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RESPONSE OF INFLUENZA-VIRUS INFECTED MICE TO SELECTED DOSES OF --ETC(U)
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INTRAPERITONEALLY OR BY AEROSOL

ARMY MEDICAL RESEARCH INSTITUTE OF
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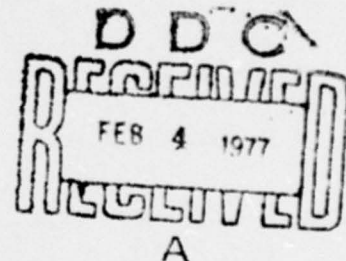
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Response of Influenza-Virus Infected Mice to Selected
Doses of Ribavirin Administered Intraperitoneally or by Aerosol

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Running title: Ribavirin Dose Effects



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In conducting the research described in this report, the investigators
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ABSTRACT

Influenza virus-infected mice were given graded doses of ribavirin intraperitoneally (i.p.) and by aerosol to determine the relative efficacy of the two routes of administration. Percent survival, lung pathology (expressed as lesion scores) and titer of virus in the lungs were determined. The ED_{90} values (in terms of survival) were 9.9 and 47.9 mg/kg/day for aerosol and i.p. routes, respectively. Lung lesion scores and titers of virus were also lower after aerosol than i.p. therapy with identical doses.

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Recently, ribavirin (1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide) was found to be effective for the treatment of type A influenza virus infections in mice (1, 6, 10, 11). Results of previous experiments also indicated that small-particle aerosols (mass median diameter: 1.4 μ m, geometric standard deviation: 1.8) of ribavirin were more effective in reducing mortality, lesions in the lung, and virus concentrations in the lungs than approximately equivalent doses of drug given intraperitoneally (i.p.) (10). However, the results of previous experiments did not indicate how much better aerosol administration was than i.p. in terms of dosage required to achieve the same effect; i.e., how much ribavirin was required to achieve an effective dose (ED_{90}) that would allow >90% of the mice to survive. This information would be helpful when considering the possible administration of ribavirin by aerosol for the treatment of humans, especially if a significant therapeutic advantage was gained by using an aerosol route.

The objective of this research has been to determine the response of influenza virus infected mice to selected doses of ribavirin given by aerosol or i.p.

METHODS AND MATERIALS

Mice. Five-week-old outbred female mice, Tac:(SW)fBR, weighing approximately 20 g, were used for all experiments. Food and water were provided ad lib. Tetracycline was added to drinking water (3.0 mg/ml) daily until the day of virus challenge. Mice were randomly assigned to treatment groups consisting of 40 mice each; 25 of the mice in each group were used for survival studies and 15 for determination of pulmonary lesions and virus concentration.

Aerosol dissemination and sampling system. Aerosols of virus and ribavirin were generated by a Collison spray device into a Henderson apparatus (5, 7). Viral infections were initiated by exposing mice to aerosols of a mouse-adapted variant of influenza A/Aichi/2/68 (H3N2) for 10 min. The preparation of the mouse-adapted variant has been described by Scott (8). The concentration of virus in the aerosols was estimated by sampling the aerosol with all-glass impingers and calculating virus concentrations as previously described (9). Inhaled dosages were then calculated by multiplying these concentrations by the total volume inhaled as determined from the formula of Guyton (3). Retained dosage was calculated by multiplying inhaled dose by 50%, based upon the values given by Hatch and Gross for retention of particles of the size disseminated in these studies (4). The mean calculated inhaled dose for the 5 replicate trials was 1.70×10^4 (SEM = 7.1×10^3) EID₅₀ for each mouse. Three-hundred sixty mice were infected weekly. After exposure, the mice were randomly assigned among 9 treatment groups of 40 animals each.

