ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1996, p. 2555–2557 Vol. 40, No. 11
0066-4804/96/$04.00+0

Phase I Study of Intravenous Ribavirin Treatment of Respiratory Syncytial Virus Pneumonia after Marrow Transplantation

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Received 30 May 1996/Returned for modification 12 July 1996/Accepted 27 August 1996

Respiratory syncytial virus (RSV) pneumonia in marrow transplant recipients is associated with significant mortality. Ribavirin is a nucleoside analog with activity against RSV and in its aerosolized formulation is the only drug approved for treatment of RSV pneumonia in the United States. The clinical use of aerosolized ribavirin has been limited by caregivers' concerns about drug exposure and potential teratogenic effects. Since there is lack of proven efficacy and safety of the aerosolized ribavirin in this setting, we performed a phase I study of intravenous ribavirin treatment. Between November 1993 and May 1994, 10 patients with clinically significant RSV pneumonia at the Fred Hutchinson Cancer Research Center were enrolled. Only 2 of the 10 survived (20%; 95% CI, 3-56). Two of the 10 patients developed acute hemolysis that necessitated discontinuation of the medication. In conclusion, treatment of marrow transplant recipients with RSV pneumonia with intravenous ribavirin did not improve mortality compared with historical controls treated with the aerosolized drug.

Respiratory syncytial virus (RSV) is being increasingly recognized as a cause of serious pneumonia following marrow transplantation. Englund et al. described six patients with RSV pneumonia after transplant, three of whom died despite treatment with aerosolized ribavirin (3), while Hertz et al. reported on six patients, four of whom died (7). Harrington et al. characterized an outbreak of RSV infection in 31 patients in a marrow transplant center, 18 (58%) of whom developed pneumonia (6). Of these 18, 13 (72%) died. More recently, Whimbey et al. described an outbreak of RSV in 42 patients in a marrow transplant center, 16 (38%) of whom developed pneumonia. Of these, 42% died (15, 16).

Ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a nucleoside analog with activity against RSV in vitro and in vivo (8, 11, 10). The aerosolized formulation is currently the only approved drug in the United States for treatment of RSV pneumonia. In some noncontrolled studies, aerosolized ribavirin appears to have improved the clinical outcome of RSV pneumonia in children with a history of cardiac or respiratory disease (5). All the patients described by Englund and Hertz received aerosolized ribavirin (3, 7). In the Harrington study, 13 of the 18 received aerosol ribavirin and all of the survivors were among those treated for more than 5 days (6). The differences in outcomes between treated and untreated patients were not statistically significant, and the high mortality in each of these studies suggests that the beneficial effect of aerosolized ribavirin, if any, is small. In the Whimbey studies, 12 of the 16 patients were treated with a combination of aerosolized ribavirin and intravenously (i.v.) administered immunoglobulin (15, 16). All of the survivors received treatment, and the patients whose treatment was initiated more than 24 h prior to intubation had more favorable outcomes (15, 16). Since these studies were neither randomized nor controlled trials, the efficacy of aerosolized ribavirin remains uncertain.

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The use of aerosolized ribavirin has been limited by caregivers' concerns about personal drug exposure and potential teratogenic effects to their unborn children (14). i.v. administration of ribavirin has been effective in treating various viral infections, such as Lassa fever (13), hemorrhagic fever with renal syndrome (9), Argentine hemorrhagic fever (4), urinary tract adenoviral infections (12), and Sabia virus (1). It is potentially attractive because it avoids the environmental contamination of the aerosolized treatment. Since there is currently no therapy proven to be of benefit in marrow transplant recipients with RSV pneumonia, we performed a phase I pilot study to determine the safety and clinical effect of i.v. administered ribavirin given for 7 days in patients with proven lower-respiratory-tract RSV infection.

