Pharmacokinetics of Ribavirin Aerosol in Mice

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The pharmacokinetics of ribavirin administered in single or multiple treatments to mice by small-particle aerosol were monitored in lung, serum, and brain tissues. Ribavirin aerosol was administered with a standard drug concentration (20 mg/ml) in the reservoir for 12 h or a high dose (60 mg/ml) for 2 or 4 h. After single or 3-day treatments, ribavirin rapidly accumulated in the lungs at concentrations sufficient to inhibit influenza virus or respiratory syncytial virus (1 to 5 mM). While peak levels of ribavirin in the lungs after the high-dose administration were about three times those found with the standard dose, ribavirin was rapidly cleared from the lungs. There was no accumulation of drug in the lungs after multiple treatments. Ribavirin cleared from the lungs was detected in the blood within 15 min. Concentrations in the serum were similar (20 to 30 μM) for standard- and high-dose treatments with either single or multiple treatments. Ribavirin clearance from the serum after treatment was similar for each regimen. Ribavirin also rapidly accumulated in the brain to a similar level (ca. 6 nmol per brain) after standard- or high-dose treatment for 3 days. In contrast to ribavirin in the serum, ribavirin in the brain appeared to be slowly cleared, allowing levels to remain relatively constant during and after treatment. With the interest in viral encephalopathies, further evaluation of the possible advantages of this method of drug administration is warranted.

Ribavirin is a broad-spectrum antiviral agent that is used to treat respiratory syncytial virus infections in infants and has been shown to effectively inhibit the replication of influenza, parainfluenza, and a number of other respiratory viruses (7–10). The current protocol recommends prolonged daily ribavirin treatment periods of 12 to 18 h. Recently, we showed that a shorter period of treatment with higher concentrations of ribavirin is as effective in reducing pulmonary viral titers in mice and cotton rats (*Sigmoden hispidus*) experimentally inoculated with influenza or respiratory syncytial virus and in preventing mortality in mice given lethal doses of influenza A or B virus (18, 19). In the present study, the pharmacokinetics of ribavirin in lung, serum, and brain tissues of mice given standard and high doses of ribavirin by continuous small-particle aerosol were monitored by using a high-performance liquid chromatography assay (15) to determine drug levels in these tissues during and after administration. Standard administration consisted of 12 h of drug treatment per day with 20 mg of ribavirin per ml in the reservoir. High-dose, short-duration administration consisted of drug treatment for 2 h twice daily with 60 mg of ribavirin per ml in the reservoir.

This report shows that after high-dose, short-duration aerosol administration, ribavirin rapidly accumulated in the lungs and was quickly cleared once treatment had ceased; that ribavirin was quickly absorbed into the blood; and that ribavirin appeared to accumulate in brain tissue.

MATERIALS AND METHODS

Mice. Six- to eight-week-old (25- to 28-g) randomly bred CD-1 mice obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass., were used in all experiments. The animals were housed in cages covered with barrier filters and were fed mouse chow and water ad libitum.

Small-particle aerosol treatment. Mice were placed in sealed plastic cages and exposed to aerosols of ribavirin as described previously (18). The aerosol generator and particle characteristics were the same as those previously described (11, 17). The average concentrations of ribavirin in the delivered aerosol were 200 and 600 μg/liter of air for reservoirs containing 20 and 60 mg of ribavirin per ml, respectively. Assuming that a mouse has a minute volume of approximately 1 ml/g of body weight (14), we estimated that a 28-g mouse exposed to aerosols generated from reservoirs containing 20 and 60 mg of drug per ml would retain 101 mg (413 μmol) and 303 mg (1,238 μmol) of ribavirin per h of exposure, respectively. With either the standard or high-dose, short-duration administration, this estimate is equivalent to 43.3 mg/kg of body weight. Additional drug may have been obtained orally from grooming of the fur.

