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Treatment of Influenza A(H1N1) Virus Infection with Ribavirin Aerosol

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In a randomized, controlled study of ribavirin aerosol treatment of influenza A(H1N1) virus infection among college students, treated patients had a significantly shorter duration of fever than control patients. There was a trend of more rapid recovery in treated patients. Virus shedding was similar in treated and control patients, declining gradually from a 50% tissue culture infective dose of 3.5 $\log_{10}$ per ml at admission to 1.8 $\log_{10}$ per ml at 53 h after admission. There was no local or systemic intolerance and no hematological or biochemical abnormalities associated with ribavirin treatment.

Ribavirin, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, a broad-spectrum antiviral agent, has been used successfully to treat respiratory syncytial virus infection in children (6, 10) and respiratory syncytial virus and parainfluenza virus infections of immunodeficient children (5) by aerosol. We previously described the therapeutic efficacy of ribavirin small-particle aerosol in the treatment of influenza A/England/333/80(H1N1) virus infection in college students (7). In that study, fever and illness disappeared more rapidly and virus shedding was less in treated patients than in control patients.

In February and March 1982, an outbreak of influenza occurred again in the same location, and a randomized, controlled study was made of ribavirin aerosol in its treatment. When diagnostic studies became available, it was learned that the outbreak had consisted of about equal numbers of patients with influenza A(H1N1) virus and influenza B virus infections. The present report will describe the findings in the treatment of patients with influenza A(H1N1) virus infection; the favorable results of the treatment of influenza B virus infection with ribavirin aerosol have been presented elsewhere (8).

MATERIALS AND METHODS

Patients. Fifty-four students with a clinical syndrome resembling influenza of <24-h duration and with fever ≥101°F volunteered for a randomized, controlled study of influenza treatment. After signing consent forms approved by the institutional review boards of Texas A & M University and Baylor College of Medicine, they were assigned to treated or control groups by a code derived from a table of random numbers. Control patients received sterile saline by aerosol. Patients were not informed whether they were to receive ribavirin or saline aerosol.

The etiology of the infection of these patients based on virus isolation was not known until after completion of treatment. Viral diagnostic studies ultimately revealed that 19 patients had influenza A/England/333/80-like virus infection, of which 8 were treated and 11 were controls. Twenty-one other patients proved to have influenza B/Singapore/222/79-like virus infection, two others had a picornavirus, and one had varicella virus. The remaining 11 were undiagnosed.

Four of eight treated patients and 5 of 11 control patients were women. One treated patient was of hispanic origin and one control patient was oriental. Six treated and 10 control patients reported having had influenza within the past 2 years. One control patient reported receiving an influenza vaccination within 2 years. The mean age of treated patients was 20.8 years, and that of control patients was 21.2 years. Except for one control patient who was 28 years of age, patients in both groups ranged in age from 18.7 to 23.2 years. A pregnancy test and a careful history revealed no women with evidence of pregnancy.

Aerosol-producing system. A Collison generator operated by compressed air was designed to operate continuously with an aerosol flow of 12.5 liters/min with an average ribavirin concentration of 190 μg/liter of air. This concentration of ribavirin is slightly greater than previously reported due to an improved method of circulation of the drug solution between the aerosol generator and the fluid reservoir (8). The particle size was 1.3 microns mass median diameter, with 95% of the particles <5 microns in diameter. The aerosol generator was connected to a Puritan Benefit Mask worn by the patient by means of smooth-flow Corr-A type II tubing. Treatment was begun within 1 h of admission and continued for 16 h continuously or until 6:00 a.m. the following morning, whichever came first. The following morning all participants were treated on a regular schedule for 4 h continuously, commencing at 7:00 a.m., 1:00 p.m., and 7:00 p.m. Treatment was continued through the first 4-h period on day 3. The retained dose of ribavirin was calculated as follows: the mean minute volume (liters) for each patient (2) each day was multiplied by 0.19 mg (mean ribavirin aerosol concentration per liter) times 60 min times the number of hours of treatment each day times 0.7 (estimated fraction of inhaled aerosol deposited in the respiratory tract) as previously described (8; B. E. Gilbert, S. Z. Wilson, V. Knight, R. B. Couch, T. L. Melhoff, H. W. McClung, G. W. Divine, D. D. Bartlett, L. C. Cohan, and T. L. Gallion, *in* R. A. Smith, V. Knight, and J. Smith (ed.), *Clinical Aspects of Ribavirin*, in press). This calculation gives the estimated milligrams of ribavirin retained per day for each patient.