MATERIALS AND METHODS

Patients. All bone marrow transplant recipients and candidates at the Fred Hutchinson Cancer Research Center with symptoms of upper-respiratory-tract infection between November 1993 to May 1994 were evaluated by pooled nasal-pharyngeal (NP) swabs for virus fluorescent antibody (FA) stain and culture (6) and for the presence of lower-respiratory-tract infection by physical exam and chest radiograph. Patients with a positive nasal swab and with auscultatory wheezes or crackles, infiltrates present on chest radiograph, or hypoxemia underwent bronchoalveolar lavage (BAL). Patients with a positive direct-FA stain or culture for RSV of BAL were eligible for i.v. treatment with ribavirin. Patients with hemolytic anemia or a known sensitivity to ribavirin were not considered eligible.

Treatment protocol. The protocol was performed in accordance with human subject guidelines and approved by the institutional review board. Informed consent was obtained from the patients or their parents or guardians. Patients received ribavirin i.v. in a loading dose of 35 mg/kg of body weight, given in three divided doses every 8 h and then a maintenance dosage of 25 mg/kg/day given in three divided doses every 8 h for an additional 6 days. Each dose was administered for 30 min. The dosing scheme was based on the manufacturer's (ICN Pharmaceuticals, Costa Mesa, Calif.) recommendation and on the report by Huggins et al. (9).

Laboratory evaluation. Viral specimen collection, transportation, culture, and FA (Bartels Immunodiagnostic Supplies, Bellevue, Wash.) of NP swab and BAL materials were performed as previously described (6). BAL, biopsy, and autopsy specimens were submitted for microbiological, cytological, and pathological examinations.

During the study, NP swabs were repeated every 3 days, and BAL was re-

TABLE 1. Patient demographics and severity of illness at time of presentation of RSV pneumonia

Patient no.	Age (yr)	Gender	Dx[a]	BMT[b]		Engraftment	Chest X-ray	Oxygenation[c]	Concomitant infections
				Day	Type				
1	49	M	MM	7	M, U	No	Diffuse	NP	VRE[d]
2	41	M	NHL	9	M, R	No	Diffuse	Ventilator	*Staphylococcus aureus*/sepsis
3	50	M	NHL	11	A	Yes	Diffuse	50% FM	None
4	22	F	AML	13	A	No	Focal	NP	None
5	60	M	RAEB	16	M, R	No	Diffuse	100% HFG	None
6	59	M	AA	76	M, R	Yes	Diffuse	70% FM	Adenovirus
7	46	F	MM	77	M, R	Yes	Focal	NP	*Aspergillus* sp./parainfluenza
8	42	M	MM	78	M, R	Yes	Focal	94% RA	Parainfluenza
9	49	F	CML	110	M, U	Yes	Diffuse	NP	*Aspergillus* sp.
10	32	M	CML	151	M, U	Yes	Diffuse	100% HFG	None

[a] DX, underlying diagnosis. MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; AML, acute myelogenous leukemia; RAEB, refractory anemia with excess blasts; AA, aplastic anemia; CML, chronic myelogenous leukemia.
[b] BMT, bone marrow transplant. Type: M, matched; U, unrelated; R, related; A, autologous; day, day after transplant.
[c] NP, Nasal prongs; FM, face mask; HFG, high-flow oxygen generator; RA, room air.
[d] VRE, vancomycin-resistant enterococcus.

peated between days 5 and 7 of treatment if the patient's clinical condition permitted. Complete blood counts, the amount of free hemoglobin, reticulocyte count, and the levels of alanine aminotransferase, lactic dehydrogenase, creatinine, and blood urea nitrogen were determined daily for 10 days and then on days 14, 21, and 28 after the start of treatment. All patients were evaluated daily during the treatment by a physical examination by a study physician and a chest radiograph.

Transplant protocols. Conditioning regimens included total body irradiation delivered as 10 Gy in a single dose or as a 12- to 15.75-Gy midline dose at 6 to 7 cGY/min in six or seven fractions without lung shielding. Methotrexate, cyclosporine, or a combination of both with or without the addition of methylprednisolone were given as graft-versus-host-disease prophylaxis to allogeneic marrow recipients.

