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Inhibition of influenza virus infections in immunosuppressed mice with orally administered peramivir (BCX-1812)

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Abstract

Experiments were run to determine the effect of oral gavage treatment with the cyclopentane influenza virus neuraminidase inhibitor peramivir (BCX-1812, RWJ-270201) in influenza A (H1N1) virus-infected mice that had their immune system suppressed by cyclophosphamide (CP) therapy or in severe combined immune deficient (SCID) mice. Treatment of CP-immunosuppressed mice with peramivir using doses of 100, 10, or 1 mg/kg/day was begun 2.5 or 8 days post-virus exposure and continued twice daily for 3 or 5 days. The 5-day therapy was more effective than the 3-day treatment, as seen by significantly increased survivor numbers, lessened decline in arterial oxygen saturation, reduced lung consolidation, and inhibition of lung virus titers. Infected SCID mice were also responsive to peramivir therapy begun 8 days after virus exposure and continued for 5 days, although antiviral effects did not include prevention of death and were dependent upon the viral challenge dose received. These data indicate that peramivir may have potential for treatment of influenza virus-infected immunosuppressed patients.

Keywords: Influenza virus; Peramivir; RWJ-270201; BCX-1812; Neuraminidase inhibitor; Immunosuppression; Antiviral

1. Introduction

Influenza virus infections can be especially serious in individuals whose immune system is compromised due to complications associated with aging, genetic factors, treatment with an anticancer drug, or by a drug used in connection with organ transplantation (Ljungman et al., 1993; Rocha et al., 1991; Whimbey and Bodey, 1992). Laughlin et al. (1991) of the National Institutes of Health have indicated that there is a need to evaluate the effects of treatments in immunosuppressed animals in order to predict these effects in human patients. It is generally assumed that the efficacy of an agent inhibiting viral replication may require the intact host immune system in order to exert a beneficial antiviral effect in that host. Thus, an influenza virus inhibitor that would be effective in an immunodeficient host would be of great value.

The cyclopentane influenza virus neuraminidase inhibitor peramivir (BCX-1812, RWJ-270201) has been established as a significant anti-influenza virus agent in vitro (Smee et al., 2001) and in immunocompetent mice (Sidwell et al., 2001a; Bantia et al., 2001) and ferrets (Sweet et al., 2002). Challenge studies in human patients have indicated treatment with this compound lowers composite symptom scores when administered orally (Hayden et al., 2000), although once-daily therapy used in Phase III clinical trials against influenza was not considered significantly efficacious (Anonymous, 2002).

It was thought important to determine the effects of treatment with peramivir in influenza virus-infected immunocompromised mice. Three groups of immunocompromised mice were used: those treated short term (through day 7) with cyclophosphamide (CP), those receiving prolonged (through 35 days) CP treatment, and severe combined immune deficient (SCID) mice, in order to mimic human patient situations. This report describes the results of these experiments.

2. Materials and methods

2.1. Compounds

Peramivir was provided by the R.W. Johnson Pharmaceutical Research Institute (Raritan, NJ). CP was obtained from Sigma Chemical Co. (St. Louis, MO). Both were prepared in sterile saline for these studies and the solutions stored at 4 °C.

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2.2. Virus and cells

Influenza A/NWS/33 (H1N1) virus was obtained from Dr. Kenneth Cochran of the University of Michigan (Ann Arbor, MI). It was used as an MDCK cell preparation and stored at −80 °C. The MDCK cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in Eagle's minimum essential medium (MEM) containing 5% fetal bovine serum (Hyclone, Logan, UT). When used with the influenza virus, the MEM was supplemented with 10 U/ml trypsin, 1 μg/ml EDTA, 0.18% $NaHCO_3$, and 50 μg/ml gentamicin. For virus titrations, 96-well flat-bottomed microplates (Corning, NY) were used.

2.3. Animals

Female, 18–21 g, specific pathogen-free BALB/c mice were obtained from B & K Universal (Fremont, CA). Female, weighing 18–21 g, SCID mice were provided by Charles River Laboratories (Wilmington, MA). The SCID mice were maintained in HEPA-filtered horizontal laminar flow ventilated animal racks and fed sterilized food and water. All animals were quarantined 24–48 h prior to use.