Three randomly chosen animals were sacrificed for each data point. Blood (ca. 0.25 ml) was removed from the orbital sinus plexus before each animal was killed. The animals were then sacrificed. Brain samples, consisting mostly of cerebral hemispheres and occasionally of some cerebellum, and then the lungs were removed. Blood was mixed with 0.25 ml of high-performance-liquid-chromatography-grade water. Lungs and brains were washed free of adhering blood and homogenized in 1 ml of high-performance-liquid-chromatography-grade water. All samples were stored at −70°C until they were processed.

Quantification of ribavirin in biological tissues. All samples of lung, blood, and brain tissues were processed by centrifugation at 13,000 × g for 5 min to remove debris, deproteinized by ultrafiltration through CF25 cones (Amicon Corp., Danvers, Mass.), and chromatographed on a phenylboronate affinity column as previously described (15, 18). Ribavirin was quantified by high-performance liquid chromatography, with monitoring at 207 nm. All measurements were made at ambient temperature on a Microsorb C18 stainless steel high-performance liquid chromatography column (particle size, 5 μm; length, 25 cm; inner diameter, 4.6 mm; Rainin Instrument Co., Emeryville, Calif.). A ribavirin (10 μg/ml) standard was periodically processed, and recovery for 14 samples was found to be 101 ± 7% (mean ± the standard
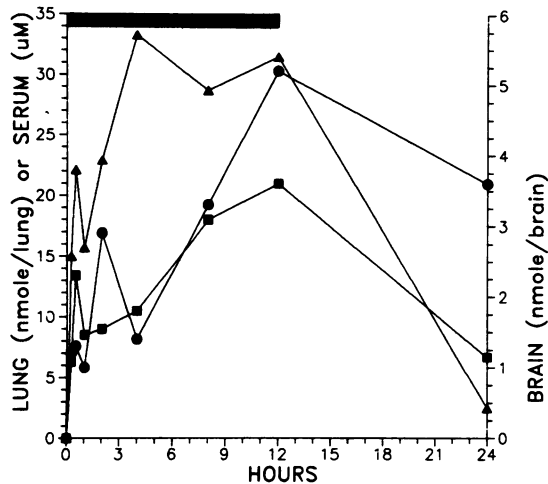
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FIG. 1. Pharmacokinetics of ribavirin in lung, serum, and brain tissues after a single administration by small-particle aerosol for 12 h with 20 mg of drug per ml in the reservoir. Tissues were obtained and processed as described in Materials and Methods. Ribavirin levels were measured by high-performance liquid chromatography. Values represent the means of data for three mice. Symbols: ▲, lung; ■, serum; ●, brain; ▬, period of aerosol administration.

deviation). This method does not measure phosphate derivatives of ribavirin.

Estimation of ribavirin concentrations in lung and brain tissues. The following assumptions were made to estimate the average tissue fluid concentration of ribavirin for a 28-g mouse. (i) The average amount of liquid in the mucus layer in lung tissue is estimated to be 1 μl/g of body weight (12). This estimate assumes an average mucus thickness of 1 μm over 70 m^2 for a 70-kg person. (ii) Hematocrit is 46%, and the total blood volume is 0.071 ml/g of body weight, or 2.0 ml (1). (iii) The volume of cerebrospinal fluid in the mouse brain is 0.040 ml, as extrapolated from the volume in the human brain, which is 100 ml (range, 90 to 150 ml/70 kg of body weight), or 1.43 μl/g of body weight; brain weight is 3% of total body weight; and plasma volume of the brain is 0.030 ml/g of tissue (1).

By using these values, the volume of serum contained within the brain was estimated to be 28 g × 3% × 0.030 ml/g, or 0.0252 ml. Ribavirin concentration in the brain, corrected for residual blood, was calculated from the following equation: corrected ribavirin concentration (μM) = [(total nmol of ribavirin per brain) − (nmol of ribavirin per ml of serum) (0.0252)]/0.040 ml of cerebrospinal fluid. This correction reduced the total nanomoles of ribavirin per brain by 8.5 ± 3.5% (mean ± the standard deviation), which is an overestimate of the amount of contamination.

