ELSEVIER

Antiviral Research 51 (2001) 179–187

Antiviral Research

www.elsevier.com/locate/antiviral

Influence of virus strain, challenge dose, and time of therapy initiation on the in vivo influenza inhibitory effects of RWJ-270201

Robert W. Sidwell [a,*], Donald F. Smee [a], John H. Huffman [a], Dale L. Barnard [a], John D. Morrey [a], Kevin W. Bailey [a], W.-C. Feng [a], Y.S. Babu [b], Karen Bush [c]

[a] *Institute for Antiviral Research, Utah State University, Logan, UT 84322-5600, USA*
[b] *BioCryst Pharmaceuticals, Inc., Birmingham, AL 35244, USA*
[c] *The R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ 08809, USA*

Received 2 March 2001; accepted 25 April 2001

Abstract

The influenza virus neuraminidase inhibitor RWJ-270201 (cyclopentane carboxylic acid, 3-[*cis*-1-(acetylamino)-2-ethylbutyl]-4[(aminoiminomethyl)amino]-2-hydroxy-[*cis*, 2*S*, 3*R*, 4*R*]) was significantly inhibitory to an infection in mice induced by influenza A/NWS/33 (H1N1) virus when oral gavage (p.o.) treatment with 10 mg/kg per day was delayed at least 60 h after virus exposure. Treatment was 5 mg/kg twice daily for 5 days. Viral challenge doses of influenza A/Shangdong/09/93 (H3N2) virus ranging from the LD_{70} to the LD_{100} did not affect the marked antiviral efficacy of 12.5 mg/kg of RWJ-270201 administered p.o. twice daily for 5 days beginning 4 h pre-virus exposure; infection by an approximate 2 LD_{100} dose (10^8 cell culture infectious doses/ml) was only weakly inhibited by the same treatment as seen by significant increase in mean day to death. Murine infections induced by influenza A/Bayern/57/93 (H1N1) and B/Lee/40 viruses were significantly inhibited by 100, 10, and 1 mg/kg per day of RWJ-270201 using the above treatment regimen; influenza A/PR/8/34 (H1N1) virus infections in mice were only moderately inhibited, the antiviral effects using this virus being lessening of arterial oxygen decline, reduced lung consolidation, and inhibition of lung virus titers primarily at the higher dosages.

Keywords: Influenza virus; Antiviral; RWJ-270201; Neuraminidase inhibitor

1. Introduction

The design, synthesis, and selective inhibition of influenza virus neuraminidase by the novel cyclopentane RWJ-270201 (Fig. 1) has been recently described by Babu et al. (2000) and by Bantia et

* Corresponding author. Tel.: +1-435-7971902; fax: +1-435-7973959.
E-mail address: rsidwell@cc.usu.edu (R.W. Sidwell).

0166-3542/01/$ - see front matter
PII: S0166-3542(01)00149-8

al. (2001). The striking in vitro influenza virus inhibitory effects of this compound have also recently been reported by Smee et al. (2001). The compound was found to have activity against a spectrum of influenza A (H1N1, H3N2, H5N1) and B viruses which was at least equivalent to that of zanamivir and oseltamivir carboxylate (GS4071). The compound also significantly inhibits influenza A/Shangdong/09/93 (H3N2), A/Victoria/3/75 (H3N2), A/NWS/33 (H1N1), A/Turkey/Mass/76XA/Beijing/32/92 (H6N2), and B/Hong Kong/05/72 virus infections of mice when administered by oral gavage (p.o.) beginning 4 h pre-virus exposure (Bantia et al., 2001; Sidwell et al., 2001). This activity generally was seen at up to 10-fold lower dosages than oseltamivir, which was run in parallel. No toxicity was observed in the mice in the study by Sidwell et al. (2001) at dosages of up to 1000 mg/kg per day.