Assessment of illness and fever. A detailed clinical assessment of each patient was made on admission and daily at 8:00 a.m. and 5:00 p.m. thereafter until discharge. The details of this examination were described in our previous report (7). In brief, a detailed history and physical examina-

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tion, concentrating on respiratory tract disease, were made and recorded on a form designed for computer input. At the conclusion of each exam, the illness of the patient was scored with a range of 0 to 3+ (increasing severity) according to the categories of rhinitis, pharyngitis, tracheobronchitis, pneumonia, and systemic illness. As in previous studies, systemic illness characterized by prostration, anorexia, headache, and muscle aches was the most prominent feature of the illness. Patients who remained confined to bed except for bathroom activities and were profoundly prostrated were classified as 3+ severity. Those remaining essentially bedridden but with less prostration were classified as 2+ severity; the major activity of this group was watching television. Students not yet asymptomatic but who were ambulatory were classified as 1+. Patients were assigned to one of four physicians for their care and evaluation. As in previous studies, each physician was trained for this assignment by a senior clinician (H. McC.) who also supervised their treatment and evaluation of patients (7, 8). Physicians were aware of the treatment status of patients. The patients were not informed of their treatment status until discharge from the hospital. Oral temperatures were taken by nurses every 4 h. Antipyretics were not routinely available; however, two treated and two control patients received a single dose of acetaminophen for temperatures exceeding 103.5°F.

Clinical laboratory studies. The following automated biochemical observations were made on serum at admission, discharge, 1 week after discharge, and 1 month after discharge: sodium, potassium, chloride, calcium, phosphorus, urea, creatinine, uric acid, cholesterol, triglycerides, total protein, globulin, albumin, glucose, total bilirubin, direct and indirect bilirubin, alkaline phosphatase, aspartate aminotransaminase, alanine transaminase, lactic dehydrogenase, and gamma glutamyltransaminase.

Hematological studies consisted of hematocrit, hemoglobin, mean cell volume, and leukocyte count with differential and platelet count. Serum for acute and convalescent antibody titration was stored at −20°C. Throat swabs for bacteria and chest roentgenograms were obtained at admission.

Virus isolation and quantification. A throat swab was collected at admission in veal infusion broth containing penicillin and streptomycin and subjected to standard virus diagnostic procedures (3). The initial isolates from all 19 patients in this study exhibited specific immunofluorescence with influenza A/Brazil/11/78(H1N1) virus (1, 4). Subsequent analysis (see below) of antibody response of the patients suggested that these strains were most closely related to influenza A/England/333/80(H1N1).

Quantification of virus in nasal secretions was performed in 24-well microtiter plates containing MDCK cell culture by a method described previously (7). Titers were calculated by the method of Karber (9) and are reported as the 50% tissue culture infective dose ($TCID_{50}$) per milliliter of original nasal wash. In the previous report (7), they were given as $TCID_{50}$ per 0.1 ml.

Hemagglutination inhibition-antibody titration. Specimens from acutely ill and convalescent patients were titrated in plastic dishes with 8 rows, each containing 12 0.25-ml conical wells (Linbro-Titerek, Hamden, Conn.), by a standard method (3). Three antigens, A/England/333/80(H1N1), A/Brazil/11/78(H1N1), and A/Texas/13/81(H1N1) were used in the tests. There was a high degree of cross-reactivity with the three antigens, but there was a slight predominance of reactivity with influenza A/England/333/80(H1N1).

Statistical analysis. The Student's *t* test and the Wilcoxon rank sum test were used as described below.