Determination of ribavirin half-life. Data were analyzed by curve-fitting programs by using a Hewlett-Packard 41 CV programmable calculator.

Statistical analysis. Data were analyzed with the aid of the clinical information data management and analysis system. P values were calculated by the Student t test, or, if data were not normally distributed, by the Wilcoxon rank sum test.

RESULTS

Single-treatment pharmacokinetics. Ribavirin levels in lung, serum, and brain tissues were determined after mice were treated with either standard-dose ribavirin (20 mg/ml in

the reservoir) for 12 h or high-dose ribavirin (60 mg/ml in the reservoir) for 2 h.

Standard-dose regimen. With the standard dose, ribavirin levels rose rapidly in the lungs and attained maximum levels (30 to 35 nmol per lung) at 2 to 3 h (Fig. 1). These levels were maintained until treatment was stopped at 12 h and the drug was cleared from the lungs. Twelve hours after treatment was ended, only 2.5 nmol of ribavirin (7.5% of the peak level) remained in the lungs. The rate of clearance was consistent with a more detailed determination (data not shown) which estimated the half-life to be about 3 to 4 h.

Part of the clearance of ribavirin involved absorption into the blood. At the earliest time point (15 min), ribavirin was detected in the serum (6.5 ± 0.4 μM [mean ± the standard deviation]). Except for an initial spike in drug levels at 30 min, which paralleled the increase seen in the lungs, levels in serum increased throughout the treatment period, reaching a maximum concentration of 21 ± 3 μM. During the 12-h period after treatment, ribavirin was cleared from the serum, with 31.9% of the peak level remaining after 24 h.

Ribavirin also was detected in brain tissue at the 15-min point. In general, drug levels in the brain mirrored those seen in the lungs and serum and increased during the treatment period to a maximum level (5.2 nmol per brain) after 12 h of treatment. During the 12-h nontreatment period, levels in the brain decreased but not at the same rate as seen in the serum (31 versus 68%, respectively).

High-dose regimen. With high-dose administration for 2 h, ribavirin levels in the lungs rapidly increased to a maximum level 3.5 times that seen at the maximum standard-dose level (116 ± 21 versus 31.5 ± 1.1 nmol per lung) (Fig. 2). This increase reflected the threefold-greater concentration of ribavirin in the reservoir. At the end of the 2-h exposure period, drug levels rapidly decreased. The initial phase of clearance had a half-life of 1.2 h, followed by a second phase with a half-life of 4 to 6 h.

Ribavirin was rapidly absorbed into the blood, reaching a serum concentration somewhat higher than that seen with the standard dose (i.e., 34 ± 6 versus 21 ± 3 μM). After aerosol exposure, levels in serum decreased, with 14.9% of the peak level remaining after 24 h.

Levels of ribavirin in the brain also increased during the high-dose regimen and reflected the pattern seen in the
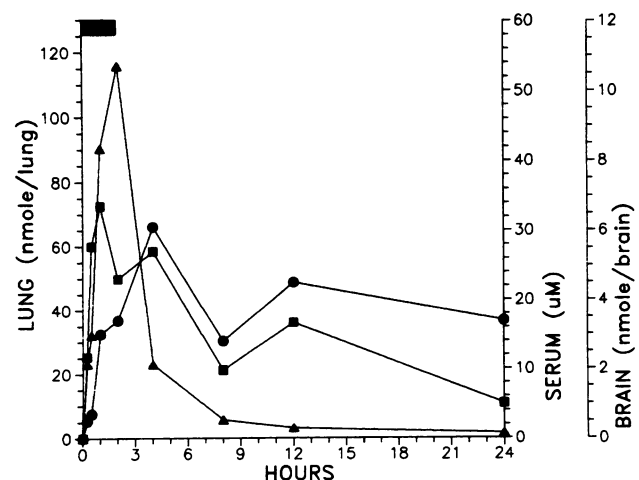