It was of interest to further evaluate the in vivo effects of RWJ-270201 against other strains of influenza virus. In addition, since it had been observed that the in vitro antiviral effects of this compound were somewhat dependent on the viral multiplicity of infection (Smee et al., 2001), an experiment was run to determine if the in vivo efficacy was affected by using various viral challenge doses. We have previously shown that the influenza virus neuraminidase inhibitor oseltamivir retained its in vivo influenza disease-inhibitory effects when therapy was delayed until 60 h after virus exposure (Sidwell et al., 1998); it therefore seemed appropriate to determine if RWJ-270201 therapy could be delayed to the same degree and still render a protective effect on the virus infection. This report describes the results of these studies.

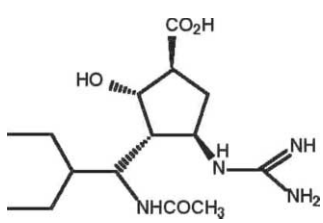

Fig. 1. Structure of RWJ-270201.

2. Materials and methods

2.1. Compound

RWJ-270201 (Fig. 1) was synthesized by BioCryst Pharmaceuticals (Birmingham, AL). It was dissolved in sterile physiological saline (PSS) for use in these studies. All solutions were stored at 4 °C until used.

2.2. Viruses

Influenza A/NWS/33 (H1N1) virus was provided by K.W. Cochran (University of Michigan, Ann Arbor, MI). Influenza A/PR/8/34 (H1N1) virus was obtained from the American Type Culture Collection (Manassas, VA). Influenza A Bayern/57/93 (H1N1) and A/Shangdong/09/93 (H3N2) virus were obtained from Helen Regnery of the Centers for Disease Control and Prevention (Atlanta, GA). The A/Bayern virus was passaged 3 times through mice to adapt it to cause lethality. The A/Shangdong virus was initially prepared in the allantoic cavity of fertilized hens' eggs, then passed 7 times through 8–10 g mice to increase its murine virulence. Influenza B/Lee/40 was obtained from F.M. Schabel of Southern Research Institute (Birmingham, AL). It was passaged twice in eggs to prepare a virus pool. Pools of all viruses were prepared in MDCK cells and titrated in mice prior to use.

2.3. Mice

Female specific pathogen-free BALB/c mice weighing 8–10 or 18–21 g, depending on the virus used, were purchased from B & K Universal (Fremont, CA). Care and housing were as previously reported (Sidwell et al., 1985).

2.4. Determination of arterial oxygen saturation (SaO_2)

Daily SaO_2 values were determined using an Ohmeda Biox 3740 pulse oximeter (Ohmeda, Louisville, OH) as previously described (Sidwell et al., 1992).

2.5. Lung virus titer determination

The mouse lungs were homogenized in minimum essential medium, the homogenates then centrifuged at $3200 \times g$ for 5 min, and varying dilutions of each supernate assayed in triplicate for infectious virus in MDCK cells as described previously (Sidwell et al., 1985; Smee et al., 2001). This centrifugation procedure prior to virus titration allowed for the quantitation of extracellular virus by eliminating cell-associated virus.

2.6. Experiment design: effect of delay of treatment on inhibition of infection

Young adult mice were anesthetized by intraperitoneal (i.p.) injection of 100 mg/kg of ketamine (Ft. Dodge Animal Health, Ft. Dodge, IA); 90 μl of influenza A/NWS/33 (H1N1) virus having a titer of 10^4 cell culture 50% infectious doses ($CCID_{50}$)/ml was placed on the nares (i.n.) of each anesthetized mouse to initiate the infection. Groups of ten mice were treated p.o., twice daily for 5 days with 5 mg/kg (10 mg/kg per day) of RWJ-270201. Treatments began 4, 24, 36, 48, or 60 h after virus exposure. As controls, 20 infected mice were treated p.o. for the same period of time with PSS beginning 4 h post-virus exposure. Five uninfected control mice were included with the study. The animals were observed for death daily for 21 days and SaO_2 levels determined on days 3–10. Five uninfected mice were treated in parallel with the compound to serve as toxicity controls; these animals were weighed prior to start of treatment and again 18 h after final treatment and observed daily for 21 days.

