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Prophylactic and Therapeutic Efficacies of Poly(IC · LC) against Respiratory Influenza A Virus Infection in Mice

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Polyriboinosinic-polyribocytidylic acid [poly(IC · LC)] was evaluated for its prophylactic and therapeutic efficacies against respiratory influenza A virus infection in mice. Two doses of poly(IC · LC) (1 mg/kg of body weight per dose) administered intranasally within 12 days prior to infection with 10 50% lethal doses of mouse-adapted influenza A/PR/8 virus fully protected the mice against the infection. Determination of virus titers by hemagglutination and plaque assays showed more than a 2-$\log_{10}$ decrease in virus titers in lung homogenates of pretreated mice compared with those in the lungs of the nonpretreated group. Treatment of infected mice with poly(IC · LC) resulted in a modest (40%) survival rate. These results suggest that poly(IC · LC) provides a highly effective prophylaxis against respiratory influenza A virus infection in mice.

Poly(IC · LC) is a synthetic, double-stranded polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine carboxymethyl cellulose and a potent immunomodulating agent (2, 6, 7, 12). In rodents and primates, poly(IC · LC) has been shown to be effective in providing protection against a number of viral infections, including those caused by yellow fever (11), Venezuelan equine encephalomyelitis (10), Rift Valley fever (5), and rabies (1) viruses. The antiviral activity of poly (IC · LC) is believed to be mediated by its ability to augment the production of alpha, beta, and gamma interferons in vivo (5, 7, 8) and to stimulate specific components of the cellular and humoral immune systems, including the activation of natural killer cells (2, 12). The antiviral potential of poly(IC · LC) for the prevention and treatment of respiratory influenza A virus infection in mice was evaluated in the present study. Poly(IC · LC), by virtue of its immunomodulating properties, may provide a broad-spectrum therapy against a number of influenza virus strains, even new strains resulting from antigenic drift.

Influenza A/PR/8 (H1N1) and A/Aichi/2 (H3N2) viruses were passaged in mice as described earlier (14). The poly(IC · LC) used in the present study was prepared by the College of Pharmacy, University of Iowa (Iowa City). Each milliliter of poly(IC · LC) contained 2 mg of poly(I · C), 1.5 mg of poly-L-lysine, and 5 mg of carboxymethyl cellulose in 0.9% sodium chloride. Poly(IC · LC) was administered to the mice by the intranasal (i.n.), intraperitoneal (i.p.), or intravenous (i.v.) route. The volumes of inoculum used were 50 μl for the i.n. route and 100 μl for the i.p. and i.v. routes. For i.n. administration, mice were anesthetized with sodium pentobarbital (50 mg/kg of body weight given i.p.). When the animals were unconscious, the antiviral agents were gently applied with a micropipette into the nostrils and were presumably inhaled into the lungs.

For the prophylaxis of influenza A virus infection in mice, groups of sodium pentobarbital-anesthetized mice (5 to 10 mice per group) were given one or two doses of poly(IC · LC) by the i.n. or i.p. route (1 mg/kg per dose). The single dose of poly(IC · LC) was administered 8 h prior to infection with influenza A virus, and two doses were given at 48 and 8 h prior to infection. The mice were then intranasally infected with 10 50% lethal doses (LD_{50}s) of the mouse-adapted influenza A/PR/8 virus. At day 14 after virus infection, the number of mice which survived the virus challenge was then recorded. For treatment studies, groups of mice were intranasally infected with 10 LD_{50}s of influenza A virus. At 8 and 48 h postinfection, the mice were treated i.v. with two doses of poly(IC · LC) (1 mg/kg per dose) or with a single dose (1 mg/kg per dose) of poly(IC · LC) administered at 8 h postinfection. At day 14 postinfection, the number of mice which survived the virus challenge was recorded. The survival rates of the control and treated mice were compared by the Mann-Whitney unpaired nonparametric one-tailed test (InStat, version 1.14; GraphPAD Software, San Diego, Calif.). Differences were considered statistically significant at $P < 0.05$.