FIG. 2. Pharmacokinetics of ribavirin in lung, serum, and brain tissues after a single administration by small-particle aerosol for 2 h with 60 mg of drug per ml in the reservoir. Methods and symbols are described in the legend to Fig. 1.
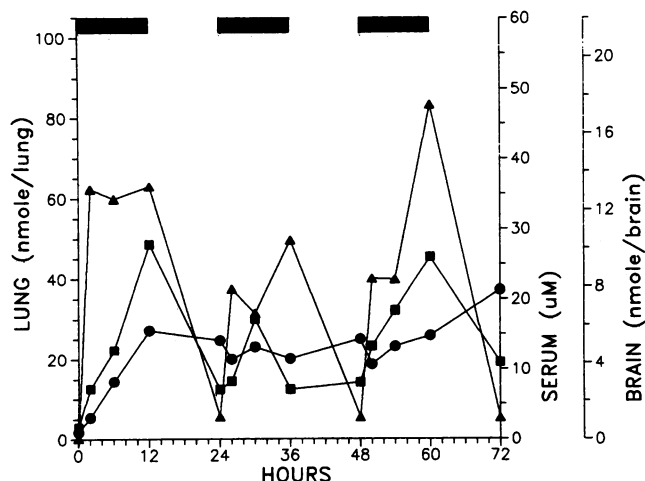
FIG. 3. Pharmacokinetics of ribavirin in lung, serum, and brain tissues after 3 days of drug administration by small-particle aerosol for 12 h daily. Concentration of drug in the reservoir, 20 mg/ml. Methods and symbols are described in the legend to Fig. 1.

serum. As with standard-dose exposure, the rate of clearance of ribavirin from the brain during the 22-h nontreatment period was slower than that seen in the serum (54 versus 83%). Thus, high-dose administration of ribavirin for just 2 h led to ribavirin concentrations in the serum and brain that were comparable to those observed with the standard-dose treatment for 12 h, but there was an approximately threefold-higher level of ribavirin in the lungs.

Multiple-dose pharmacokinetics. To determine if multiple exposures of mice to aerosols of ribavirin would alter the drug distribution pattern, ribavirin was given for 3 consecutive days. Lung, serum, and brain samples were obtained from mice treated continuously for 12 h/day with 20 mg of ribavirin per ml in the reservoir or for 2 h twice daily with 60 mg of ribavirin per ml in the reservoir. These two regimens give equivalent amounts of drug (i.e., 43.3 mg/kg of body weight).

Standard-dose regimen. With the standard dose, pulmonary patterns of ribavirin levels on each of the 3 days were similar to those seen in the single-dose study described above (Fig. 3), although average maximum levels were higher in these experiments than previously determined (see above; 11, 18). However, in each instance ribavirin was cleared from the lungs and at 24 h was reduced to the same low level. There did not appear to be any accumulation of drug in the lungs.

Levels in serum again reflected the pattern seen in the lungs; however, there appeared to be some accumulation of ribavirin over each 24-h period. Concentrations in serum at 24, 48, and 72 h were 7.1 ± 1.6, 8.1 ± 0.8, and 11.0 ± 1.7 μM, respectively, but were not statistically significant ($P > 0.08$; two-tailed Student t test).

Ribavirin levels in the brain reflected those seen in the serum, with the exception that declines in drug levels between treatment periods were not as noticeable as those observed in the lungs and serum. Having reached a maximum on the first day, the level tended to be maintained over the 3-day period.

High-dose regimen. The pattern of ribavirin deposition in the lungs was remarkably consistent for each of the six 2-h treatment periods with the high-dose regimen (Fig. 4). Maximum values for the six groups ranged from 92 to 143 nmol of ribavirin per lung. Moreover, whether 12- or 24-h intervals

were evaluated, drug clearance occurred rapidly and little remained in the lungs at this time; there did not appear to be any accumulation of the drug even when larger amounts were given.