2.4. Determination of arterial oxygen saturation (SaO_2)

An Ohmeda Biox 3740 pulse oximeter (Ohmeda, Louisville, OH) was used to determine daily SaO_2 levels in mice as we have previously described (Sidwell et al., 1992).

2.5. Determination of lung virus titers

Each mouse lung was homogenized to ∼10% (w/v) suspension in cell culture medium, centrifuged at 1000 × *g* for 10 min and the supernatant assayed in MDCK cells in 96-well microplates using triplicate wells for each 10-fold dilution. Viral cytopathic effect determined visually was used as an endpoint.

2.6. Procedure for antiviral study with peramivir in short-term CP-treated mice

BALB/c mice were treated intraperitoneally (i.p.) with 100 mg/kg of CP at 24 h pre-virus exposure and again on days +3 and +7. Infection was achieved by intranasal (i.n.) administration of 90 μl of $10^{4.4}$ cell culture 50% infectious doses ($CCID_{50}$) of virus to anesthetized mice. This dose of virus was an approximate LD_{90} challenge, based on a preliminary titration in similarly immunosuppressed mice. Anesthesia was achieved by i.p. injection of 100 mg/kg of ketamine (Ft. Dodge Animal Health, Ft. Dodge, IA). The animals were divided into groups of 10 (20 for saline-treated controls) which were treated by oral gavage (p.o.) with peramivir at dosages of 100, 10, or 1 mg/kg/day twice daily for 3 or 5 days beginning 24, 42, or 60 h after virus exposure. Two sets of saline-treated controls were used. One group was treated twice daily for 3 days, and the other for 5 days beginning 24 h after virus exposure. The mice were observed for death daily for 23 days; SaO_2 levels were ascertained on days 3–11 in these animals.

2.7. Procedure for antiviral study with peramivir in prolonged CP-treated mice

BALB/c mice were treated i.p. with 100 mg/kg of CP on days −1, +3, +7, +11, +15, +19, +23, +27, +31, and +35. They were infected i.n. as described above on day 0 with $10^{4.4}$ $CCID_{50}$/ml of virus. Groups of 20 infected mice were treated p.o. with peramivir at doses of 100, 10, or 1 mg/kg/day twice daily for 5 days beginning at 60 h (2.5 days) or 192 h (8 days) after virus exposure. Ten of these animals were observed for 35 days for occurrence of death, and SaO_2 levels were determined on days 10–20. Five mice in each group were killed after 3-day treatment with peramivir and five additional were killed 18 h after the end of treatment. The lung of each was assigned a consolidation score ranging from 0 (normal) to 4 (maximal plum coloration), weighed, one third of selected lungs placed in formalin, and the remainder assayed for virus titer. A group of 68 infected mice were treated with saline as above beginning 60 h after virus exposure. Twenty of these controls were assayed for SaO_2 in parallel with the drug-treated mice and observed for death through 35 days. Eight additional saline-treated animals were killed 60 h after virus exposure (immediately before therapy began) and 8, 11, and 13 days after virus exposure. The lungs of each mouse were processed similarly to those treated with drug. A group of five normal (untreated, uninfected) mice were observed daily for 35 days and the SaO_2 levels measured as above. Three additional normal controls were killed the same days as above and their lungs processed similarly to provide baseline values.

2.8. Procedure for antiviral study with peramivir in SCID mice

The effect of therapy with peramivir was determined in SCID mice infected with four varying one-half $\log_{10}$ challenge doses of influenza virus ranging from an approximate three LD_{100} dose to an LD_{25} dose (10^6, $10^{5.5}$, 10^5, and $10^{4.5}$ $CCID_{50}$). The animals were treated p.o. with peramivir at doses of 100, 10, and 1 mg/kg/day twice daily for 5 days beginning at 8 days post-virus exposure. Ten mice were used for each drug dose, with 20 used for saline-treated controls. All were observed for death for 35 days, and SaO_2 levels were ascertained on days 14–26. Five uninfected, untreated SCID mice were run in parallel.

2.9. Statistical analysis

Increases in survivor numbers were analyzed by chi-square analysis with Yates' correction. Changes in mean day to death, lung weights, SaO_2 levels, and virus titers

were evaluated using the Student's *t*-test. Lung scores were analyzed using the Wilcoxon ranked sum analysis.