Patients who were not allergic to sulfamethoxazole and trimethoprim received these drugs for 2 weeks before transplant surgery and for 6 months after transplant surgery as a prophylaxis against *Pneumocystis carinii* pneumonia. Cytomegalovirus-seronegative marrow recipients received cytomegatovirus-seronegative blood products. Ganciclovir prophylaxis was started either at engraftment or for cytomegalovirus antigenemia in both seropositive recipients and allogeneic seronegative recipients with seropositive donors.

RESULTS

Between November 1993 and May 1994 at the Fred Hutchinson Cancer Research Center, 28 patients had NP swabs positive for RSV by FA. Of these, 16 (57%) had signs and symptoms limited to the upper respiratory tract, and all of these patients survived. None of these patients received therapy for RSV. Twelve patients (43%) had signs and symptoms of lower-respiratory-tract disease and BAL results demonstrating RSV infection. Ten of these were enrolled in the i.v. administered ribavirin protocol. Of the remaining two, one declined to participate and the other was not offered treatment because of imminent death with complications of severe graft-versus-host disease.

The demographics for the patients are shown in Table 1. The average age of the 10 patients in the study was 50 years (median, 49 years; range, 22 to 60 years) (Table 1). The mean time after marrow infusion at onset of treatment was 55 days (median, 46 days; range, 7 to 151 days). Eight patients developed infection before day 100, and six were engrafted at the time of enrollment in the study. Renal function was abnormal (serum creatinine of >2.0 mg/dl) in two patients, while hepatic function was abnormal (alanine aminotransferase level of >50 mg/dl) in four patients.

Seven of 10 patients with lower-respiratory-tract RSV infection had diffuse, bilateral infiltrates on chest radiographs. One of these patients also was concomitantly diagnosed with an *Aspergillus* infection by BAL. The remaining three had localized radiographic infiltrates. Two of these three patients were documented by BAL to have concomitant infections: both *Aspergillus* and parainfluenza virus type 3 infections in one case and a parainfluenza virus type 3 infection in the other.

Table 2 shows clinical outcomes. Two of the marrow recipients treated i.v. with ribavirin survived (mortality, 80%; 95% confidence interval, 44 to 97). Five patients, including the two survivors, completed the full 7 days of i.v. ribavirin treatment. Three patients died before completing therapy. In two patients, treatment was stopped prematurely because of acute hemolysis. In one patient (no. 3), the free hemoglobin level began to rise on day 3 of treatment and peaked at 346 mg/dl (normal, <5 mg/dl) on day 4 without a decline in hematocrit. Ribavirin was stopped at this time. Within 3 days the free hemoglobin level returned to normal, but the patient subsequently died of respiratory failure. The autopsy revealed diffuse alveolar damage with RSV infection. The other patient (no. 9) developed hemolysis on day 5 of i.v. ribavirin treatment (hematocrit dropped from 29 to 19.9% and the free hemoglobin level increased from <5 to 32.6 mg/dl). Ribavirin was stopped, and the patient expired the next day, with respiratory failure and suspected disseminated *Aspergillus* infection. No autopsy was performed. At the time that hemolysis developed, neither patient had significant hepatic or renal dysfunction. Both of the patients who were not enrolled in the study died.

TABLE 2. Clinical and microbiological outcomes of patients with RSV pneumonia

Patient no.	Survival (days)	Cause of death	Final viral result		
			Culture	FA[a]	Toxicity
1	18	VRE[b]	−	+	
2	5	Respiratory failure	−	ND[c]	
3	5	Respiratory failure	Toxic	+	Hemolysis
4	>365	NA[d]	−	−	
5	2	Respiratory failure	−	ND	
6	7	Respiratory failure	−	ND	
7	8	Respiratory failure	+	+	
8	>365	NA	+	+	
9	6	Respiratory failure	+	+	Hemolysis
10	29	Multiple	+	+	

[a] FA, fluorescent antibody staining for RSV.
[b] VRE, vancomycin-resistant enterococcus.
[c] ND, not done.
[d] NA, not applicable.