To determine the effect of pretreatment on the virus titers in the lungs of infected mice, the lungs from each group were pooled and homogenized in 4 ml of sterile phosphate-buffered saline, and the tissue homogenates were centrifuged at 5,000 × *g* for 15 min. The clear supernatants were then used for virus determinations by hemagglutination and plaque assays as described previously (3, 14).

The prophylactic and therapeutic efficacies of poly(IC · LC) against a lethal respiratory influenza A/PR/8 virus infection in mice are summarized in Table 1. All untreated control mice challenged with the virus dose died from the respiratory infection. The mean survival time in these infected mice was 7 to 8 days (data not shown). For prophylaxis, mice pretreated with two i.n. doses of 1 mg/kg per dose were fully protected from the intranasal challenge with 10 LD_{50}s of mouse-adapted influenza A virus (100% survival rate) ($P < 0.01$ versus the controls). However, i.p. administration of poly(IC · LC) was found to be less effective than i.n. administration at protecting the mice against the virus infection ($P < 0.05$). Delivery of poly(IC · LC) to the lungs by the i.n. route may elicit a more concentrated antiviral defense in the lungs compared with that elicited by delivery by the i.p. route, thereby resulting in greater antiviral efficacy. In addition, in mice given two doses of poly(IC · LC) by the i.n. route, the minimal effective dose was observed to be

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TABLE 1. Prophylactic and therapeutic efficacies of poly(IC · LC) against respiratory influenza A/PR/8 virus infection in mice

Group	No. of survivors[a]/total no.	% Survival	P vs control
Untreated control	0/10	0	
Prophylaxis against influenza A/PR/8 virus			
Two 1-mg/kg doses of poly(IC · LC) by[b]:			
i.n. route	10/10	100	<0.01
i.p. route	4/10	40	>0.05
Effect of dose (two doses [μg/kg] given i.n.)			
500	5/5	100	<0.01
250	5/5	100	<0.01
50	2/5	40	>0.05
No. of doses, dose (μg/kg) given i.n.			
Two,[b] 500	5/5	100	<0.01
One,[c] 1,000	4/5	80	<0.05
One,[c] 500	3/5	60	>0.05
Against influenza A/Aichi/2 (H3N2) virus, two i.n. doses[b] (1 mg/kg/dose)	10/10	100	<0.01
Comparison with interferon (two i.n. doses,[b] 100,000 U/kg/dose):			
Gamma (mouse recombinant)	5/10	50	<0.05
Alpha (mouse fibroblast)	5/10	50	<0.05
Carboxymethyl cellulose control, two i.n. doses (2.5 mg/kg/dose)[b]	0/5	0	>0.05
Postexposure treatment (no. of doses, μg/kg/dose)			
Against influenza A/PR/8 virus			
Two,[b] 1,000	4/10	40	>0.05
One,[c] 1,000	1/10	10	>0.05
Against influenza A/Aichi/2 virus			
Two,[b] 1,000	4/10	40	>0.05
One, 1,000	ND[d]		

[a] Number of survivors determined at day 14 postinfection.
[b] Given at 48 and 8 h prior to infection (for prophylaxis) or postinfection (for treatment) with 10 LD_{50}s of influenza A virus.
[c] Given at 8 h prior to infection (for prophylaxis) or postinfection (for treatment) with 10 LD_{50}s of influenza A virus.
[d] ND, not determined.

approximately 250 μg/kg per dose. In mice pretreated with doses of less than 250 μg/kg the survival rate decreased rapidly from a 100% survival rate for those receiving 250 μg/kg per dose to 40% for those receiving 50 μg/kg per dose. Mice pretreated with a single dose (1 mg/kg) of poly(IC · LC) had a slightly lower survival rate (80%) compared with that for mice pretreated with two doses of 500 μg/kg (100% survival rate). In the same way, mice pretreated with a single 500-μg/kg dose had a lower survival rate (60%) compared with that for mice pretreated with two 250-μg/kg doses. Poly(IC · LC) was equally effective in the prophylactic protection of mice against influenza A/Aichi/2 virus. All mice pretreated with the two i.n. doses of poly(IC · LC) were protected against 10 LD_{50}s of influenza A/Aichi/2 virus. Carboxylmethyl cellulose, which was administered in two i.n. doses at 2.5 mg/kg per dose, provided the mice no protection against influenza A/PR/8 virus infection. Compared with the prophylactic efficacy of mouse recombinant gamma interferon and alpha interferon, two i.n. doses (1 mg/kg per dose) of poly(IC · LC) were found to be more efficacious than two i.n. doses (100,000 U/kg per dose) of gamma interferon or alpha interferon in protecting mice against influenza A/PR/8 virus infection ($P < 0.05$).