3. Results

3.1. Effect of peramivir therapy in short-term CP-treated mice

The results of this experiment are summarized in Table 1. The influenza virus infection in these animals proceeded at a slower rate than seen in normal mice, with the mean day to death being approximately 17 days in the CP-treated animals compared to 8 days in the immunocompetent host. Treatment for 3 days with peramivir was only weakly inhibitory to the infection in the CP-treated mice, with the maximum survival rate (40%) seen in animals receiving 100 mg/kg/day of the compound beginning 60 h post-virus exposure. When therapy was continued through 5 days, the compounds' influenza disease-inhibitory effects were significantly enhanced, with 90–100% of the infected mice treated with the 100 mg/kg/day dose surviving. Significant prevention of death was also seen using the 10 mg/kg/day dose. The lowest dose, 1 mg/kg/day, administered for 5 days only resulted in significant increases in mean day to death. Significant declines in SaO_2 were not observed in the infected mice; the values were only obtained up to day 11, which is usually adequate in infected immunocompetent mice (Sidwell et al., 2001a), but the delay in death occurring in the CP-treated animals suggests that the SaO_2 parameter should be studied for a longer period of time.

3.2. Effect of peramivir therapy in prolonged CP-treated mice

The survival and mean day 20 SaO_2 data for this experiment are summarized in Table 2. The daily SaO_2 levels are seen in Fig. 1. In this experiment, only 5-day peramivir therapy was used, starting either 60 or 192 h after virus exposure. Treatment with all doses of the compound, began at either time point, resulted in significant prevention of deaths and in lessened decline in SaO_2 values. Effects on lung parameters for this experiment are shown in Table 3. Prior to start of treatment at 60 h, the lungs exhibited little

Table 1
Effect of 3- and 5-day oral treatment[a] with peramivir on influenza A/NWS/33 (H1N1) virus infection in short-term CP-immunosuppressed[b] mice

Compound	Dose (mg/kg/day)	Time of therapy initiation (h)	3-day treatment			5-day treatment		
			Dead/total	MDD[c] ± S.D.	Mean day 11 SaO_2 (% ± S.D.)	Dead/total	MDD[c] ± S.D.	Mean day 11 SaO_2 (% ± S.D.)
Peramivir	100	24	8/10	20.9 ± 1.0**	86.3 ± 2.6	0/10***	>22.0 ± 0.0***	88.1 ± 2.9*
	10		9/10	20.3 ± 0.7**	87.7 ± 2.1	3/10***	20.7 ± 1.5*	86.8 ± 2.9
	1		10/10	19.0 ± 1.1	89.0 ± 2.3	9/10	19.4 ± 2.0*	85.6 ± 3.5
	100	42	7/10*	20.4 ± 1.1**	86.5 ± 2.6	1/10***	19.0 ± 0.0	87.8 ± 2.7*
	10		8/10	20.6 ± 1.7**	87.9 ± 2.0	7/10	20.6 ± 1.4**	87.3 ± 2.4*
	1		9/10	18.7 ± 20.2	87.1 ± 2.0	10/10	19.8 ± 1.1**	86.9 ± 2.6
	100	60	6/10**	20.0 ± 1.3*	87.4 ± 1.6	1/10***	17.0 ± 0.0	87.0 ± 2.7
	10		1/10	20.1 ± 1.4**	87.6 ± 2.5	5/10*	20.2 ± 1.3*	87.1 ± 2.1
	1		10/10	18.4 ± 1.3	87.7 ± 2.7	8/10	20.0 ± 1.3**	87.0 ± 2.5
Saline	–	24	19/19	17.3 ± 2.8	87.8 ± 3.5	18/20	16.7 ± 2.9	84.0 ± 4.7

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to saline-treated controls.
[a] Bid for 3 or 5 days beginning at times indicated after virus exposure.
[b] Treated with 100 mg/kg of cyclophosphamide on days −1, +3, and +7.
[c] Mean day to death of mice dying prior to day 22.

