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Prophylactic and therapeutic activities of 7-thia-8-oxoguanosine against Punta Toro virus infections in mice

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Summary

The biological response modifier 7-thia-8-oxoguanosine was evaluated in mice against the hepatotropic Adames strain of Punta Toro virus. When administered intraperitoneally in divided doses, significant protection from death was conferred at doses of 50 and 100 mg/kg/day given 24 and 17 h pre-virus inoculation, 25–100 mg/kg/day administered 4 h pre- and 3 h post-virus challenge, and 12.5 to 100 mg/kg/day administered 24 and 31 h after virus inoculation. These doses preventing death reduced liver icterus scores, serum alanine aminotransferase and aspartate aminotransferase levels, and liver and serum virus titers relative to placebo controls. Full daily doses administered at 24 h were somewhat less protective to mice than divided daily doses starting at the same time. The initiation of treatment could be delayed as late as 36 h after virus inoculation, resulting in complete protection from mortality at 100 mg/kg/day. This prevention of death occurred despite the acute nature of the infection which resulted in deaths by 96 h in the placebo-treated controls. These results show that 7-thia-8-oxoguanosine has both prophylactic and therapeutic potential as an anti-Phlebovirus agent. Interferon induction appears to be the reason for antiviral activity in this model, since up to 10000 units of interferon/ml were induced in mice 1 h after treatment with 100 mg 7-thia-8-oxoguanosine per kg, and antibody to interferon α/β administered shortly after treatment with the nucleoside negated the antiviral effect.

Phlebovirus; Nucleoside; Interferon inducer

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Introduction

Phleboviruses are members of the family Bunyaviridae, which are negative stranded RNA viruses (Cash et al., 1979). They are important pathogens in the Mediterranean area, parts of Africa, and South America (Sabin, 1948; Travassos da Rosa et al., 1983). Representatives of this group, Rift Valley fever virus and Sandfly fever virus, can be considered important targets of studies to devise treatments for the debilitating diseases they cause. A major epidemic of Rift Valley fever in 1985 was responsible for thousands of cases of infection and over 500 deaths in Egypt (Meegan et al., 1981). Since this particular strain of virus can be quite dangerous to man, we have developed the closely related Punta Toro virus (PTV) model as a less virulent virus for evaluating anti-Phlebovirus agents. The antiviral agents ribavirin (Sidwell et al., 1988b) and ribamidine (Sidwell et al., 1988a) were effective in treating the hepatotropic form of this disease in mice.

Part of our antiviral evaluations using the PTV model involves studying biological response modifiers which exert their effects on cells of the immune system. These agents do not usually show antiviral activity in cell culture because non-immune cells are predominantly used for viral assays. The nucleoside analog 7-thia-8-oxoguanosine is a novel biological response modifier of this type that is known to induce interferon (Smee et al., 1990c). It was reported to inhibit a broad spectrum of RNA and DNA viruses in animal infection models (Smee et al., 1989; Smee et al., 1990b). One of these, San Angelo virus, is in the same family as PTV but belongs to the California group of bunyaviruses (Sather and Hammon, 1967) instead of the *Phlebovirus* group. 7-Thia-8-oxoguanosine was effective in preventing death by San Angelo virus in mice when administered prior to or within 24 h after virus challenge (Smee et al., 1989; Smee et al., 1990b). In the present report 7-thia-8-oxoguanosine is shown to be effective in the treatment of hepatotropic PTV infections in mice. Interferon induction and action appear to be responsible for the biological activity of the compound.

Materials and Methods

Compounds and reagents

5-Amino-3-β-D-ribofuranosylthiazolo[4,5-d]pyrimidine-2,7(3H,7H)-dione (7-thia-8-oxoguanosine) was provided in dry powder form by the U.S. Army Medical Research Institute of Infectious Diseases via Technassociates, Inc. (Rockville, MD). Since the compound is rather insoluble in saline at neutral pH (Smee et al., 1989), it was prepared in 2% sodium bicarbonate (pH 8.6–8.9) for injection into mice. Bicarbonate also served as the placebo control for animal studies. Antibody to interferon α/β was purchased from Lee Biomolecular, San Diego, CA; it was diluted in sterile water for injection.