2.7. Experiment design to determine the effect of virus challenge dose on antiviral effect

Groups of ten young adult mice were challenged i.n. with 90 μl of varying concentrations of influenza A/Shangdong ranging from $10^{6.4}$ to $10^{8.0}$ $CCID_{50}$/ml. These viral challenge inocula induced an infection which ranged from a 70% lethal dose (LD_{70}) to approximately 2 LD_{100} doses. The mice were treated p.o. with RWJ-270201 at a dose of 25 mg/kg per day twice daily for 5 days beginning 4 h pre-virus-exposure. As controls, groups of 20 mice similarly infected with each virus dose were treated in parallel with PSS. The animals were observed for death daily for 21 days, and SaO_2 levels determined on days 3–10.

2.8. Experiment design to determine the in vivo sensitivities of influenza A/Bayern/57/93, A/PR/8/34 and B/Lee/40 viruses to RWJ-270201

Groups of 20 mice were infected i.n. with influenza A/Bayern, A/PR/8, or B/Lee viruses and treated p.o. twice daily for 5 days with 100, 10, or 1 mg/kg per day of RWJ-270201 beginning 4 h pre-virus exposure. The viral concentrations were $10^{5.8}$, $10^{4.3}$, and $10^{2.9}$ $CCID_{50}$/ml for the A/Bayern, A/PR/8, and B/Lee viruses, respectively. Young adult mice were used for the A/PR/8 and B/Lee viruses, and weanling animals were used for the A/Bayern virus. This induced a 95–100% mortality in the mice with a mean day to death of 7.4–10.6 days. On days 3 and 6, five mice in each group were killed and their lungs assigned a consolidation score ranging from 0 (normal) to 4 (maximal plum coloration), weighed, and assayed for virus titer. The remaining mice were observed daily for 21 days and SaO_2 ascertained on days 3–10. Thirty animals similarly infected with each virus were treated in parallel with PSS. Five mice in these control groups were killed on days 3 and 6 and lungs removed and handled as above concomitantly with the drug-treated animals, and the remaining 20 were observed for 21 days, with SaO_2 values determined as above. Fifteen uninfected control mice were held in parallel, with five animals killed on days 3 and 6 and the remainder observed 21 days and SaO_2 values obtained in parallel with the infected animals.

2.9. Statistical evaluations

Increases in numbers of survivors were evaluated using χ^2 analysis with Yates' correction. Differences in mean day to death, mean SaO_2 values, and mean lung virus titers were analyzed by t-test. The Wilcoxon ranked sum analysis test was used to compare mean lung scores.

Table 1
Effect of delay of initiation of oral therapy[a] with RWJ-270201 on an influenza A/NWS/33 (H1N1) virus infection in mice

Compound	Start of treatment (h, relative to virus exposure)	Infected, treated mice		
		Survivors/total	Mean day to death[b] ± S.D.	Mean Day 10 SaO_2[c] (% ± S.D.)
RWJ-270201 (10 mg/kg/day)	4	10/10[d]	>21.0 ± 0.0[d]	88.5 ± 2.1[d]
	24	9/9[d]	>21.0 ± 0.0[d]	88.6 ± 1.5[d]
	36	10/10[d]	>21.0 ± 0.0[d]	87.9 ± 1.4[d]
	48	10/10[d]	>21.0 ± 0.0[d]	88.4 ± 1.8[d]
	60	10/10[d]	>21.0 ± 0.0[d]	88.5 ± 1.5[d]
PSS	4	6/20	10.1 ± 1.5	80.2 ± 5.6

[a] bid × 5.
[b] Mean day to death of mice dying prior to day 21.
[c] Normal (uninfected) control SaO_2 value on day 10 was 89.2 ± 1.3.
[d] $P < 0.001$, compared to PSS-treated controls.
Note: Toxicity controls treated with 10 mg/kg per day of RWJ-270201 all survived, gained 0.7 g during treatment, and displayed no adverse signs suggesting lack of toxicity.

