close observation for a week. During this period, a careful clinical follow up revealed no neurological abnormalities. Except for a mild anemia, a routine clinical laboratory analysis showed no other alterations. A lumbar puncture was performed in this patient, with the finding of 28 cells per mm^3 (lymphocytes) and Ab specific for JV by PRNT with titers of 1:640 in the CSF. A serum sample taken simultaneously showed Ab specific for JV by PRNT with a titer of 1:2560. The patient was discharged afebrile, and prior to discharging, auditory evoked responses of brain stem were evaluated. This patient showed abnormalities in the evoked responses consisting of a prolonged time of central conduction and modifications in the morphology of the components.

The 3 survivors of the study are still on follow-up, and have not presented any other alterations.

Discussion

Immune plasma is very effective in reducing mortality in AHF when given within the first 8 days after the onset of symptoms, but it is of no benefit in patients treated after this time [13]. This treatment is complicated by the potential risk of transfusion-borne diseases and by the presentation of an LNS in 10% of treated survivors [6,13]. Furthermore, immune plasma is somewhat difficult to obtain, and expensive to test and store properly.

These considerations led us to initiate studies with ribavirin, a broad spectrum antiviral drug with proven efficacy in several related viral diseases [8,11].

The clearance of viremia and decrease in IFN titers observed in the 6 AHF cases in this study demonstrate that ribavirin has an antiviral effect against JV. In addition, JV was not isolated from the 3 fatal AHF cases. In this respect, it should be noted that JV can be isolated from blood in almost 80% of terminal AHF cases at the time of death [14]. In the 3 fatal cases treated with ribavirin the average time to death appears to be prolonged, since the mean time to death in AHF is 12 days, with only 13% of cases dying after day 13.

In spite of the activity of ribavirin against JV, the administration of this drug to patients with advanced AHF was not effective in decreasing mortality. It is noteworthy that the available evidence suggests that the hematologic, vascular and neurologic alterations seen in severe advanced 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 the virus itself [2,7,10,15,20]. If this is the case, no beneficial therapeutic effect should be expected from the antiviral action of ribavirin when given late in the course of illness. From this study, and from the efficacy of ribavirin in the early treatment of patients with Lassa Fever, we infer that administration of this drug early after the onset of symptoms might also be effective in AHF.

The only adverse effect observed in the 6 patients treated with ribavirin was the development of anemia, which has been documented previously among the secondary reactions of this drug [3,18]. AHF is characterized by an acute and transient inhibition of global hematopoiesis of varying degree. This manifest itself as a decreased cellularity in the bone marrow, without persistent or permanent hematological abnormalities [17]. In the patients described here, anemia was easily managed and the recuperation period was within the expected time, suggesting that hematologic alterations present in AHF are not a contraindication for the use of ribavirin.

One of the 3 survivors 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 and 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. The combination of the CSF alterations and the abnormalities in the evoked responses detected in this patient have been described in AHF patients with LNS [6,4]. On the other hand, late onset neurological manifestations have also been described in

experimental models of arenavirus infections (JV and Machupo virus) treated with ribavirin [8,9,22]. The patient in question had no neurological alterations, hence, does not fulfill the criteria for consideration as a case with LNS. Nonetheless, this is the first report of alterations in evoked responses and abnormalities in the CSF in a patient that was not treated with immune plasma. Although this may be a coincidental finding, we believe that this observation deserves mentioning and needs further confirmation.

The results described here demonstrate that ribavirin has an antiviral effect in advanced cases of AHF, and that anemia, the only secondary effect observed, can be easily managed. Based on these results, we postulate that ribavirin may be effective in the treatment of AHF if given during the first days of illness.

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