Virus

The Adames strain of PTV as described by Sidwell et al. (1988b) was used. The virus was originally isolated from patients presumably infected in Panama. It was twice plaque purified in a derivative strain of continuously passaged LLC-MK_2 cells, virus pools were made, and the virus titrated in mice.

Animals

Specific pathogen-free female C57BL/6 mice weighing 10–12 grams were obtained from Simonsen Laboratories (Gilroy, CA) for these studies. Larger mice become resistant to lethal infection. The animals were quarantined 24–48 h prior to use, housed five or ten to a cage, and fed Wayne Laboratory Chow and tap water ad libitum.

Interferon (IFN) titrations

Disposable 96-well microplates containing confluent monolayers of L929 cells were exposed to half-$\log_{10}$ dilutions (0.1 ml/well) of mouse serum samples presumed to contain interferon. After one day, 100–320 cell culture infectious doses of vesicular stomatitis virus were added to each well. After 2–3 days the cells were examined for virus-induced cytopathic effect (CPE). Interferon titers were expressed as the maximum dilution of supernatant fluid which inhibited CPE by 50%. This assay was developed by Sidwell and Huffman (1971).

Toxicity determinations

Uninfected mice were weighed prior to treatment with high doses (100 to 700 mg/kg) of 7-thia-8-oxoguanosine administered in half-daily increments 7 h apart. The animals were weighed the next day and deaths recorded for 21 days. From these results non-toxic doses were selected for antiviral experiments.

In vivo chemotherapy studies

Mice were inoculated subcutaneously (s.c.) with 10 50% lethal doses of PTV (approximately 10^5 cell culture infectious doses in LLC-MK_2 cells). 7-Thia-8-oxoguanosine was evaluated at several doses, with intraperitoneal (i.p.) treatments given in single or 2 injections (7 h apart) at various times pre- or post-virus inoculation. Other studies with this compound have shown that multiple days of dosing do not enhance antiviral activity (Smee et al., 1989). Initially there were 20 mice in each treated group and 40 mice in the placebo controls. Five sham-infected animals were treated with each drug dosage as toxicity controls, while uninfected untreated mice served as normal controls. The toxicity and normal control mice were maintained in an area remote from the infected mice. These were weighed prior to initial treatment and again 18 h after the last treatment. Four days after virus inocu-

lation, 10 infected animals from each treatment group and 20 placebo-treated control mice were killed, bled, and their livers removed. Hepatic icterus, characterized by discoloration of the liver, was assigned a score from 0 (normal) to 4 (maximal discoloration). These livers were frozen at −70°C and later assayed for virus titer in LLC-MK_2 cells as described previously (Sidwell et al., 1988b). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) determinations were made using colorimetric kits (Sigma Chemical Co., St. Louis, MO). Animals not killed on day 4 were held for 21 days after virus inoculation, and deaths were recorded daily.

Survivor increases were evaluated using chi square analysis with Yates' correction. The Student's *t*-test was used to analyze increases in mean survival times of animals that died before day 21, reductions in ALT, AST and reduced PTV titers in livers and sera. Liver score inhibition was compared using ranked sum analysis. In all cases, values of statistical significance were determined by comparing treated groups to respective placebo controls. The thresholds of statistical significance were $P<0.05$ and $P<0.01$, using two-tailed analyses.

Results

Toxicity of 7-thia-8-oxoguanosine in uninfected animals

Several doses of 7-thia-8-oxoguanosine from 100 to 700 mg/kg given one day only were evaluated for acute toxicity in mice (Table 1). Divided daily doses of ≥400 mg/kg caused death and/or severe weight loss. The pattern of death was somewhat erratic at these doses, probably because the compound was administered as a suspension. The maximum tolerated dose using this treatment regimen was 300 mg/kg.

TABLE 1

Acute toxicity of 7-thia-8-oxoguanosine in C57BL/6 mice

Dose[a] (mg/kg)	Survivors/total	Body weight change[b] (g)
0	5/5	+0.3
100	5/5	+0.2
200	5/5	0.0
300	5/5	+0.6
400	2/5	−1.5
500	5/5	−2.1
600	3/5	−1.7
700	4/5	−2.0

[a]Given as a divided dose 7 h apart.
[b]Determined 24 h after the last treatment. Values are relative to initial weights.

