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Intranasal treatment of picornavirus and coronavirus respiratory infections in rodents using 7-thia-8-oxoguanosine

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Summary

Since common cold viruses are responsive to interferon, we developed two animal models to test the efficacy of the interferon inducer 7-thia-8-oxoguanosine: (i) an intranasal coronavirus infection in suckling rats; and (ii) an intranasal encephalomyocarditis (EMC) virus infection in adult mice. Concentrations of 0.3 and 1% 7-thia-8-oxoguanosine delivered intranasally to rats 24 and 18 hours before virus inoculation were highly protective against the otherwise lethal coronavirus infection. A 1% concentration of drug administered 4 and 8 hours after virus challenge increased mean survival times of rats but did not increase numbers of survivors. Intranasal treatment of an EMC infection produced moderate improvements in mean survival times and survival. Titrations of EMC virus indicated >300-fold reductions in nasal titres in drug-treated relative to placebo control animals on days 2–4 following virus challenge. The distribution of [^{14}C]-7-thia-8-oxoguanosine was determined shortly after intranasal delivery to mice and rats. Approximately 50% of total doses were deposited in the inner noses and mouths of both species. Most of the rest was found on/in the outer noses, stomachs, tracheas, oesophagi, and lungs. By analogy, the infecting viruses were deposited on/in the same organs and tissues of each species. The results suggest that containment of the viruses primarily occurred in the nasopharyngeal area prior to their spread to the lungs (rat coronavirus) or the brain (EMC), where fatal pathologies were manifest. Intranasal application of an interferon-inducing nucleoside analogue represents a new approach for the study of treatment of the common cold.

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Introduction

Rhinoviruses and coronaviruses account for 50% and 20% of common colds in humans, respectively (Tyrrell, 1988). The development of antiviral agents has primarily focused on rhinoviruses as targets. Certain types of compounds have been identified which inhibit the uncoating of rhinovirus particles (Al-Nakib and Tyrrell, 1987; Diana *et al.*, 1989; Kelley *et al.*, 1988), thus preventing virus replication. One of these substances was recently shown in a clinical study to suppress common colds when given prior to virus challenge (Al-Nakib *et al.*, 1989). Interferon has been given prophylactically to persons in settings in which other family members were afflicted with colds (Douglas *et al.*, 1986; Hayden *et al.*, 1986; Monto *et al.*, 1989), but side-effects from such treatments are common and efficacy is questionable (Monto *et al.*, 1989).

We recently reported that the novel immune modulator, 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3H,6H)-dione (7-thia-8-oxoguanosine, Fig. 1) is a broad-spectrum antiviral agent (Smee *et al.*, 1989; 1990a), inhibiting many DNA and RNA viruses *in vivo*. Depending upon the infection model, efficacy of the compound can be achieved between 10 and 200 mg kg^{-1} when administered prophylactically. The nucleoside induces interferon and activates natural killer cells *in vivo* (Smee *et al.*, 1990b), but does not inhibit the growth of cultured cells at ≤500 μM. In mice, the 50% lethal dose of 7-thia-8-oxoguanosine is 320 mg kg^{-1} when given as a single intraperitoneal (i.p.) injection (Smee *et al.*, 1989). Divided daily doses of 200 mg kg^{-1} or less do not appear to be deleterious to mice, even when administered for 10 or more consecutive days. By i.p. administration, 7-thia-8-oxoguanosine was active against coronavirus infections in suckling rats (initiated by intranasal virus inoculation) and against encephalomyocarditis (EMC) virus infections in mice (when the virus challenge was i.p.).