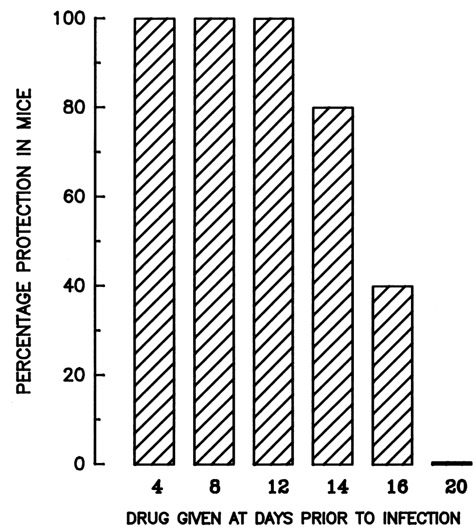

FIG. 1. Relationship between time of poly(IC · LC) administration prior to infection and survival rates.

To determine the duration of protection provided by poly(IC · LC), mice were pretreated with two i.n. doses (1 mg/kg per dose) of poly(IC · LC) on days 1 to 20 prior to infection with 10 LD_{50}s of virus, and the survival rates were determined at day 14 postinfection (Fig. 1). Mice pretreated on day 12 or earlier were fully protected from the infection. The survival rates decreased to 80 and 40% for mice pretreated on day 14 and day 16, respectively. Poly(IC · LC) administered on day 20 prior to infection provided no protection. Infectious virus particles in lung homogenates of the pretreated groups on day 4 postinfection were below the detectable limits of the assays (0 hemaglutination units per 0.05 ml of lung supernatant and $<1.4 \times 10^4$ 50% tissue culture infective doses per mouse lung). The control nonpretreated group of mice had high virus titers (64 hemagglutination units per 0.05 ml of lung supernatant and 2.8×10^6 50% tissue culture infective doses per mouse lung). In general, poly(IC · LC) was shown to be less effective in the postexposure treatment of influenza virus infection than it was for prophylaxis. For mice treated with two i.v. doses (1 mg/kg per dose) of poly(IC · LC), there was a small increase in the survival rate (40%) compared with that for the untreated control mice ($P = 0.064$).

The ability of poly(IC · LC) to modulate the immune responses including interferon induction (5, 7, 8) and activation of natural killer cells (2, 12) may present many advantages for influenza prophylaxis. The antiviral activity of interferons and/or activation of natural killer cells induced by poly (IC · LC) may result in a nonspecific antiviral defense against a number of viral agents and may therefore provide a broad-spectrum antiviral effect against influenza viruses, regardless of the strain or subtype involved. The prolonged protection provided by poly(IC · LC) seen in mice in the present study might provide a short-term prophylactic measure against influenza virus. However, multiple high doses of poly(IC · LC) given i.v. have been known to produce toxic reactions in humans (9). Current efforts in our laboratory are directed at reducing the toxicity of poly(IC · LC) by encapsulating it in liposomes. The slow sustained-release and specific targeting characteristics of liposomes may significantly reduce the potential side effects of poly(IC · LC). Liposomes have successfully been used to enhance the prophylactic and therapeutic effectiveness of anti-influenza agents, including antiviral antibody (14), gamma interferon (13), and ribavirin (6). By using liposomes as a drug delivery system for poly(IC · LC), a low-dose, nontoxic but therapeutically active formulation of poly(IC · LC) may be achievable.

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