Susceptibility of clinical isolates to ribavirin. To determine whether clinical isolates of influenza virus varied significantly from year to year in their susceptibility to ribavirin, 61 samples obtained over a 3-year period from patients in our studies at Texas A & M University were tested. Pre- and posttreatment influenza virus isolates were passaged once from tissue culture harvests (1981 and 1982) or from nasal wash specimens (1983) into embryonated eggs. Egg pools were collected and virus was quantitated ($TCID_{50}$ per milliliter) as previously described (7). Drug sensitivity was determined by addition of 10 $TCID_{50}$ (0.1 ml) of virus to confluent monolayers of MDCK cells, adsorption for 1 h at 34°C, and replacement of the inoculum with fresh minimal essential medium with Worthington trypsin (2 μg/ml) containing 0, 10, 20, 30, 40, 60, 80, or 100 μM ribavirin (three wells per dilution). After 3 days of incubation at 34°C, the presence of viral replication at each dilution of ribavirin was determined by the hemadsorption of guinea pig erythrocytes (0.075%) at 4°C. The $TCID_{50}$ of ribavirin was calculated by plotting the number of positive wells showing hemadsorption against the log of the ribavirin concentration. In addition, the MIC of ribavirin was determined as the last well not demonstrating hemadsorption. A standard laboratory strain of influenza A/Bangkok/1/79(H3N2) was assayed with each replicate set of tests. The $TCID_{50}$ determined for eight assays was 32.5 ± 28.2 μM. Probability (*P*) values were calculated by the Student's *t* test, two-tailed.

RESULTS

Febrile response in treated and control patients. Nineteen patients had influenza A(H1N1) virus infection, of which 11 were controls and 8 were treated. The mean time of illness before treatment was 2.25 h greater in control than in treated patients (17.00 and 14.75 h, respectively), a difference greater than in previous studies. The period from the start of treatment to a sustained afebrile state was less in treated (31.4 h) than in control (42.7 h) patients but was of borderline significance. However, if the calculation is made from onset of illness to the sustained afebrile state (<100°F), the difference is significant (treated: 46.1 h; control: 59.7 h; $P = 0.045$, Student's *t* test, two tailed).

In Table 1, the mean maximum daily temperatures for treated and control patients show a trend to lower temperature in treated patients on day 2.

Illness in treated and control patients. Table 2 shows the assessment of rhinitis and systemic illness in treated and control patients. Rhinitis was more severe before treatment in control patients and, thereafter, was about equal in the two groups until day 2 when treated patients improved more rapidly than control patients. Systemic illness was about the same severity in treated and control patients except on the afternoon of day 2 when it was significantly less in treated patients. In both groups, the p.m. values on day 2 were inconsistently low. Tracheobronchitis and pharyngitis were not severe, and there were no significant differences between treated and control patients. There was no pneumonia in either group.

Virus shedding in treated and control patients. All 19 patients had influenza A(H1N1) virus isolated from a throat swab at admission. In one treated patient, however, virus shedding was never detected in any of the nasal wash specimens. Virus titers in treated and control groups declined from a mean $TCID_{50}$ of 3.5 $\log_{10}$ per ml at admission to 1.8 $\log_{10}$ per ml at 53 h after admission. Virus titers of nasal secretions obtained from treated and control patients at admission and at 9, 19, 31, 43, and 53 h after admission were

TABLE 1. Mean maximum daily temperatures in treated and control patients

Patient group (no.)	Mean maximum daily temp (°F) on day:			
	0	1	2	3
Treated (8)	103.1	102.8	99.4	98.2
Control (11)	102.9	102.2	100.3	98.4
P- value	0.150[a]	0.106	0.070	0.150

[a] Student's *t* test, one-tailed.

not significantly different ($P > 0.3$; Student's *t* test, two-tailed).

Hemagglutination inhibition antibody titers of treated and control patients. The geometric mean serum antibody titer to influenza A/England/333/80 at the time of admission was 1:2.8 and 1:2.7 in treated and control patients, respectively, whereas 1 month later the titers in treated and control patients were 1:13 and 1:21, respectively. The differences in titers in treated and control patients at admission or in convalescence were not statistically significant (Wilcoxon rank sum test, two-tailed).

Hematological findings. The hematological profiles in treated and control patients were very similar at all times. The leukocyte series revealed the expected low normal values at onset of this illness. At discharge, total counts were still lower duè to a further reduction in polymorphonuclear cells. Absolute lymphocyte counts were lowest at admission and increased slightly thereafter. Platelet counts remained within a normal range throughout the study. The hematological findings in both groups reflect the normal course of influenza infection.