For the present studies, the intranasal route of virus challenge was used with rat coronaviruses and EMC viruses as animal models for the common cold. The rat coronavirus infection is probably the animal model closest to coronavirus-induced common colds (Parker *et al.*, 1970). Human rhionoviruses have not been adapted to rodents, except for one report in which unusual and tedious procedures were necessary to document virus replication in mice (Yin and Lomax, 1986). The EMC virus,

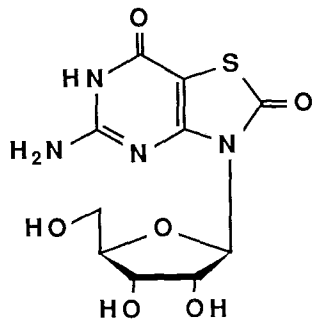

Fig. 1. Structure of 7-thia-8-oxoguanosine.

which is a picornavirus related to human rhinoviruses, has been used by others to evaluate the efficacy of interferon preparations (Sim and Cerruti, 1987), presumably as an indicator of antirhinovirus activity. Since mice receiving EMC virus die of encephalitis, this is an imperfect model for rhinovirus-induced common colds. However, we have observed that EMC, when administered intranasally, replicates in the nose and lungs of infected mice. Suppression of EMC virus replication in the nose would therefore be akin to inhibition of rhinovirus replication in the upper respiratory tract. In this communication, 7-thia-8-oxoguanosine is shown to have antiviral activity in the intranasal EMC and rat coronavirus models when administered intranasally.

Results

For rat coronavirus infection, suckling rats were treated intranasally with 7-thia-8-oxoguanosine (Table 1). Prophylactic treatments of 0.3 and 1% solutions were significantly effective at increasing the numbers of survivors relative to the placebo control. A more moderate effect was achieved using the 0.1% solution, and 0.03% was inactive. Some increases in mean survival times were evident in treated groups of rats that died. A second study was conducted to determine whether intranasal treatments given after virus challenge would still be protective to rats (Table 2). Prophylactic treatment with a 1% solution of compound starting at −24 hours produced a substantial cure rate from the infection, but treatments starting four hours after virus challenge did not. However, this therapy caused significant increases in mean survival times relative to the respective placebo control. There were differences in the severity of infection in the two placebo groups (39% survival for rats pretreated with placebo compared to 0% survival for animals receiving virus first), which may have affected the results.

For intranasal EMC virus infection, intranasal treatment with 7-thia-8-oxoguanosine provided moderate protection from mortality and some increase in mean survival times of mice that died (Table 3). For comparative purposes, the immune modulator was also given by i.p. administration to the mice. At 100mg kg^{-1}, statistically significant increases in survivor numbers were achieved. Increases in mean survival times were evident at 50 and 100mg kg^{-1}. These results were fairly consistent regardless of whether 10 or 32 LD_{50} (50% lethal dose) of virus was used as challenge.

As a follow-up to the results shown in Table 3, EMC virus was titrated from organs and tissues of mice treated with compound or placebo (Fig. 2). There were >300-fold reductions in virus titres in the noses of treated mice on days two to four, indicating a marked antiviral effect in the upper respiratory tract. Spleen and brain virus concentrations were also suppressed in mice receiving the nucleoside. By day six, the virus broke through to produce high titres in the brain, which resulted in mortality thereafter. Surprisingly, 7-thia-8-oxoguanosine did not produce a strong suppressive effect on lung virus titres.

Table 1. Dose-response intranasal treatment of coronavirus-infected rats with 7-thia-8-oxoguanosine.

% Solution[a] given (mM)	Dose (mg kg^{-1})	Survivors/ Total (%)	Mean survival time[b] (days)
Placebo	0	0/15 (0)	9.0 ± 1.5[c]
0.03 (1)	2.2	0/5 (0)	10.4 ± 0.9
0.1 (3)	6.6	5/13 (38)[d]	10.8 ± 1.4[e]
0.3 (10)	22	11/13 (85)[d]	11.0 ± 2.8
1 (30)	66	9/13 (69)[d]	11.3 ± 1.5[e]

a. Concentration of 7-thia-8-oxoguanosine in bicarbonate buffer, 20 µl of which was administered intranasally to each rat 24 and 18 hours before intranasal virus challenge. The dose corresponding to each amount is given in the adjacent column.
b. Mean survival time (days) of rats that died. Survivors lived through 21 days.
c. Standard deviation.
d. Statistically significant ($p < 0.05$), determined by the two-tailed Fisher exact test.
e. Statistically significant ($p < 0.02$), determined by the two-tailed *t*-test.