3. Results

3.1. Effect of delay of treatment initiation on an A/NWS virus infection

The results of this study are summarized in Table 1. Treatment with 10 mg/kg per day of RWJ-270201 could be delayed at least 60 h after virus exposure and still prevent any deaths from occurring in the infected mice. The SaO_2 values in the infected, treated animals remained near normal (uninfected) levels. No toxicity was observed in the controls used in this experiment.

3.2. Effect of viral challenge dose on antiviral activity of RWJ-270201

This experiment using A/Shangdong virus is summarized in Table 2, with daily mean SaO_2 values shown in Fig. 2. Treatment with 25 mg/kg per day of RWJ-270201 significantly prevented deaths in the mice at all virus challenge doses but the highest, considered to be approximately 2 LD_{100}. At this latter viral challenge dose, the drug-treated mice all died but the mean day to death was significantly prolonged. SaO_2 decline at this high virus challenge dose was only moderately inhibited (Fig. 2A) compared to inhibition observed in the other groups. When the viral challenge dose was lowered to induce a relatively non-lethal infection, the SaO_2 decline in the placebo-treated mice was insufficient for significant differences to be consistently seen in the drug-treated group (Fig. 2E).

3.3. Sensitivity of in vivo A/Bayern, A/PR/8, and B/Lee virus infections to RWJ-270201

The results of the experiments with each virus are seen in Table 3. The A/Bayern and B/Lee virus infections were highly sensitive to RWJ-270201 therapy, with a significant prevention of

Fig. 2. Effect of p.o. treatment with RWJ-270201 on arterial oxygen saturation decline in mice challenged with varying doses of influenza A/Shangdong/09/93 (H3N2) virus. ●: Infected, treated bid × 5 with 25 mg/kg per day of RWJ-270201. ○: Infected, treated bid × 5 with saline. □: Uninfected controls. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to saline-treated control. (A) Viral challenge = 10^8 $CCID_{50}$/ml; (B) viral challenge = $10^{7.6}$ $CCID_{50}$/ml; (C) viral challenge = $10^{7.2}$ $CCID_{50}$/ml; (D) viral challenge = $10^{6.8}$ $CCID_{50}$/ml; (E) viral challenge = $10^{6.4}$ $CCID_{50}$/ml.