Prophylactic antiviral activity of 7-thia-8-oxoguanosine

Based upon previous studies against other types of viruses (Smee et al., 1989; Smee et al., 1990b) prophylactic treatments with the nucleoside were administered 24 and 17 h before virus challenge. Doses of 50 and 100 mg/kg/day were significantly effective in reducing mortality (Fig. 1A), whereas 12.5 and 25 mg/kg were inactive. In Fig. 1B are seen survival data for mice treated at −4 and +3 h relative to virus inoculation. Using this regimen, the compound was more effective, with doses of 25 to 100 mg/kg providing nearly complete protection from the otherwise lethal infection.

Other disease parameters for these groups of mice are presented in Table 2. For treatments starting at −24 h, liver icterus scores, ALT and AST values, and liver and serum virus titers were substantially reduced at 50 and 100 mg/kg. Likewise, these same parameters were reduced in mice treated with nucleoside starting at −4 h using doses as low as 25 mg/kg. Generally, doses significantly reducing these disease parameters were the same as those reducing mortality. Additional antiviral effects were observed on liver scores at 25 mg/kg (−24 and −17 h treatments) and 12.5 mg/kg (−4 and +3 h treatments), and on AST values and liver virus titers at 12.5 mg/kg (−4 and +3 h treatments) that did not correlate with increases in survival.

Therapeutic antiviral activity of 7-thia-8-oxoguanosine

Treatments were initiated after virus inoculation at +24 and +31 h. Doses from 12.5 to 100 mg/kg/day were highly effective in preventing death compared to placebo controls (Fig. 2A). Because of these encouraging results, another experiment

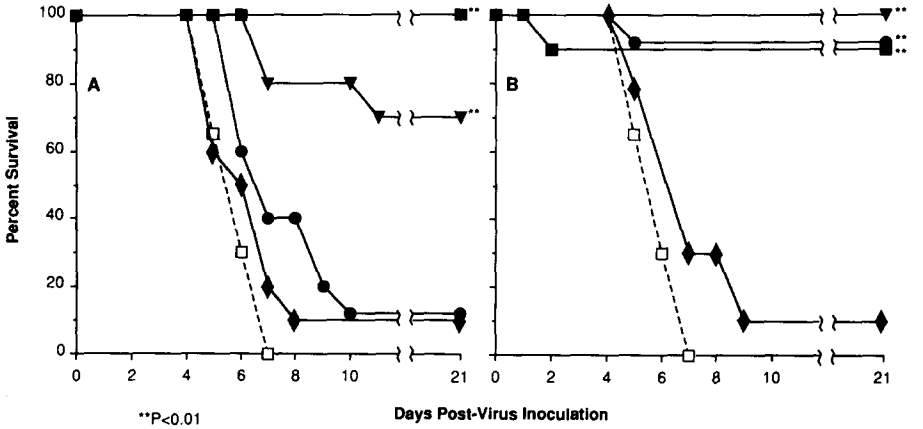

Fig. 1. Effect of divided prophylactic treatments with 7-thia-8-oxoguanosine on PTV-induced mortality in mice. A: treatments given 24 and 17 h before virus inoculation; B: treatments given 4 h before and 3 h after virus inoculation. Symbols: placebo (□); 7-thia-8-oxoguanosine at 12.5 (◆), 25 (●), 50 (▼), and 100 (■) mg/kg/day.

TABLE 2

Effect of prophylactic treatments with 7-thia-8-oxoguanosine on PTV infections in mice