Table 2. Effects of timing of 7-thia-8-oxoguanosine treatment on a rat coronavirus infection in suckling rats.

Dose (mg kg^{-1})	Times of treatment[a]	Survivors/ Total (%)	Mean survival time[b] (days)
Placebo	−24, −18	11/28 (39)	10.5 ± 1.4[c]
66	−24, −18	26/28 (93)[d]	12.0 ± 1.4
Placebo	+4, +8	0/28 (0)	6.7 ± 1.3
66	+4, +8	0/27 (0)	9.3 ± 1.4[e]

a. Administered intranasally at the times indicated relative to virus challenge.
b. Mean survival time (days) of rats that died. Survivors lived through 21 days.
c. Standard deviation.
d. Statistically significant ($p < 0.001$), determined by the two-tailed Fisher exact test.
e. Statistically significant ($p < 0.001$), determined by the two-tailed *t*-test.

Table 3. Effects of intranasal and intraperitoneal treatments of 7-thia-8-oxoguanosine on an intranasal EMC virus infection in mice.

Virus LD_{50}	Dose[a] (mg kg^{-1})	Treatment route[b]	Survivors/ Total (%)	Mean survival time[c] (days)
10	Placebo	i.n.	6/16 (38)	5.4 ± 1.5[d]
	20	i.n.	12/16 (75)	8.0 ± 1.4[e]
	Placebo	i.p.	2/16 (13)	4.9 ± 1.1
	50	i.p.	7/16 (44)	8.6 ± 4.0[e]
	100	i.p.	12/16 (75)[f]	9.0 ± 2.4[e]
32	Placebo	i.n.	1/16 (6)	3.7 ± 0.7[e]
	20	i.n.	4/16 (25)	5.7 ± 1.6[e]
	Placebo	i.p.	1/16 (6)	4.9 ± 1.2
	50	i.p.	5/16 (31)	7.1 ± 2.3[e]
	100	i.p.	12/16 (75)[f]	8.0 ± 1.6[e]

a. Half-daily doses were administered 24 and 18 hours before virus inoculation.
b. i.n. = intranasal (20 µl of a 1% solution of 7-thia-8-oxoguanosine); i.p. = intraperitoneal.
c. Mean survival time (days) of mice that died. Survivors lived through 21 days.
d. Standard deviation.
e. Statistically significant ($p < 0.005$), determined by the two-tailed *t*-test.
f. Statistically significant ($p < 0.002$), determined by the two-tailed Fisher exact test.

The distribution of [^{14}C]-7-thia-8-oxoguanosine on/in various organs and tissues following intranasal administration was determined (Table 4). In both mice and rats, the inner noses and mouths received about 50% of the total dose. Mice which were anaesthetized for drug administration received 9% of the dose in the lungs and 11% in the stomach. In contrast, unanaesthetized rats got 20% of the dose in their stomachs and only 1% in lungs. The deposition of virus-containing media in mice and rats following intranasal dosing would be similar to the distribution of 7-thia-8-oxoguanosine since similar inoculation techniques were used.

Discussion

These results demonstrate that a topically applied interferon-inducing immune modulator provided protection to mice and rats infected with viruses related to those causing common colds in humans. From the rat studies we showed that prophylactic, or at best very early, treatments were necessary to provide a benefit. This suggests that prophylactic application such as in household settings may be appropriate to achieve clinical efficacy. This is the strategy researchers are using to evaluate interferon preparations (Douglas *et al.*, 1986; Hayden *et al.*, 1986; Monto *et al.*, 1989) for example.