Table 2
Effect of delayed-initiation oral treatment[a] with peramivir on influenza A/NWS/33 (H1N1) virus infection in prolonged CP-immunosuppressed[b] mice

Compound	Dose (mg/kg/day)	Time of therapy initiation (h)	Dead/total	MDD[c] ± S.D.	Mean day 20 SaO_2 (% ± S.D.)
Peramivir	100	60	0/10***	>36.0 ± 0.0***	85.5 ± 1.3**
	10	60	3/10**	17.0 ± 0.0	82.2 ± 4.2**
	1	60	6/10*	15.2 ± 1.5	79.0 ± 4.2
	100	192	0/10***	>36.0 ± 0.0***	86.2 ± 1.3***
	10	192	1/10***	14.0 ± 0.0	84.8 ± 3.6**
	1	192	3/10**	16.0 ± 1.2	81.3 ± 3.6*
Saline	–	60	20/20	16.3 ± 1.9	75.0 ± 0.0

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to saline-treated controls.
[a] Bid × 5 days beginning at the times indicated after virus exposure.
[b] Treated with 100 mg/kg of cyclophosphamide on days −1, +3, +7, +11, +15, +19, +23, +27, +31, and +35.
[c] Mean day to death of mice dying prior to day 22.

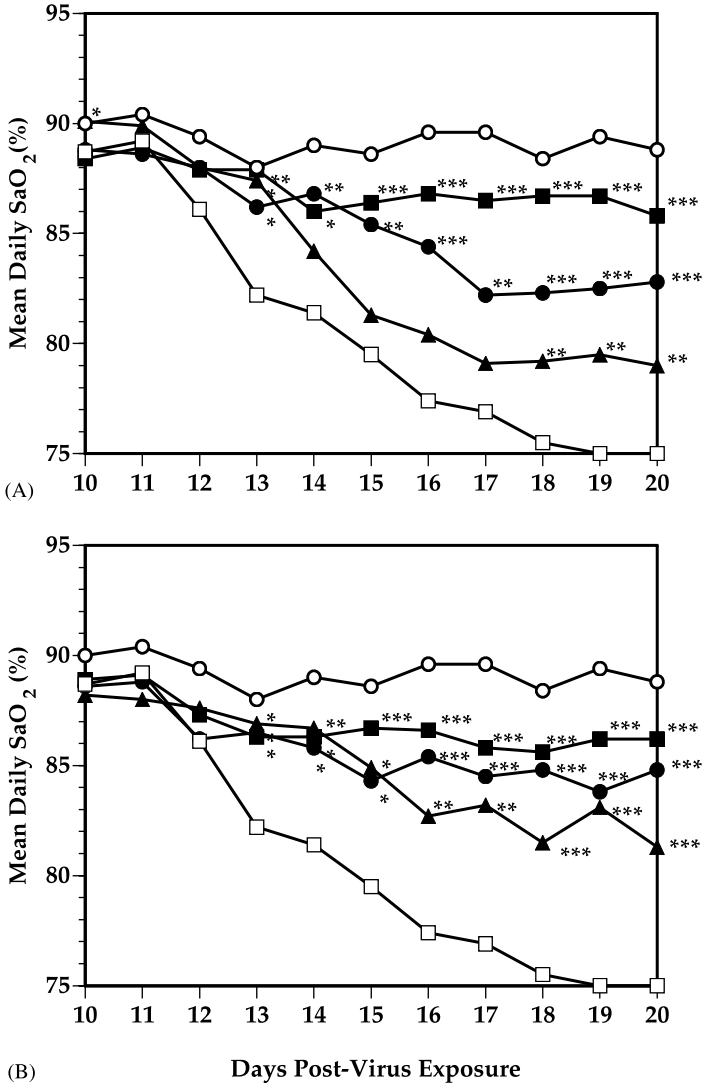

Fig. 1. Effect of early (A)- and delayed (B)-initiation treatment with peramivir on arterial oxygen saturation (SaO_2) decline in prolonged CP-immunosuppressed mice infected with influenza A (H1N1) virus. (■) Peramivir, 100 mg/kg/day; (●) peramivir, 10 mg/kg/day; (▲) peramivir, 1 mg/kg/day; (□) saline; (○) normal controls. Note: all S.D.'s were ≤6.0.

evidence of lung consolidation, with a mean score of 0.3 and lung weight of 138 mg, but virus titers were relatively high ($10^{5.3}$ $CCID_{50}$/g). Treatment with peramivir begun either 60 or 192 h after virus exposure had little inhibitory effect on lung scores or lung weight increases at either 3 or 6 days after start of treatment, but significant inhibition of lung virus titers was seen throughout the experiment. Histopathological examination of lung sections taken during this experiment indicated infiltration of lymphocytes, neutrophils, and macrophages into the infected lungs by days 11 and 13 in both peramivir- and saline-treated animals (data not shown).