Biochemical tests in treated and control patients. The mean values of 22 biochemical tests in treated and control patients were within a normal range. Some differences in means within a normal range were encountered that approached significance. These were examined individually (triglycerides, albumin, and gamma glutamyltransaminase) and considered to be of no clinical significance.

Ribavirin aerosol dosage. Table 3 shows the estimated retained dosage of ribavirin. From the table, it can be calculated that the dose per hour of treatment per patient was 51.6 mg or 0.79 ± 0.04 mg/kg per h. The total dose was 1.9 g during 37.3 h of treatment, largely administered during the first 60 h in the hospital. This is very similar to dosages of ribavirin aerosol in other studies (7, 18). There was no local or systemic intolerance to the treatment.

Susceptibility of influenza viruses to ribavirin. Pre- and posttreatment influenza virus isolates from 3 consecutive years of observation of the college students were tested for susceptibility to ribavirin. Among influenza B virus isolates in 1982, the $TCID_{50}$ in posttreatment specimens was about twofold greater than in the pretreatment specimens (Table 4). Influenza A virus isolates during the 3 years did not show an increase in the inhibitory concentration of ribavirin in posttreatment specimens, nor was there any difference in isolates obtained from control or treated patients. In a limited number of paired pre- and posttreatment samples, ribavirin susceptibility was similar (data not shown).

TABLE 2. Rhinitis and systemic illness in treated and control patients

Illness in patient group	Range of severity (mean score) on day:						
	0	1		2		3	
		a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
Rhinitis							
Treated	0.9	1.6	1.5	1.0	0.6	0.5	0.5
Control	1.7	1.6	1.6	1.5	1.5	1.1	1.0
P value[a]	0.025	0.43	0.28	0.070	0.002	0.042	0.060
Systemic illness							
Treated	2.4	2.3	1.8	1.8	0.5	1.5	0.3
Control	2.4	2.5	1.6	1.5	0.9	1.8	0.5
P value[a]	0.463	0.175	0.428	0.186	0.026	0.138	0.204

[a] Wilcoxon rank sum test, one-tailed.

TABLE 3. Estimated mean retained dose of ribavirin

Day	No. of patients receiving	No. of h administered per day	Estimated mean retained dose of ribavirin	
			mg/day	mg/kg per day
0	7	4.7	251	3.7
1	8	18.3	998	14.8
2	8	11.3	564	8.4
3	1	3.0	112	2.5

DISCUSSION

This study has described a favorable effect of ribavirin aerosol in the treatment of influenza A(H1N1) virus infection in a group of college students. Fever, systemic illness, and rhinitis improved more rapidly in treated patients than in randomly selected control patients who were given saline aerosol.

Virus disappeared from nasal wash fluids at about the same rate in treated and control patients. This is in contrast to results of a previous study of influenza A(H1N1) virus infection a year earlier at the same site and a study of influenza B virus infection concurrent with the present study (7, 8). Virus titrations of nasal secretions in the present influenza A(H1N1) study were performed >6 months after the specimens were collected, and the treated and control patients, though randomly selected, were not equal in number. Moreover, the mean periods of illness before treatment were dissimilar, both variations being introduced because of the unanticipated occurrence of both influenza A(H1N1) virus and influenza B virus infections in the same outbreak. These events may have influenced virus shedding patterns.

As in two other studies of the treatment of influenza (7, 8), there was no detectable respiratory intolerance, and no hematological, liver function, or blood chemical abnormalities associated with treatment.

The limited therapeutic effect was apparently not the result of increased resistance of the virus to treatment. The only increase in resistance to ribavirin after treatment occurred in influenza B isolates and that increase was not considered to be of clinical significance. The variations in susceptibility and in the standard deviation were most likely due to variations inherent in virus quantification. Since drug susceptibility to ribavirin is virus concentration dependent, errors in 10-fold dilutions of virus used to calculate the appropriate $TCID_{50}$ values (i.e., ± 0.25 $\log_{10}$) would be reflected in two- or threefold errors in determining drug susceptibility levels.