The schedule of treating in divided daily doses was worked out in many viral systems (Smee *et al.*, 1989; 1990a) and appears to be optimal. Neither more-frequent treatments nor divided doses given for several days in succession increases efficacy. There may be some merit to intermittent treatments (divided daily doses given every

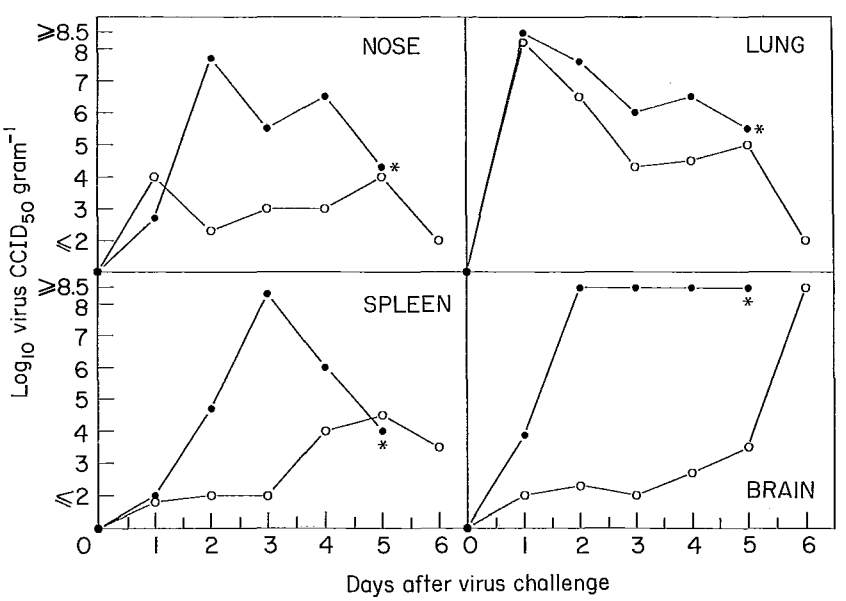

Fig. 2. EMC virus titres in organs or tissues of mice treated with 7-thia-8-oxoguanosine. Intranasal treatments were the same as in Table 3. Each data point represents the arithmetic mean titre from five mice. (●—●); (○—○), nucleoside at 20 mg kg^{-1}. (*) = all mice in the placebo group were dead by day six.

Table 4. Distribution of [^{14}C]-7-thia-8-oxoguanosine after intranasal treatment of adult mice and suckling rats.

Species[a]	Percent of Total Administered Dose ± Standard Deviation					
	Outer nose	Inner nose	Mouth	Trachea/ Oesophagus	Lungs	Stomach
Mouse	5 ± 3	30 ± 9	19 ± 6	3 ± 1	9 ± 7	11 ± 6
	(2–9)[b]	(15–44)	(10–27)	(2–4)	(4–24)	(4–13)
Rat	12 ± 10	30 ± 12	21 ± 7	4 ± 2	1 ± 1	22 ± 13
	(3–27)	(19–44)	(13–30)	(2–7)	(0–3)	(10–41)

a. Animals were sacrificed 5 minutes after administration of compound and then autopsied. Values are averages for six mice or five rats. Mice were anaesthetized for intranasal treatment whereas rats were not.
b. Range.

three or four days), however, but more studies are necessary to confirm this.

For these studies, virus challenges of 10 times the LD_{50} were used, which generally created severe infections. It took a larger dose than this to kill mice with EMC virus using the intranasal route of virus challenge coupled with intranasal drug treatment (Table 3). In the design of these studies, we wanted to get high percentages of mortality in the placebo control, since it is more difficult to achieve statistical significance with fewer deaths in this group. In so doing, the effects of the antiviral agent may also be reduced in these severe infections. This is illustrated by comparing the activity of intranasal 7-thia-8-oxoguanosine at 10 and at 32 LD_{50} (Table 3). The overall number of survivors was increased in treated mice receiving 10 LD_{50} of virus. It should also be noted that the virus dose did not seem to make a difference to the intraperitoneal activity of 7-thia-8-oxoguanosine in the same study.