3.3. Effect of peramivir therapy in SCID mice

The survival and mean SaO_2 data from the SCID mouse experiment using four viral challenge doses are summarized in Table 4. In this experiment using these more profoundly immunosuppressed mice, 5-day treatment with peramivir was unable to prevent influenza-associated deaths, although the highest (100 mg/kg/day) dosage did delay the mean day to death and significantly lessened the mean SaO_2 decline. The lung parameters were studied in the mice infected with the lowest ($10^{4.5}$ $CCID_{50}$) virus challenge dose (Table 5). A significant inhibition of lung score, but not of lung weight, was seen on treatment day 6, and a significant inhibition of lung virus titer was seen using all doses of peramivir on treatment day 3.

The mean day to death of the saline-treated infected SCID mice was similarly delayed compared to infection in immunocompetent mice as were seen in the saline-treated CP-immunosuppressed mice. These ranged from 13.6 to 20.7 days in the SCID mice, whereas in normal influenza virus-infected mice infected with this same virus strain, the

Table 3
Effect of delayed-initiation oral therapy[a] with peramivir on lung disease parameters in influenza A (H1N1) virus[b] infection in prolonged CP-immunosuppressed[c] mice

Compound	Dose (mg/kg/day)	Time of therapy initiation (h post-virus exposure)	Mean lung parameters					
			Day 3[d]			Day 6[d]		
			Score ± S.D.	Weight (mg ± S.D.)	Virus titer (log_{10}/g ± S.D.)	Score ± S.D.	Weight (mg ± S.D.)	Virus titer (log_{10}/g ± S.D.)
Peramivir	100	60	0.4 ± 0.2	160 ± 13	2.9 ± 2.2**	1.0 ± 0.4	146 ± 9*	3.6 ± 0.6***
	10	60	0.3 ± 0.2	154 ± 13	4.2 ± 0.8***	0.7 ± 0.4	174 ± 30	3.5 ± 2.0**
	1	60	0.7 ± 0.4	168 ± 13	4.9 ± 0.5**	0.8 ± 0.4	188 ± 43	5.2 ± 0.6*
Saline[e]	–	60	0.2 ± 0.4	169 ± 18	5.9 ± 0.5	0.6 ± 0.2	180 ± 32	6.0 ± 0.4
Peramivir	100	192	1.8 ± 0.2	216 ± 39**	3.2 ± 2.3**	2.4 ± 0.9	308 ± 31	1.0 ± 1.4***
	10	192	2.0 ± 0.0	252 ± 39	4.3 ± 2.7	3.3 ± 0.4	256 ± 17	0.7 ± 1.6***
	1	192	1.2 ± 0.2	260 ± 47	5.1 ± 0.9	3.2 ± 0.2	296 ± 26	4.4 ± 2.5
Saline	–	60	2.1 ± 0.5	298 ± 53	5.8 ± 0.3	2.8 ± 0.4	303 ± 32	5.7 ± 0.5
Normal controls	–	–	0	133 ± 15	–	0	147 ± 5.9	–

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to saline-treated controls sacrificed at the same time.

[a] Bid × 5 days beginning at the times indicated after virus exposure.

[b] A/NWS/33.

[c] Treated with 100 mg/kg of cyclophosphamide i.p. on days −1, +3, +7, +11, +15, +19, +23, +27, +31, +35.

[d] Relative to treatment initiation.

[e] Mean lung parameters of saline-treated controls on day 2.5: score, 0.3 ± 0.2; weight, 138 ± 18; virus titer (log_{10}/g), 5.3 ± 1.0.