Patterns of ribavirin levels in serum were similar to those seen in the lungs. However, concentrations in serum appeared to increase slightly over each 24-h period, as was seen with sera from animals given the small dose. Concentrations in serum at 24, 48, and 72 h were 4.8 ± 0.5, 6.8 ± 0.4, and 11.8 ± 3.4 μM, respectively, but again were not statistically significant ($P = 0.1$; two-tailed Wilcoxon rank sum test). As with the standard-dose regimen and unlike what was observed in the lungs and serum, ribavirin levels in the brain appeared to reach a maximum amount (ca. 6 nmol per brain) and remained constant over the 3 days.

In summary, the multiple-dose treatment of mice with high doses for 2 h twice daily yielded drug levels in the lungs that were transient but higher than those observed with the single-dose treatment. Levels in the serum and brain were similar to those seen with the standard dose which was given continuously for 12 h. A summary of some of the ribavirin pharmacokinetic parameters is presented in Table 1.

Estimated concentrations of ribavirin in lung and brain tissues. A 28-g mouse is estimated to have 28-μl of liquid on the epithelial surface of the lungs in which ribavirin could dissolve (see Materials and Methods). On the basis of this value, the ribavirin concentrations in the lungs at peak levels should be in the range of 1 to 5 mM, with minimal levels of about 100 μM for each 24-h period.

Concentrations in the brain for each regimen were determined, and corrections for blood contamination were made as described in Materials and Methods. By using these values, ribavirin levels were estimated to increase to 120 to 140 μM over the first 12 to 14 h of treatment irrespective of which treatment protocol was used (Fig. 5). This concentration remained essentially constant, indicating very slow drug clearance upon cessation of aerosolization.

DISCUSSION

Administration of ribavirin by a small-particle aerosol has been approved by the Food and Drug Administration for the
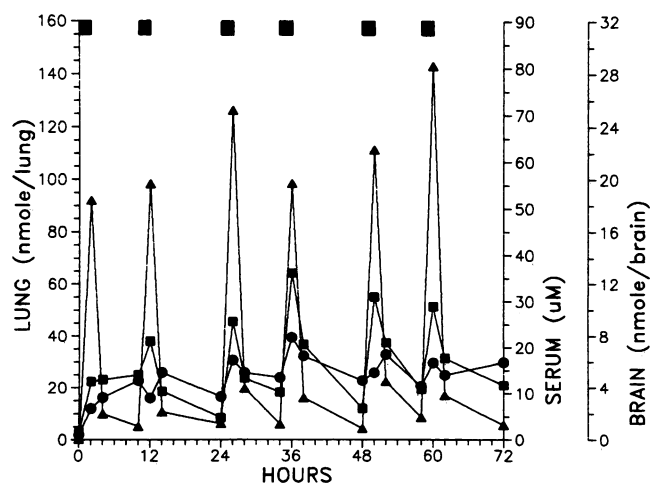

FIG. 4. Pharmacokinetics of ribavirin in lung, serum, and brain tissues after 3 days of drug administration by small-particle aerosol for 2 h twice daily. Drug concentration in the reservoir, 60 mg/ml. Methods and symbols are described in the legend to Fig. 1.
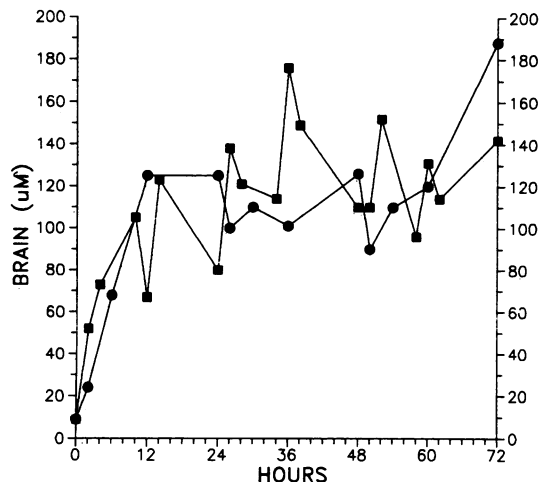
FIG. 5. Estimation of ribavirin concentration in brain fluid during drug administration by small-particle aerosol for 3 days for either 12 h of daily treatment with 20 mg of drug per ml in the reservoir or 2 h twice daily with 60 mg of drug per ml in the reservoir. Estimates were based on calculations as discussed in Materials and Methods. Symbols: ●, 12 h with 20 mg/ml; ■, 2 h twice daily with 60 mg/ml.