From the EMC virus studies it was evident that systemic (i.p.) administration of the compound was superior to the intranasal route for overall efficacy (especially at the high input of 32 LD_{50} of EMC per mouse). This also applies to studies we reported earlier with the rat coronavirus model (Smee *et al.*, 1989). For i.p. delivery, higher doses of compound could be given and more extensive drug distribution achieved, which probably leads to greater immune activation and interferon induction. In addition, 7-thia-8-oxoguanosine is very poorly absorbed orally, at least in rodents (larger animals have not been examined yet). The nucleoside would clear from the nose and mouth via the stomach and intestinal tract, where it would leave the body without having any systemic effect. All of these factors may account for superior efficacy of i.p. relative to intranasal administration. From the standpoint of safety and practicality, however, it makes sense to treat the common cold topically. Locally induced interferon coupled with activation of immune cells may be all that is required to achieve efficacy, whereas whole-body induction of interferon and immunopotentiation could have significant side-effects.

Whether or not EMC virus is a relevant model for the common cold is debatable, since the mice die from encephalitis. The results presented here show that substantial decreases in nasal virus titres were achieved by treatments with 7-thia-8-oxoguanosine, which is akin to inhibition of common cold viruses in the respiratory tract. What ultimately kept the mice alive longer than placebo controls was the suppression of virus replication in the brain. From unpublished studies we have conducted with Semliki Forest virus, the effect of a single day's treatment with the nucleoside causing some reduction in mortality lasts no more than five days. These data correlate well with those shown in Fig. 2 which illustrate the suppression of EMC virus in the brain for about five days, after which time virus breakthrough occurred, leading to mortality.

In the experiment reported in Table 2, there was a marked difference in survival between placebo groups (39% survival in one group versus 0% in the other). In the first group having 39% survival, the placebo was given on day three and the virus on day four. The second group received virus on day three followed by placebo treatment four hours later. The increased survival of the first group may have resulted from a placebo effect, or from increased resistance of the host toward the pathogen since susceptibility to infection is age-dependent (Parker *et al.*, 1970), or from a combination of these factors. It is also possible that administration of intranasal fluid after virus challenge may have increased the severity of the infection. We did not anticipate these results when the experiment was designed.

Results of radioactivity distribution studies of [^{14}C]-7-thia-8-oxoguanosine showed that 50% of the recovered compound went into the nose and mouth. Thus, the nucleoside was well targeted to exert its antiviral effect. We used 20-gram rats for these experiments instead of 6-gram rats for infections to facilitate autopsy. The distribution of compound in 6- versus 20-gram animals is probably similar, except that less compound probably can be administered internally to the smaller rats (i.e. more compound may be deposited on the outer nose or

sneezed away). In mice, only about 10% of available drug went into the lungs, which may be part of the reason why EMC virus titres were only moderately suppressed in lung tissue. The fact that more compound went to mouse lungs than rat lungs can be attributed to the use of anaesthesia in mice, which made it easier for them to breathe nucleoside into their lungs.

Virus titrations of rat coronavirus-infected lungs or nasal areas were not conducted because of the unavailability of a suitable cell-culture system. We have had very poor success producing the primary rat kidney cells, mentioned by Parker *et al.* (1970), that are only susceptible to virus infections when used within one week after initial preparation of the cells from rat kidneys. The LBC cell line that supports the replication of rat coronavirus could not be obtained from those who developed it (Hirano *et al.*, 1985). It is assumed that the *in vivo* antiviral activity against rat coronavirus is correlated with decreases in virus titres, as was shown for EMC virus.