Table 4
Effect of oral treatment[a] with peramivir on infections induced in SCID mice challenged with various doses of influenza A (H1N1) virus

Treatment	Dose (mg/kg/day)	Viral challenge dose	Survival/total	MDD[b] ± S.D.	Mean[c] SaO_2 (% ± S.D.)
Peramivir	100	$10^{-5.0}$	0/10	13.3 ± 3.3	80.0 ± 2.5
	10		0/10	13.7 ± 3.3	80.6 ± 2.6
	1		0/10	15.8 ± 3.3	80.8 ± 2.3
Saline[d]	–		0/20	13.6 ± 2.2	79.8 ± 1.8
Peramivir	100	$10^{-5.5}$	0/10	26.2 ± 6.2***	84.8 ± 1.5*
	10		0/10	20.2 ± 2.9	82.0 ± 2.3
	1		0/10	19.8 ± 4.6	82.2 ± 2.1
Saline	–		0/19	18.1 ± 2.4	81.0 ± 1.3
Peramivir	100	$10^{-6.0}$	0/10	28.9 ± 5.5***	86.0 ± 2.4**
	10		0/9	24.8 ± 5.0*	84.3 ± 2.3
	1		0/9	24.0 ± 4.7*	84.0 ± 2.4
Saline	–		3/19	20.7 ± 3.6	82.9 ± 1.7
Peramivir	100	$10^{-6.5}$	7/8	36.0 ± 0.0	85.9 ± 1.2*
	10		7/9	26.0 ± 1.8	84.0 ± 1.9
	1		7/8	17.0 ± 0.0	86.0 ± 0.9*
Saline	–		13/18	28.0 ± 9.0	83.7 ± 1.6

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to saline-treated controls receiving the same virus challenge dose.
[a] Bid × 5 beginning 8-day post-virus exposure.
[b] Equivalent to $10^{6.0}$, $10^{5.5}$, $10^{5.0}$, $10^{4.5}$ $CCID_{50}$/ml of virus.
[c] Mean day to death of mice dying before day 45.
[d] Days 14–26.

mean day to death ranged from 8 to 10 days (Sidwell et al., 1996).

4. Discussion

Treatment with the potent influenza virus neuraminidase inhibitor peramivir appeared capable of significantly ameliorating the influenza virus infection in mice immunosuppressed by short-term or prolonged CP therapy, particularly if the twice-daily treatment was continued for 5 days. This effect was seen as significant inhibition of deaths, lessening of decline in SaO_2, and reduction of lung virus titers. Importantly, treatment was effective if begun as late as 8 days after virus exposure.

The disease-inhibitory effect of therapy with this compound apparently requires more than 3 days of therapy in these immunosuppressed animals. This may be because the infection induced in these studies was relatively slow to develop and thus required the presence of the compound for a longer period of time. We have not yet examined the influence of a similar 3-day treatment in immunocompetent mice, however. The immunocompromised animals were challenged with a relatively low dose of virus compared to that used in normal mice, and this slowly progressing infection may explain why the infection would still respond to therapy begun at 8 days after virus exposure.

The infection in SCID mice did not respond as readily as did the CP-treated animals to peramivir therapy. The SCID mouse, or C.B-17 *scid/scid* mouse, is a congenic partner strain of BALB/cAnIcr, which lacks functional T or B cells (Schultz and Sidman, 1987). Consequently, the animals are hypogammaglobulinemic and poor mitogen responders, and they fail to reject skin grafts. Other hematopoietic cell types (monocytes, granulocytes, and natural killer cells), however, are present and function normally (Schultz and Sidman, 1987). The fact that the mice died of the influenza infection despite the presence of these hematopoietic cells would suggest that these cells do not play an important role in protecting the host from such an infection. CP treatment as used in this study has been shown to be markedly suppressive to cellular immune factors, with an almost total inhibition of natural killer cell response, T- and B-cell proliferation, and significant lessening of total T, T-helper, T-suppressor, and B cells reported previously by us using the same treatment schedule (Smee et al., 1997). The compound is also a profound suppressor of antibody production and reactions of delayed-type hypersensitivity (for a review, see Berd et al., 1984). The CP treatment regimen used in the present studies is identical to that reported by Selgrade et al. (1982) and later also used by us (Smee et al., 1991) to immunosuppress mice used in cytomegalovirus experiments. The immunosuppression induced by i.p. injection of 100 mg/kg of CP has been shown to persist approximately 4 days, requiring repeated injections of the same dosage to maintain the immune suppression (Selgrade et al., 1982). Despite the repeated CP treatments, the histopathological examination of the infected animal's lungs indicated an infiltration of lymphocytes, neutrophils, and macrophages by days 11 and 13, suggesting the treatment did not wholly suppress these important immune factors and may explain why the treatment with peramivir was more effective in these animals than in the SCID mice.