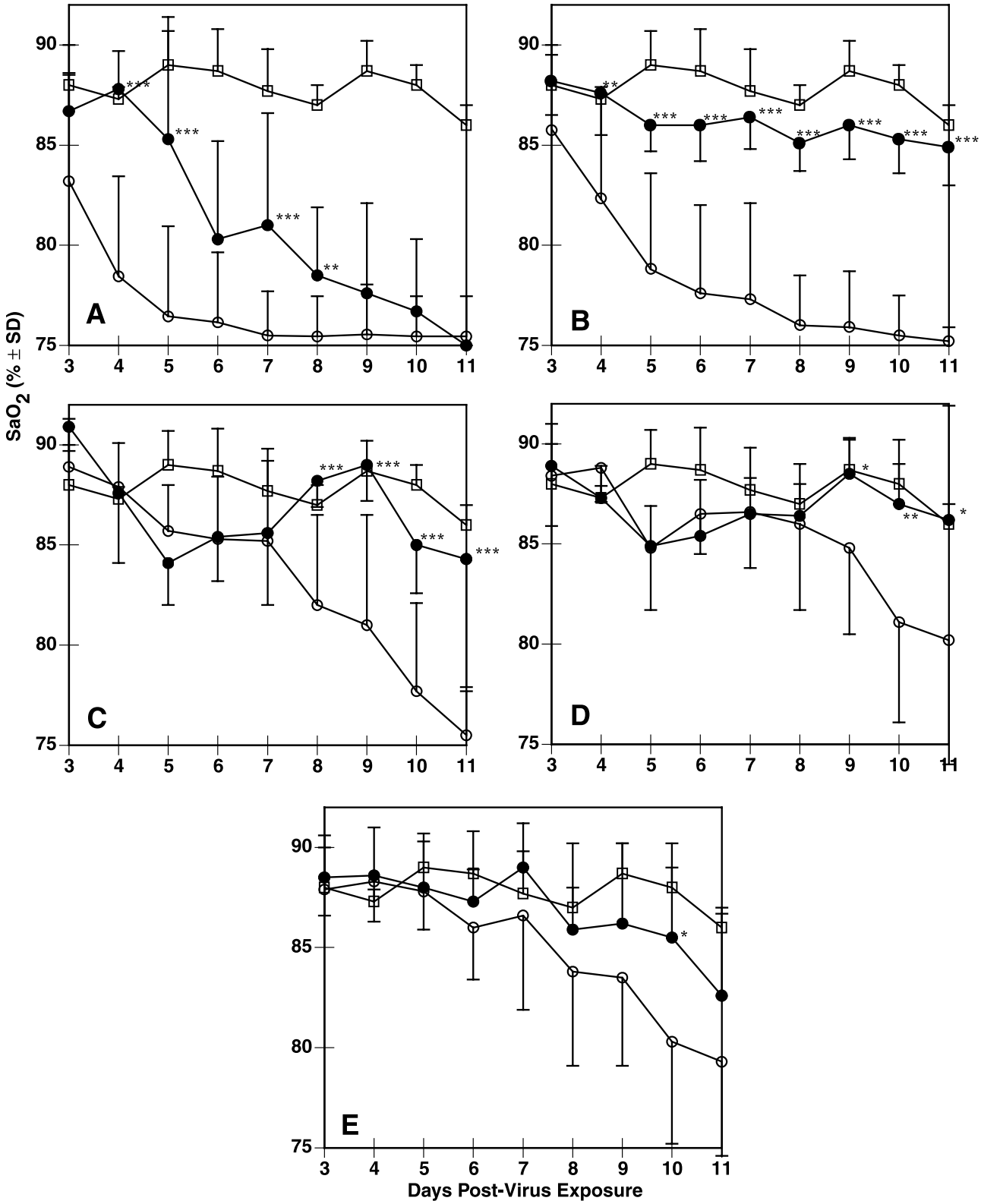

Fig. 2.

death and lessening of SaO_2 decline seen at all doses of the compound. Lung disease parameters were generally also inhibited at all doses and on both days 3 and 6, although no significant inhibition in lung virus titer reduction was seen. The A/PR/8 virus infection appeared less sensitive, with only a 25% increase in survivors seen in any group treated with RWJ-270201. SaO_2 values were generally higher in the drug-treated groups, however, and lung disease parameters generally moderately inhibited.

4. Discussion

This report provides additional information on the in vivo efficacy of the new influenza virus neuraminidase inhibitor RWJ-270201. The results indicate this compound has the potential for treating an influenza virus infection at a time when symptoms begin to appear, since protection against lethal effects of the virus was seen when therapy began 60 h after virus exposure.

We have previously shown (Sidwell et al., 2001) that high virus titers are found in the mouse lung by 24 h after exposure to virus, and lung consolidation, as evidenced by at least 25% plum coloration in the lung and 20% lung weight increase, is seen by 48 h. As we have previously reported (Sidwell et al., 2001), the greatest inhibition of lung virus titers in influenza A virus-infected mice rendered by RWJ-270201 treatment occurred 24 h after the start of treatment (earliest time studied), so it is apparent that such virus titer inhibition is sufficient to prevent the animals from dying of the influenza infection. In that earlier study, a much lesser inhibition in lung virus titers was seen in the drug-treated groups by day 3 than by day 1, which is consistent with the observations made with the influenza A viruses in the present study. Until a similar virus yield reduction study is run using the 60 h delay in therapy, we cannot speculate on why the protective effect seen occurred.