The concept of using intranasally applied interferon inducers to treat virus infections is not a new one but goes back over two decades. De Clercq and Merigan (1969) reported beneficial effects of topically applied poly I:poly C against intranasal vesicular stomatitis virus infections in mice. Aerosol administration of poly I:poly C was found to be active against mouse influenza infections (Gerone *et al.*, 1971). Against respiratory diseases in humans, intranasal poly I:poly C moderately suppressed common cold symptoms caused by rhinovirus 13 and type A2 influenza virus (Hill *et al.*, 1972). A double-stranded RNA of fungal origin (referred to by some investigators as Statolon) was effective intranasally against influenza virus in mice (Kleinschmidt and Streightoff, 1971), but was later shown only to delay the onset of symptoms and to cause slight reductions in virus titre against experimental rhinovirus type 4 infections in humans (Aoki *et al.*, 1978). More recently, the interferon inducer, bropirimine, significantly reduced symptoms of experimental infectious bovine rhinotracheitis virus disease in cattle when given as a prophylactic intranasal medication (Wierenga, 1985). To date, no interferon inducer has been approved for the treatment of any viral infections in humans or animals, however. The present results using the water-soluble nucleoside, 7-thia-8-oxoguanosine, suggest that this agent deserves further consideration with a view to treatment of the respiratory infections caused by rhinoviruses and coronaviruses.

Materials and Experimental procedures

Compound

7-thia-8-oxoguanosine was synthesized using the published procedure (Nagahara *et al.*, 1990). It was soluble in 2% sodium bicarbonate (pH 8.6–8.8) up to 10 mg ml^{-1} (Smee *et al.*, 1989). The placebo used for animal studies was a solution of 2% sodium bicarbonate. [^{14}C]-7-thia-8-oxoguanosine was custom-synthesized by Moravek Biochemicals (Brea, CA).

Viruses and cells

Rat coronavirus (8190 strain) and encephalomyocarditis virus (EMC strain) were purchased from the American Type Culture Collection (ATCC; Rockville, MD). L929 cells were also obtained from ATCC. EMC virus was propagated in L929 cells (Smee *et al.*, 1989), whereas rat coronavirus was passaged in three-day-old suckling rats (Parker *et al.*, 1970), and the virus was harvested from rat lungs at six days.

Animal experiments

Swiss Webster female mice and pregnant Fischer rats were purchased from Charles River Labs, Wilmington, MA. Viruses were pre-titrated in these animals to identify doses which were 10 times the 50% lethal dose (LD_{50}). Each experiment was conducted with 10 LD_{50}, except where indicated. Twenty-gram mice were treated with Ketamine (Park Davis Co., Morris Plains, NJ) at 100 mg kg^{-1} prior to intranasal inoculation of 20 µl of EMC virus. Three- to four-day-old (about six grams) Fischer rats were not anaesthetized for intranasal instillation (Parker *et al.*, 1970) of 20 µl of virus. Suckling rats appear to be more sensitive than adult mice to fluid which may get into their lungs when anaesthetized, and many of them die from such treatment. In these models, the mice die from EMC encephalitis (Sim and Cerruti, 1987) and the rats die from coronavirus pneumonia (Parker *et al.*, 1970).

For drug efficacy studies, mice received i.p. doses of compound at 50–100 mg kg^{-1} or 20 µl of a 1% solution of nucleoside (20 mg kg^{-1}). The rats received 20 µl of nucleoside intranasally as 0.03–1% solutions corresponding to 2–66 mg kg^{-1}. The mg per kg dose calculations for intranasal treatments assumes complete inhalation of the compound. Intranasal administration of the immune modulator or 2% sodium bicarbonate was conducted the same way as the virus challenge. For the EMC tests there were initially 16 animals per group. We were unable to predict how many Fischer rats would be born for the coronavirus studies, and uneven group sizes resulted.

All experiments ran for 21 days, at which time the animals were considered cured from the lethal phase of the infections. Statistical evaluations compared drug-treated groups with placebo controls. Increases in survival numbers were evaluated by the two-tailed Fisher exact test. Mean survival time increases were statistically analysed by the two-tailed Student's *t*-test.