treatment of respiratory syncytial virus infection in infants. The standard treatment protocol calls for administration of 20 mg of ribavirin per ml in the reservoir of the aerosol generator for 12 to 18 h. The long treatment period initially was used to obtain and maintain optimal levels of drug in light of the need for a virustatic drug to be continually

TABLE 1. Summary of ribavirin levels in lung, serum, and brain tissues as a function of treatment protocol

Treatment protocol[a] and sample	Ribavirin levels in:		
	Lungs (nmol/organ)	Serum (μM)	Brain (nmol/organ)
Single treatment			
Standard-dose regimen			
Peak	33.3	21.0	5.2
At 24 h after start of treatment	2.5	6.7	3.6
High-dose regimen			
Peak	115.8	33.5	6.1
At 24 h after start of treatment	1.6	5.0	3.4
Multiple treatment			
Standard-dose regimen			
Mean (± SD) peak[b]	65.4 ± 17.0	23.7 ± 5.7	6.2 ± 1.4
Mean (± SD) at start of next treatment[c]	5.5 ± 0.2	8.7 ± 2.0	6.1 ± 1.5
High-dose regimen			
Mean (± SD) peak[d]	111.6 ± 19.7	26.2 ± 7.8	6.1 ± 1.2
Mean (± SD) at start of next treatments[e]	6.1 ± 1.5	9.8 ± 3.4	4.6 ± 0.9

[a] Standard-dose regimen, 12 h of continuous treatment with 20 mg of ribavirin per ml in the reservoir; high-dose regimen, 2 h of treatment with 60 mg of ribavirin per ml in the reservoir.
[b] Mean of the maximum ribavirin level for three 12-h treatment periods.
[c] The next treatment began 24 h after the start of the previous treatment.
[d] Mean of the maximum ribavirin level for six 2-h treatment periods.
[e] The next treatments began 10 and 24 h after the start of the previous treatment for each day of treatment. Since there was no difference in ribavirin levels at 10 and 24 h, these values represent the means of all six time points.

present during critical periods of viral replication. With this protocol, ribavirin aerosol has been shown to be effective without toxicity in the treatment of respiratory syncytial virus bronchiolitis and pneumonia in infants (7, 10). However, over the past few years there has been interest in reducing the period of treatment so that this modality of treatment might be used in a broader clinical setting. We recently reported that in mice, death from influenzal pneumonia could be as effectively prevented by a shorter duration of treatment with 60 mg of ribavirin per ml in the reservoir as by the standard and longer duration with 20 mg of ribavirin per ml (18). While the total amounts of ribavirin delivered to the lungs of these mice should have been similar, the pharmacokinetics of ribavirin treatment following these two protocols was not studied in detail. Such knowledge could greatly facilitate efficacy and toxicity evaluations.