The efficacy of RWJ-270201 appeared moderately influenced by dose of virus challenge, since at a viral exposure dose of approximately twice the LD_{100}, therapy of the infected mice by the compound did not prevent death, although the mean day to death was significantly prolonged. At all lower viral challenge doses, however, RWJ-270201 therapy was highly inhibitory to the infection as evidenced by prevention of death and significantly lessened SaO_2 decline. We have previously shown that the in vitro influenza virus-inhibitory activity of both RWJ-270201 and the influenza virus neuraminidase inhibitor os-

Table 2
Effect of varying viral challenge dose on the influenza A/Shangdong/09/93 (H3N2) viral disease-inhibitory effects of orally administered[a] RWJ-270201

Treatment	Viral challenge dose (log_{10} $CCID_{50}$/ml)	Survivors/total	Mean day to death[b] ± S.D.	Day 8 mean SaO_2 (% ± S.D.)
RWJ-270201	8.0	0/10	8.5 ± 2.9**	78.5 ± 3.4**
PSS		0/20	4.5 ± 1.1	75.5 ± 2.0
RWJ-270201	7.6	10/10***	>21.0 ± 0.0***	85.1 ± 1.4***
PSS		0/20	6.0 ± 2.1	76.1 ± 2.1
RWJ-270201	7.2	10/10***	>21.0 ± 0.0***	87.5 ± 1.3***
PSS		1/20	9.6 ± 1.4	82.6 ± 4.2
RWJ-270201	6.8	10/10***	>21.0 ± 0.0**	86.9 ± 2.2
PSS		3/19	11.1 ± 1.9	86.6 ± 4.1
RWJ-270201	6.4	9/10**	8.0 ± 0.0	85.7 ± 3.9
PSS		6/20	10.6 ± 2.8	83.7 ± 4.3
Normal controls	–	5/5	>21.0 ± 0.0	87.0 ± 1.0

[a] 25 mg/kg per day, p.o. bid × 5 beginning 4 h pre-virus exposure.
[b] Mean day to death of mice dying prior to day 21.
*$P<0.05$; **$P<0.01$; ***$P<0.001$, compared to PSS-treated controls in same viral challenge group.

Table 3
Effect of orally administered RWJ-270201[a] on influenza virus infections in mice

Virus	Treatment	Dose (mg/kg per day)	Surv/total	MDD[b] ± S.D.	Mean day 10 SaO_2 (% ± S.D.)	Mean lung parameters					
						Day 3			Day 6		
						Score ± S.D.	Wt (mg ± S.D.)	Virus titer (log_{10}/g ± S.D.)	Score ± S.D.	Wt (mg ± S.D.)	Virus titer (log_{10}/g ± S.D.)
A/Bayern/57 /93 (H1N1)	RWJ-270201	100	6/9***	10.0 ± 0.0	85.9 ± 6.0**	0.1 ± 0.2*	118 ± 15*	6.7 ± 0.4	1.3 ± 0.8	188 ± 41	6.2 ± 0.4
		10	8/10***	9.0 ± 1.4	85.4 ± 7.3***	0.1 ± 0.2*	124 ± 15	6.8 ± 0.2	1.8 ± 0.2	184 ± 17	6.3 ± 0.5
		1	4/10*	10.2 ± 2.6	81.2 ± 6.8*	0.2 ± 0.3	128 ± 8*	6.7 ± 0.1	2.0 ± 0.4	170 ± 15	6.6 ± 0.2
	PSS	0	1/20	9.5 ± 2.1	76.3 ± 3.9	0.5 ± 0.4	138 ± 5	6.8 ± 0.0	2.4 ± 0.8	190 ± 35	6.6 ± 0.1
A/PR/8/34 (H1N1)	RWJ-270201	100	3/10	11.7 ± 1.1	82.4 ± 4.0*	0.0 ± 0.0	140 ± 10*	4.3 ± 0.6**	0.2 ± 0.3*	203 ± 12	4.5 ± 1.0*
		10	1/10	12.7 ± 3.0	81.2 ± 4.3	0.0 ± 0.0	133 ± 15*	3.9 ± 0.9*	0.5 ± 0.0*	210 ± 30	5.6 ± 0.1
		1	3/10	11.3 ± 0.5	84.3 ± 2.2***	0.0 ± 0.0	140 ± 10*	5.8 ± 0.6	0.5 ± 0.0*	210 ± 30	5.6 ± 0.1
	PSS	0	1/20	10.6 ± 2.0	78.3 ± 4.6	0.3 ± 0.3	158 ± 8	5.7 ± 0.4	1.2 ± 0.4	208 ± 13	5.7 ± 0.3
B/Lee/40	RWJ-270201	100	8/10***	9.0 ± 5.7	86.1 ± 4.3***	1.0 ± 0.0**	134 ± 13***	0.4 ± 0.8*	1.0 ± 0.0**	214 ± 8.6*	0.8 ± 0.9
		10	3/10*	7.6 ± 3.4	79.6 ± 6.1*	1.1 ± 0.4**	158 ± 26	0.4 ± 0.8*	1.0 ± 0.4**	226 ± 39	2.0 ± 1.1
		1	5/10***	9.4 ± 6.3	83.2 ± 6.4**	1.3 ± 0.6*	176 ± 34	0.0 ± 0.0**	2.3 ± 0.4	248 ± 22	0.4 ± 0.9*
	PSS	0	0/20	7.4 ± 3.2	76.7 ± 3.8	2.2 ± 0.4	182 ± 13	1.9 ± 1.1	2.5 ± 0.5	255 ± 39	1.9 ± 0.2