Titrations of EMC virus from organs

At various times after infection, the nasal areas of mice were removed, and 1:10 homogenates were made using an Omnimixer (Dupont Co., Newtown, CT). Freon 113 at 20% concentration was added to supernatants during homogenization to separate viruses (which are Freon-resistant) more efficiently from cell debris. Virus titrations were conducted by end-point dilution methods (Reed and Muench, 1938) in 96-well plates (Smee *et al.*, 1982) of L929 cells using four wells per dilution. Virus concentrations are expressed as $\log_{10}$ cell culture infectious doses ($CCID_{50}$) per gram of tissue.

Distribution of [^{14}C]-7-thia-8-oxoguanosine in animals

Twenty-gram mice or 20-gram suckling Sprague Dawley rats (Charles River Labs) received an intranasal 20 μl dose of 7-thia-8-oxoguanosine spiked with 10 μCi [^{14}C]-nucleoside ml^{-1}. We used the Sprague Dawley strain instead of Fischer rats as a cost-effective measure. Only one animal at a time was dosed and then autopsied, to reduce time variability. After each mouse or rat appeared to completely inhale all of the compound, three minutes of time was allowed to elapse. Then the rodent was placed in a chamber containing anaesthesia grade ether and was allowed to expire. About two minutes later, when the animal was dead, the autopsy began; at 10 minutes the autopsy was over. First, compound on the outer surface of the nose was rinsed into a scintillation vial using about 2 ml of 70% ethanol. The mouth was rinsed and fluid collected in a vial which also contained the tongue and lower jaw. Other tissues such as the nose, oesophagus/trachea (removed as a unit), lungs, and stomach were dissected and placed in vials. The tissues were incubated overnight in 2 ml of methylbenzethonium hydroxide (Sigma Chemical Co., St. Louis, MO). Each sample was vigorously sonicated for 2–5 minutes using a Heat Systems-Ultrasonics (Farmingdale, NY) sonicator. Samples were acidified with 0.3 ml of concentrated HCl in scintillation fluid in order to neutralize chemiluminescence before counting.

References

Al-Nakib, W., and Tyrrell, D.A.J. (1987) A 'new' generation of more potent synthetic antirhinovirus compounds: comparison of their MICs and their synergistic interactions. *Antiviral Res* **8:** 179–188.

Al-Nakib, W., Higgins, P.G., Barrow, G.I., Tyrrell, D.A.J., Andries, K., Vanden Bussche, G., Taylor, N., and Jannsen, P.A.J. (1989) Suppression of colds in human volunteers challenged with rhinovirus by a new synthetic drug (R61837). *Antimicrob Agents Chemother* **33:** 522–525.

Aoki, F.Y., Reed, S.E., Craig, J.W., Tyrrell, D.A.J., and Lees, L.J. (1978) Effect of a polynucleotide interferon inducer of fungal origin on experimental rhinovirus infections in humans. *J Inf Dis* **137:** 82–86.

De Clercq, E., and Merigan, T.C. (1969) Local and systemic protection by synthetic polyanionic interferon inducers in mice against intranasal vesicular stomatitis virus. *J Gen Virol* **5:** 359–368.

Diana, G.D., Cutcliffe, D., Oglesby, R.C., Otto, M.J., Mallamo, J.P., Akullian, V., and McKinlay, M.A. (1989) Synthesis and structure-activity studies of some disubstituted phenylisoxazoles against human picornaviruses. *J Med Chem* **32:** 450–455.

Douglas, R.M., Moore, B.W., Miles, H.B., Davies, L.M., Graham, N.M.H., Ryan, P., Worswick, D.A., and Albrecht, J.K. (1986) Prophylactic efficacy of intranasal alpha$_2$-interferon against rhinovirus infections in the family setting. *N Engl J Med* **314:** 65–70.

Gerone, P.J., Hill, D.A., Appell, L.H., and Baron, S. (1971) Inhibition of respiratory virus infections in mice with aerosols of synthetic double-stranded RNA. *Infect Immun* **3:** 323–327.