No patient requiring more than minimal supplemental oxygen support (i.e., nasal prong O_2) survived. Both survivors had minimal radiographic infiltrates. Other factors such as age, engraftment, time after transplantation, and concomitant organ failure did not appear to be associated with survival, although the number of patients studied was small.

Follow-up viral cultures or FA results were available from NP and/or BAL from all patients during treatment. Viral cultures became negative by the end of the treatment for five patients. Seven patients had BAL or autopsies at the completion of treatment or death. Lower-respiratory-tract cultures from three of these patients became negative and three remained positive. The remaining culture was toxic to the fibroblast monolayer and was therefore uninterpretable. FA staining for RSV protein became negative for only one of six patients who could be evaluated.

Clearance of viral excretion did not correlate with survival. At the end of the 7-day treatment period, one of the two survivors had an NP swab that was negative by both FA and culture for RSV, while the other remained positive by BAL.

DISCUSSION

In this study, 10 patients with BAL-proven lower-respiratory-tract RSV infection were given ribavirin i.v. Two of the 10 survived (20%; 95% confidence interval, 3 to 56), a rate comparable to that for patients treated with the aerosolized drug (3, 7, 6). Because of the small sample size and design limitations of this study, only large differences could be detected with certainty.

Two of the 10 patients developed significant acute hemolysis. The hemolysis resolved after cessation of the therapy in one patient, while the other expired shortly after therapy was stopped. Hemolysis has been reported as a toxicity of i.v. administered ribavirin (2). As much as a 20% decline in hematocrit was reported during a 12-day course for treatment of Lassa fever (13). In that study, transfusions were not required and the anemia reversed after completion of treatment. Similarly, evidence of hemolysis, noted by a rise in indirect bilirubin levels, was seen in all males treated i.v. with ribavirin for 7 days for hemorrhagic fever with renal syndrome (9). No patients were removed from that study because of anemia. It is unclear whether the hemolysis that we saw after marrow transplantation associated with i.v. ribavirin treatment was substantially more severe than that reported among other patient populations. Prior transfusions, as well as the marrow transplant itself, could make marrow recipients more susceptible to erythrocyte injury and acute hemolytic episodes.

There appeared to be no correlation between cessation of viral excretion and survival. Although five of nine evaluable patients became culture negative for RSV, only one of these five survived. The measurements of study drug concentrations in blood and BAL were not available during the study. As a result, we cannot discern whether the decrease in viral excretion was the result of i.v. ribavirin therapy or the result of host immunity. Viral antigen persisted in respiratory secretions in six of seven patients tested by FA stains at the end of therapy, including one of the survivors. Ribavirin may have had an inhibitory effect on the culture of respiratory secretions while viable virus persisted in vivo. In contrast, since FA staining can detect viral antigens in the absence of viable virus, i.v. ribavirin treatment truly may have had an antiviral effect. If so, cessation of viral replication was not sufficient to reverse the underlying lung injury and ultimate fatality. Since only patients with evidence of limited lung injury (i.e., mild hypoxemia) were among the survivors, successful treatment of RSV pneumonia may depend on the early recognition of infection and treatment prior to the development of severe lower-respiratory-tract disease. The studies by Whimbey et al. (15, 16) support this hypothesis.

In conclusion, treatment of marrow transplant recipients with RSV pneumonia with ribavirin i.v. did not improve mortality compared with that of historical controls treated with the aerosolized drug. The incidence of associated hemolysis in this and other populations raises concerns about the safety of i.v. administration of ribavirin after marrow transplantation. Future investigations should focus on alternative treatments of established RSV pneumonia (such as immunoglobulins or cytokine inhibitors) and, possibly, preemptive treatment strategies for early infection.

ACKNOWLEDGMENTS

This study was funded by the following grants: NIH 5T32-HL-07287, an American Lung Association Fellowship Award, and a Poncin Research Award (D.M.L.); NCI P01-CA-18029 and NIH HL3644 (R.A.B.); and NCI P01-CA-18029 (S.W.C.).

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