[a] bid × 5 beginning 4 h pre-virus exposure.
[b] Mean day to death.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared to PSS-treated controls run with the same virus.

eltamivir carboxylate (GS4071) were dependent on the viral multiplicity of infection (m.o.i.) (Smee et al., 2001). Such an m.o.i.-dependent antiviral effect is common among antiviral drugs (Burlington et al., 1983). The observation that treatment with RWJ-270201 was highly effective against a lethal influenza virus infection is quite significant, since most infections occurring in the clinic will be of less severity. The actual dose of the compound that will be used in the clinic is not yet fully resolved; in the present study using multiple viral challenge concentrations, a single dose of RWJ-270201, 25 mg/kg per day, was used, while the maximum tolerated dose in mice is >1000 mg/kg per day (Sidwell et al., 2001), which suggests a broad therapeutic window for this compound when used in the human patient.

We have previously reported that RWJ-270201 therapy was highly effective against influenza A/Shangdong/09/93 (H3N2), A/Victoria/3/75 (H3N2), A/NWS/33 (H1N1), and B/Hong Kong/05/72 virus infections in mice (Sidwell et al., 2001). The data obtained in the present study also indicate deaths induced by influenza A/Bayern/57/93 (H1N1) and B/Lee/40 viruses were also significantly prevented by the same treatment regimen. Influenza A/PR/8/34 (H1N1) virus infections in mice appear to be less sensitive, although the usual SaO_2 decline, lung consolidation, and lung virus titers were inhibited. This experiment with the A/PR/8/34 virus has been repeated with similar findings seen (data not shown). It is interesting that RWJ-270201 was significantly inhibitory to this virus in vitro, the 50% effective (virus-inhibitory) concentration (EC_{50}) versus this virus being 1.5 μM; the EC_{50} versus A/Bayern/07/95 was 1.0 μM, against B/Lee/40 the EC_{50} was 3.2 μM (Smee et al., 2001). IC_{50} values for neuraminidase inhibition were lowest for the A/PR/8/34 and A/NWS/33 viruses (0.09 and 0.1 nM, respectively) and highest for the B/Lee 40 virus (11 nM) (Bantia et al., 2001), indicating that response in the mouse infection model did not correlate with the observed in vitro activity. The A/Bayern/57/93 neuraminidase inhibition has not yet been studied. It is noted that the virus concentration, expressed as cell culture infectious doses, that induced similar lethality in the mice varied considerably with the virus used, with the B/Lee/40 virus being most potent.

Earlier reported studies compared the in vivo anti-influenza effects of RWJ-270201 with the influenza virus neuraminidase inhibitor oseltamivir, FDA approved for use in humans. In general, RWJ-270201 appeared up to 10-fold more potent than oseltamivir in mice. We have previously reported oseltamivir to also be inhibitory to influenza A/NWS/33 virus infections when therapy was delayed up to 60 h after virus exposure (Sidwell et al., 1998). We are unaware of any reports on the in vivo effect of oseltamivir against the A/Bayern, A/PR/8, and B/Lee viruses.