Hayden, F.G., Albrecht, J.K., Kaiser, D.L., and Gwaltney, J.M. (1986) Prevention of natural colds by contact prophylaxis with intranasal alpha$_2$-interferon. *N Engl J Med* **314:** 71–75.

Hill, D.A., Baron, S., Perkins, J.C., Worthington, M., Van Kirk, J.E., Mills, J., Kapikian, A.Z., and Chanock, R.M. (1972) Evaluation of an interferon inducer in viral respiratory disease. *J Am Med Assoc* **219:** 1179–1184.

Hirano, N., Ono, K., Sada, Y., Inoue, A., Murakami, T., and Takamaru, H. (1985) Replication of rat coronavirus in a rat cell line LBC. *Arch Virol* **85:** 301–304.

Kelley, J.L., Linn, J.A., Krochmal, M.P., Selway, J.W.T. (1988) 9-Benzyl-6-(dimethylamino)-9H-purines with antirhinovirus activity. *J Med Chem* **31:** 2001–2004.

Kleinschmidt, W.J., and Streightoff, F. (1971) Inhibition of influenza virus and interferon response intranasally with statolon. *Infect Immun* **4:** 738–741.

Schwartz, S.A., and Albrecht, J.K. (1989) Ineffectiveness of postexposure prophylaxis of rhinovirus infections with low-dose intranasal alpha 2b interferon in families. *Antimicrob Agents Chemother* **33:** 387–390.

Nagahara, K., Anderson, J.D., Kini, G.D., Dalley, N.K., Larson, S.B., Smee, D.F., Jin, A., Sharma, B.S., Jolley, W.B., Robins, R.K., and Cottam, H.B. (1990) Thiazolo[4,5-*d*]pyrimidine nucleosides. The synthesis of certain 3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidines as potential immunotherapeutic agents. *J Med Chem* **33:** 407–415.

Parker, J.C., Cross, S.S., and Rowe, W.P. (1970) Rat coronavirus (RCV): a prevalent, naturally occurring pneumotropic virus of rats. *Arch gesamte Virusforsch* **31:** 293–302.

Reed, L.J., and Muench, M. (1938) A simple method of estimating fifty percent end points. *Am J Hyg* **27:** 493–498.

Sim, I.S., and Cerruti, R.L. (1987) Recombinant interferons alpha and gamma: comparative antiviral activity and synergistic interaction in encephalomyocarditis virus infection of mice. *Antiviral Res* **8:** 209–221.

Smee, D.F., Sidwell, R.W., Clark, S.M., Barnett, B.B., and Spendlove, R.S. (1982) Inhibition of rotaviruses by selected antiviral agents: mechanisms of viral inhibition and *in vivo* activity. *Antimicrob Agents Chemother* **21:** 66–73.

Smee, D.F., Alaghamandan, H.A., Cottam, H.B., Sharma, B.S., Jolley, W.B., and Robins, R.K. (1989) Broad-spectrum *in vivo* antiviral activity of 7-thia-8-oxoguanosine, a novel immunopotentiating agent. *Antimicrob Agents Chemother* **33:** 1487–1492.

Smee, D.F., Alaghamandan, H.A., Cottam, H.B., Jolley, W.B., and Robins, R.K. (1990a) Antiviral activity of the novel immune modulator 7-thia-8-oxoguanosine. *J Biol Response Mod*, in press.

Smee, D.F., Alaghamandan, H.A., Jin, A., Sharma, B.S., and Jolley, W.B. (1990b) Roles of interferon and natural killer cells in the antiviral activity of 7-thia-8-oxoguanosine against Semliki Forest virus infections in mice. *Antiviral Res*, in press.

Tyrrell, D.A.J. (1988) Hot news on the common cold. *Annu Rev Microbiol* **42:** 35–47.

Wierenga, W. (1985) Antiviral and other bioactivities of pyrimidinones. *Pharmac Ther* **30:** 67–89.

Yin, F.H., and Lomax, N.B. (1986) Establishment of a mouse model for human rhinovirus infection. *J Gen Virol* **67:** 2335–2340.