Ribavirin rapidly accumulated in the lungs after either the standard- or high-dose regimen. Within minutes of the start of aerosolization, the concentration of ribavirin in the lungs of exposed mice approached millimolar levels which should be sufficient to inhibit the replication of influenza A or B or respiratory syncytial virus (10, 16). Ribavirin concentrations after high-dose, short-duration treatment were two to three times higher at peak levels than those seen during treatment with the standard dosage. However, immediately upon cessation of treatment, ribavirin was cleared from the lungs so that these high levels were present only for a relatively short period. The rapid clearance was most dramatic with the high-dose regimen in which the initial phase of clearance had a half-life of 1.2 h. Subsequent clearance was similar to that of the standard-dose protocol. After multiple treatments, a similar pattern was observed, with little or no accumulation of ribavirin with each subsequent treatment. At the end of 24 h, the concentration of ribavirin remaining in the lungs (ca. 100 μM) was greater than the 50% inhibitory dose for clinical isolates of influenza viruses and respiratory syncytial virus (10, 16). This rapid accumulation and clearance with the high-dose, short-duration regimen may explain the effective antiviral activity seen without any histological evidence of toxicity (18, 19).

Clearance of ribavirin from the lungs is mediated through mucociliary activity and absorption into the blood. Within minutes, free ribavirin was detected in the serum, indicating the rapidity with which clearance begins. Since the assay used in these studies does not measure the phosphorylated derivatives of ribavirin, total levels of ribavirin and metabolites in the blood are significantly higher than indicated by our assay due to the accumulation of ribavirin triphosphate by erythrocytes (3). Levels of ribavirin in serum with the drug administered as an aerosol were similar (20 to 30 μM) to those reported for plasma of mice injected intraperitoneally with 40 mg of ribavirin per kg, as measured by a radioimmunoassay (2). Thus, ribavirin concentrations in mouse serum appear to be two to three times higher than those found in humans (1 to 10 μM) (5; B. E. Gilbert, unpublished results). In part, the higher levels in mouse serum may be due to the constant preening of mice, which would result in the ingestion of ribavirin, 45% of which would be systemically absorbed from the intestinal tract (8).

The clearance of free ribavirin from serum after either treatment protocol was similar to that previously reported (2, 4). With multiple doses over 3 days, there appeared to be a small accumulation of ribavirin in serum at the end of each day's treatment. Sequestering and clearing of ribavirin from serum would be accomplished by the incorporation of riba-

virin triphosphate into erythrocytes (3) and by renal excretion (3, 6), respectively. Although we did not measure ribavirin levels in the urine, previous studies in rats have shown that ribavirin is rapidly excreted in the urine (82% is excreted in 24 h) (6).

Ribavirin levels in the brain were proportional to those in the serum at each time point. However, in contrast to the observed decrease in concentrations in serum, levels appeared to be sustained at an optimal level even when treatment had ended. An estimation of the ribavirin concentration in cerebrospinal fluid, most of which would bathe the brain, indicates that the concentration is four to five times higher than that in serum (e.g., 140 versus 30 μM). This concentration of ribavirin is in the range necessary for the inhibition of those viruses for which ribavirin is indicated, including human immunodeficiency virus (5, 13). It appears that aerosol administration of ribavirin allows the drug to go directly to the brain via the carotid arteries, while the parenterally administered drug must first pass the liver, where much of it is eliminated from the serum. Accumulation of ribavirin in the brain suggests that ribavirin is trapped within the aqueous compartments of the brain and has a greater half-life than it does in serum. Since the active intracellular form of ribavirin is ribavirin triphosphate, it is important to determine if the higher, sustained levels of ribavirin also are reflected in higher ribavirin triphosphate levels.

High-dose, short-duration administration of ribavirin appears to be an alternative method for the treatment of respiratory syncytial virus infections. Although higher levels in the lungs were attained, once treatment was over ribavirin was rapidly cleared without apparently causing pulmonary tissue damage. Clearly, the advantage of higher drug levels and rapid clearance is the rapid attainment of drug levels which can inhibit viral replication without additional toxicity. While levels in serum were similar to those attained by other routes of administration, ribavirin reached higher concentrations in the brain which were sustained during the nontreatment period. With the interest in treating viral encephalopathies associated with human immunodeficiency virus and herpesviruses, further evaluation of the possible advantages of this method of drug administration is warranted.

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