The aerosol dosage of ribavirin was controlled by adjusting both concentration of the drug in the spray fluid (25 or 100 mg/ml) and time of exposure (40 or 80 min). Among the 4 combinations of spray fluid concentration and exposure time, calculated theoretical doses of 2.5, 5.0, 10.0 and 20.0 mg/kg/day were given. Impinger samples were collected for measuring actual ribavirin concentrations from which the final doses were ascertained. Ribavirin concentrations in impinger fluids or injection samples were determined spectrophotometrically as previously described (10). Dosages calculated from impinger samples agreed with theoretical values within limits of $\pm 20\%$. Intraperitoneal doses of 5.0, 10.0, 20.0 and 40.0 mg/kg/day were prepared by dilution of weighed ribavirin in sterile water.

Experimental plan. Treatment was initiated at 6 hr after exposure to the virus and was then repeated daily for a total of 5 days. Intraperitoneal injections were given once daily at the same time. A virus-control group received no treatment. This group was included to insure that mortality exceeded 90% in infected untreated mice. On the fourth day after exposure, 3 mice from each group were killed by cervical dislocation, the thoracic cavity was opened and the degree of gross pathology in the lungs was scored as previously described (11). The concentration of virus in the lungs was also determined according to previously-described methods (10). On the seventh day after exposure, 5 additional mice in each group were killed and lung-lesion scores were recorded. Virus titers were not determined at this time.

Survival was recorded daily for 21 days on 25 mice that were randomly allocated for this purpose from each treatment group. Five replicate experiments were performed. The survival data were analyzed by probit analysis (2), and the doses of ribavirin yielding 50 and 90% survival (ED_{50} and ED_{90} , respectively) were calculated together with 95% confidence limits. The lung-virus data were evaluated by analysis of variance. Significance of differences between dose-groups was determined by the method of least significant differences. The effect of aerosol therapy was compared with that of intraperitoneal injection; no statistical comparisons were made with the virus control group. Differences between the lung-lesion scores of the various groups were analyzed by Wilcoxon's rank-sum test.

RESULTS

Survival. The mean percent survival of each treatment group is given in Table 1 together with ED_{50} and ED_{90} values. The ratio of the ED_{90} after aerosol treatment to that after i.p. administration was 4.8 to 1, indicating significant differences in efficacy between the 2 treatment groups. ED_{50} values for these experiments were 3.3 and 15.8 mg/kg/day for aerosol and i.p. treatments, respectively. Also of interest was the "threshold" effect that was noted with aerosolized ribavirin; raising the dosage from 2.5 to 5.0 mg/kg increased survival by a factor of 144%, while raising the dosage from 5.0 to 10.0 mg/kg increased survival by only 4%. This effect, illustrated clearly in Fig. 1A, was not seen after i.p. therapy.

Titer of virus in lungs. The concentration of virus in the lungs is given in Table 2 and Fig. 1B. Most impressive was the observation that successful therapy was accompanied by a very small decrease in lung virus concentration. The differences between aerosol and i.p. administration were highly significant ($P < 0.001$) when compared at equivalent doses (5, 10 and 20 mg/kg), but even at an ED_{90} dosage (9.9 mg/kg) the aerosolized drug only reduced the virus titer by approximately $0.5 \log_{10}$ in comparison to the controls. Also of interest was the apparent threshold effect seen with i.p. administration of ribavirin; 40 mg/kg was no more effective than 20 mg/kg.

Lung lesions. Four and 7-day lung-lesion scores are given in Table 3. In addition, the 7-day scores are presented graphically in Fig. 1C. Although none of the doses given by either route completely eliminated

the development of lesions in the lungs of all mice, aerosols had considerably more therapeutic effect than the equivalent amount of ribavirin given i.p.

DISCUSSION

We have shown that about one-fifth the amount of ribavirin is required to prevent most mortality in influenza-infected mice when the drug is administered as aerosol than when it is administered intraperitoneally. Aerosol therapy was also significantly more effective in reducing the amount of virus and extent of lesions in the lungs than was i.p. treatment when equivalent doses were compared. Our observation of the superiority of aerosol over i.p. therapy, therefore, confirms and extends those reported earlier by Stephen et al. (10) and Walker et al. (11).