We have noted elsewhere (Gilbert et al., in press) that the duration of illness with influenza A(H1N1) virus infection is less than that with influenza A(H3N2) or B virus infection. Thus, there is less time in which to show a therapeutic effect. In 1981, despite a short duration of illness with A(H1N1) infection, the ribavirin aerosol treatment was quite effective (7). The shorter period of illness may be part of the explana-

TABLE 4. Susceptibility of clinical isolates of influenza virus to ribavirin

Virus	Year	$TCID_{50}$ (μM ± SD)[a]			P value[b] (pre- vs posttreatment)
		Pretreatment	Posttreatment	All isolates	
H3N2	1983	39.6 ± 12.7 (5)		39.6 ± 12.7 (5)	
B	1982	40.6 ± 10.4 (5)	78.6 ± 32.2 (8)	64.0 ± 31.8 (13)	0.032
H1N1	1983	57.0 ± 30.2 (7)	42.4 ± 15.0 (7)	49.7 ± 24.2 (14)	0.279
	1982	42.8 ± 38.7 (6)	58.7 ± 32.4 (6)	53.4 ± 34.5 (12)	>0.6
	1981	39.8 ± 36.3 (17)		39.8 ± 36.3 (17)	

[a] Number of isolates tested.
[b] Student's *t* test, two-tailed.

tion for the limited therapeutic effort observed in the present study, but additional factors may contribute.

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LITERATURE CITED

1. **Baxter, B. D., R. B. Couch, S. B. Greenberg, and J. A. Kasel.** 1977. Maintenance of viability and comparison of identification methods for influenza and other respiratory viruses of humans. J. Clin. Microbiol. **6**:19–22.
2. **Comroe, J. H., Jr., R. E. Forster III, A. B. Dubois, W. A. Briscoe, and E. Carlsen.** 1962. Clinical physiology and pulmonary function tests, 2nd ed., p. 328. Year Book Medical Publishers, Inc., Chicago.
3. **Dowdle, W. A., A. P. Kendal, and G. R. Hokle.** 1979. Influenza viruses, p. 585–609. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial and chlamydial infections. American Public Health Association, Inc., Washington, D.C.
4. **Frank, A. L., R. B. Couch, C. A. Griffis, and B. D. Baxter.** 1979. Comparison of different tissue cultures for isolation and quantitation of influenza and parainfluenza viruses. J. Clin. Microbiol. **10**:32–36.
5. **Gelfand, E. W., D. McCurdy, C. P. Rao, and P. J. Middleton.** 1983. Ribavirin treatment of viral pneumonitis in severe combined immunodeficiency disease. Lancet **ii**(8352):732–733.
6. **Hall, C. B., J. T. McBride, E. E. Walsh, D. M. Bell, C. L. Gala, S. Hildreth, L. G. TenEyck, and W. J. Hall.** 1983. Aerosolized ribavirin treatment of infants with respiratory syncytial viral infection. N. Engl. J. Med. **308**:1443–1447.
7. **Knight, V., H. W. McClung, S. Z. Wilson, B. K. Waters, J. M. Quarles, R. S. Cameron, S. E. Greggs, J. M. Zerwas, and R. B. Couch.** 1981. Ribavirin small-particle aerosol treatment of influenza. Lancet **ii**(8253):945–949.
8. **McClung, H. W., V. Knight, B. E. Gilbert, S. Z. Wilson, J. M. Quarles, and G. W. Divine.** 1983. Ribavirin aerosol treatment of influenza B virus infection. J. Am. Med. Assoc. **249**:2671–2674.
9. **Rhodes, A. J., and C. E. Van Rooyen.** 1953. Textbook of virology, ed. 2. The Williams & Wilkins Co., Baltimore, p. 66–69.
10. **Taber, L. H., V. Knight, B. E. Gilbert, H. W. McClung, S. Z. Wilson, H. J. Norton, J. M. Thurston, W. H. Gordon, R. L. Atmar, and W. R. Schlaudt.** 1983. Ribavirin aerosol treatment of bronchiolitis associated with respiratory syncytial virus infection in infants. Pediatrics **72**:613–618.