The lung disease parameters evaluated in this study provided some conflicting data; they showed that therapy significantly inhibited virus titer development in the lungs both

Table 5
Effect of delayed oral treatment[a] with peramivir on lung parameters in SCID mice challenged with a low dose[b] of influenza A (H1N1) virus

Compound	Dosage (mg/kg/day)	Mean lung parameters								
		Day 3			Day 6			Day 41		
		Score ± S.D.	Weight (mg ± S.D.)	Virus titer (log_{10}/g ± S.D.)	Score ± S.D.	Weight (mg ± S.D.)	Virus titer (log_{10}/g ± S.D.)	Score ± S.D.	Weight (mg ± S.D.)	Virus titer (log_{10}/g ± S.D.)
Peramivir	100	0.1 ± 0.2	134 ± 6	0.0 ± 0.0*	0.0 ± 0.0*	145 ± 6	0.9 ± 1.8**	0.1 ± 0.2	146 ± 10*	0.0 ± 0.0
	10	0.4 ± 0.2	150 ± 12	0.0 ± 0.0*	0.3 ± 0.3	163 ± 22	3.7 ± 2.9	0.4 ± 0.9	184 ± 50	0.0 ± 0.0
	1	0.2 ± 0.3	142 ± 11	0.0 ± 0.0*	0.1 ± 0.2*	136 ± 9	1.5 ± 1.6	0.4 ± 0.2	160 ± 20	0.0 ± 0.0
Saline	0	0.4 ± 0.2	150 ± 34	3.75 ± 2.5	0.8 ± 0.4	146 ± 30	3.8 ± 2.9	0.2 ± 0.4	163 ± 17	0.3 ± 0.6
Normal controls	0	0.0 ± 0.0	143 ± 32	0.0 ± 0.0	0.0 ± 0.0	133 ± 12	0.0 ± 0.0	0.0 ± 0.0	140 ± 10	0.0 ± 0.0

* $P < 0.05$, ** $P < 0.01$ compared to saline-treated controls.

[a] Bid × 5 beginning 8 days post-virus exposure.

[b] $10^{-6.5}$ dilution ($10^{4.5}CCID_{50}$/ml of virus).

3 days after start of treatment and 24 h after the end of the 5-day treatment. Despite this virus titer inhibition, however, the lung consolidation as evidenced by lung score and increased lung weight were generally only moderately reduced in the CP-treated animals. Since influenza virus-infected mice usually die of viral pneumonia as shown by lung consolidation, the observation that this parameter was not significantly improved by peramivir treatment leaves the question of why they would survive. In conflict with the occurrence of substantial lung consolidation was the significant lessening of SaO_2 decline in these mice. Such SaO_2 data suggest that consolidation was not sufficient to prevent the lungs from exerting their gas transference capacity. It is recognized that lung consolidation primarily occurs due to a combination of viral damage to alveolar cells causing necrosis of the capillary walls, leading to lung hemorrhage, as well as to late-occurring vascular phenomena resulting from immune response to the infection (Hers et al., 1962; Raut et al., 1975; Wyde et al., 1977; Leung and Ata, 1980).

We have reported earlier (Sidwell et al., 2001b) that high viral challenge doses lessen the inhibitory effects of orally administered peramivir. That work was done in normal, immunocompetent mice. The present study, run in SCID mice, again showed viral challenge dose to be a factor in the antiviral efficacy of this compound, with mice receiving overwhelmingly lethal challenge doses of the influenza virus to be poorly responsive to treatment, whereas as the viral challenge lessened, a more disease-inhibitory effect was seen.

Potential practical applications of these data are apparent. Human patients may become immunosuppressed through a number of factors, and the extent of immunosuppression occurring will vary considerably. Our data indicate that immunosuppression will allow the host to be susceptible to lower viral challenge doses, and that oral therapy with peramivir, initiated well after the infection has become established, has the potential to ameliorate such infections. However, the failure of this drug, when used once daily in the clinic, has apparently curtailed its further development at the present time (Anonymous, 2002); so, these findings using peramivir may not have an immediate practical application. Since peramivir has anti-influenza efficacy that closely resembles that of oseltamivir (Sidwell et al., 2001a), it is possible that these data could be applied to the latter drug, which is in active use throughout the world.

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