Toxicity controls were not included in all of the present studies because it had previously been shown (Sidwell et al., 2001) that p.o.-administered RWJ-270201 was well tolerated in mice at doses of at least 1000 mg/kg per day (highest dose evaluated). The treatment schedule used in those toxicity studies was twice daily for 5 days, which was the same as used in the present studies.

RWJ-270201 is currently in Phase III clinical trials against wild type influenza; challenge studies in human volunteers recently reported (Hayden et al., 2000) showed the drug to have significant antiviral activity in the treatment of experimental human influenza A and B infections and to be well tolerated. The present in vivo studies provide additional data indicating the potential of this compound to have clinical utility.

Acknowledgements

Supported by Contract NO1-AI-65291 from the Virology Branch, NIAID, NIH, and by a grant from The R. W. Johnson Pharmaceutical Research Institute.

References

Babu, Y.S., Chand, D., Bantia, S., Kotian, P., Dehghani, A., El-Kattan, Y., Liu, T.N., Littutchinson, T., Elliott, A.J., Parker, C.D., Ananth, S.L., Itorn, L.L., Laver, G.W., Montgomery, J.A., 2000. BCX-1812 (RWJ-270201): discovery of a novel, highly potent, orally active, and selective influenza neuraminidase inhibitor through structure-based drug design. J. Med. Chem. 43, 3482–3486.

Bantia, S., Parker, C.D., Ananth, S.L., Horn, L.L., Andries, K., Chand, P., Kotian, P.L., Dehghani, A., El-Kattan, Y., Lin, T., Hutchinson, T.L., Montgomery, J.A., Kellog, D.L., Babu, Y.S., 2001. Comparison of the antiinfluenza virus activity of RWJ-270201 with those of oseltamivir and zanamivir. Antimicrob. Agents Chemother. 45, 1162–1167.

Burlington, D.B., Meiklejohn, G., Mostow, S.R., 1983. Anti-influenza A activity of combination of amantadine and ribavirin in ferret tracheal ciliated epithelium. J. Antimicrob. Chemother. 11, 7–14.

Hayden, F.G., Treanor, J., Qu, R., Fowler, C., 2000. Safety and efficacy of an oral neuraminidase inhibitor RWJ-270201 in treating experimental influenza A and B in healthy adult volunteers. Poster presented at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 17–20, 2000; Toronto, Canada. Abstract no. 1156.

Sidwell, R.W., Huffman, J.H., Cull, E.W., Alaghamandan, H., Cook, P.D., Robins, R.K., 1985. Activity of selenazofurin against influenza A and B virus infections in mice. Antiviral Res. 6, 343–353.

Sidwell, R.W., Huffman, J.H., Gilbert, J., Moscon, B., Petersen, G., Burger, R., Warren, R.P., 1992. Utilization of pulse oximetry for the study of the inhibitory effects of antiviral agents on influenza virus in mice. Antimicrob. Agents Chemother. 36, 473–476.

Sidwell, R.W., Huffman, J.H., Barnard, D.L., Bailey, K.W., Wong, M.H., Morrison, A., Syndergard, T., Kim, C.U., 1998. Inhibition of influenza virus infections in mice by GS4104, an orally effective influenza virus neuraminidase inhibitor. Antiviral Res. 37, 107–129.

Sidwell, R.W., Smee, D.F., Huffman, J.H., Barnard, D.L., Bailey, K.W., Morrey, J.D., Babu, Y.S., 2001. In vivo influenza-inhibitory effects of the cyclopentane neuraminidase inhibitor RWJ-270201. Antimicrob. Agents Chemother. 45, 749–757.

Smee, D.F., Huffman, J.H., Morrison, A.C., Barnard, D.L., Sidwell, R.W., 2001. Cyclopentane neuraminidase inhibitors with potent in vitro anti-influenza virus activity. Antimicrob. Agents Chemother. 45, 743–748.