Our observation of the effect of ribavirin on the concentration of virus in lungs is consistent with those of Stephen et al. (10) and Walker et al. (11). Although the effect of aerosol treatment differed significantly from that of the same dosage given intraperitoneally ($P < 0.001$) the magnitude of difference was very small and 90% survival was achieved despite the presence of almost 10^7 EID₅₀ of virus in the lungs. Although we have not attempted to delineate growth curves, it seems unlikely that the activity of ribavirin (in vivo) is based primarily upon its antiviral properties.

The importance of the anti-inflammatory effect of ribavirin claimed by several authors (1, 10, 11) is not clear. A dramatic decrease in lung-lesion scores was seen with increasing dose, particularly in aerosol-treated mice, but it is noteworthy that >80% of the mice survived with lung consolidation of 55-87.5% (lesion scores of 2.2 and 2.7, respectively). Further investigation with pathological examination at

frequent periods after treatment would be necessary to resolve this point.

Although ribavirin was significantly more effective after aerosol administration than after i.p., the question of the effect of more frequent administration by the i.p. and other routes must be addressed before recommendations can be made concerning the utility of aerosol therapy with this particular drug. Also, the i.p. route of administration is a good one for routine screening; but attention must be given to the routes that are commonly employed in clinical situations, e.g., oral or i.m. Also, pharmacokinetic studies must be carried out to provide information on the dosage schedules that would maintain optimum drug levels in the lungs.

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TABLE 1. Survival of influenza virus-infected mice after ribavirin therapy.

Route of Therapy	Daily Dose (mg/kg)	Percent Survival*	ED ₅₀	95% Conf. Limits	ED ₉₀	95% Conf. Limits
Aerosol	2.5	32.8				
	5.0	80.0	3.3	2.5-4.3	9.9	7.6-12.9
	10.0	83.2				
	20.0	99.2				
Intra-peritoneal	5.0	16.0				
	10.0	28.8	15.8	12.7-19.5	47.9	38.7-59.2
	20.0	56.0				
	40.0	95.2				
None	0.0	16.0				

* Mean of 5 replicate experiments of 25 mice/dose level/experiment.

TABLE 2. Virus concentrations in the lungs of ribavirin-treated mice.

Route of Therapy	Dose (mg/kg/day)	Virus Conc. in Lungs, $\log_{10}$ ($\pm$ SEM)
Aerosol	2.5	7.31 (0.07)
	5.0	7.15 (0.09)
	10.0	7.02 (0.09)
	20.0	6.86 (0.11)
Intraperitoneal	5.0	7.48 (0.09)
	10.0	7.28 (0.06)
	20.0	7.09 (0.07)
	40.0	7.08 (0.06)
None	0.0 (Control)	7.48 (0.08)

TABLE 3. Lung lesions in ribavirin-treated mice.

Route of Therapy	Dose (mg/kg/day)	Mean Lung-Lesion Score	
		4 day (+ SEM)	7 day (+ SEM)
Aerosol	2.5	1.97 (0.67)	3.28 (0.14)
	5.0	1.13 (0.26)	2.70 (0.23)
	10.0	0.37 (0.10)	2.20 (0.17)
	20.0	0.03 (0.03)	0.40 (0.11)
Intra-peritoneal	5.0	1.96 (0.39)	3.10 (0.16)
	10.0	1.08 (0.18)	3.25 (0.16)
	20.0	0.70 (0.13)	2.65 (0.18)
	40.0	0.20 (0.07)	1.20 (0.22)
None (control)	0.0	1.17 (0.18)	3.07 (0.23)

FIG. 1 Survival (1-A), virus titer in lungs (1-B) and lung lesion scores (1-C) of influenza-virus infected mice. Statistical comparisons were carried out between comparable aerosol and i.p. doses. Arrows at the top indicate doses for aerosol and i.p. ED₉₀ values.