Dosage (mg/kg/day)	Disease parameter[a]				
	Liver score[b] (mean ± SD)	ALT[c] (mean ± SD)	AST[d] (mean ± SD)	Mean liver virus titer (log_{10} ± SD)	Mean serum virus titer (log_{10} ± SD)
Treatments given 24 and 17 h before virus inoculation					
100	1.8 ± 0.8[2]	716 ± 792[2]	545 ± 487[2]	3.5 ± 1.7[2]	4.1 ± 1.7[2]
50	1.6 ± 1.3[2]	1708 ± 1404[2]	1630 ± 2112[2]	2.5 ± 2.7[2]	4.5 ± 1.6[2]
25	2.3 ± 1.7[1]	4888 ± 4224	6416 ± 5506	5.3 ± 2.4	6.0 ± 1.0
12.5	3.1 ± 1.7	7927 ± 3120	9376 ± 4632	5.7 ± 2.4	6.3 ± 0.7
0	3.5 ± 0.8	6534 ± 3738	8268 ± 5357	6.1 ± 1.6	6.2 ± 0.6
Treatments given 4 h before and 3 h after virus inoculation					
100	0.0 ± 0.0[2]	170 ± 108[2]	76 ± 58[2]	1.4 ± 2.3[2]	2.4 ± 2.8[2]
50	0.0 ± 0.0[2]	286 ± 199[2]	227 ± 327[2]	2.7 ± 1.9[2]	4.2 ± 2.3[2]
25	0.6 ± 1.3[2]	1166 ± 1327[2]	1036 ± 1046[2]	3.2 ± 2.3[2]	3.2 ± 2.8[2]
12.5	2.7 ± 1.4[1]	4262 ± 3279	3338 ± 2825[2]	4.7 ± 1.7[1]	5.6 ± 2.1
0[e]	3.5 ± 0.8	6534 ± 3738	8268 ± 5357	6.1 ± 1.6	6.2 ± 0.6
Normals	0.0 ± 0.0	225 ± 221	54 ± 29	0.0 ± 0.0	0.0 ± 0.0

[a]Determined 4 days after virus challenge.
[b]Score of 0 (normal liver) to 4 (maximum discoloration).
[c]Serum alanineaminotransferase activity (Sigma-Fraenkel units/ml).
[d]Serum aspartateaminotransferase activity (Sigma-Fraenkel units/ml).
[e]This is the same placebo control as was used for −24 and −17 h treatments, since the studies were run concurrently.
[1]$P<0.05$; [2]$P<0.01$.

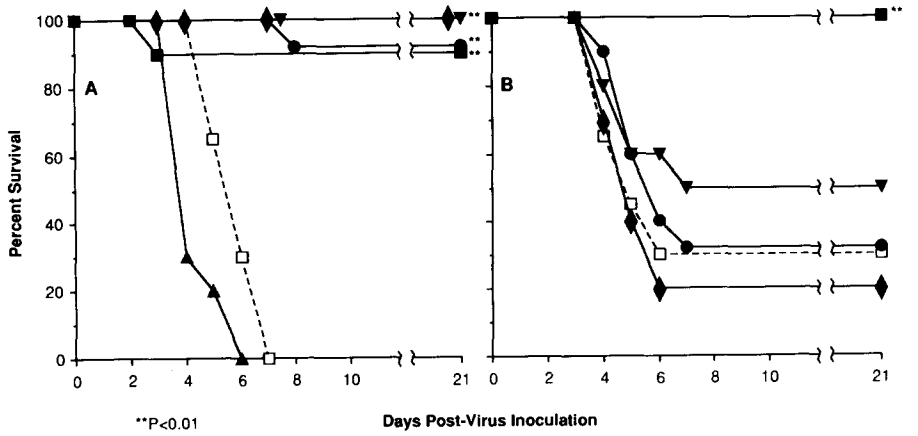

Fig. 2. Effect of divided therapeutic treatments with 7-thia-8-oxoguanosine on PTV-induced mortality in mice. A. treatments given 24 and 31 h after virus inoculation; B: treatments given 36 and 43 h after virus inoculation. Symbols: placebo (□); 7-thia-8-oxoguanosine at 6.25 (▲), 12.5 (◆), 25 (●), 50 (▼), and 100 (■) mg/kg/day.

TABLE 3

Effect of therapeutic treatments with 7-thia-8-oxoguanosine on PTV infections in mice

Dosage (mg/kg/day)	Disease parameter[a]				
	Liver score[b] (mean ± SD)	ALT[c] (mean ± SD)	AST[d] (mean ± SD)	Mean liver virus titer (log_{10} ± SD)	Mean serum virus titer (log_{10} ± SD)
Treatments given 24 and 31 h after virus inoculation					
100	0.4 ± 0.2[2]	174 ± 85[2]	71 ± 27[2]	1.6 ± 1.8[2]	1.9 ± 2.3[2]
50	0.1 ± 0.2[2]	127 ± 98[2]	46 ± 20[2]	0.8 ± 1.3[2]	0.0 ± 0.0[2]
25	1.1 ± 1.5[2]	1060 ± 1906[2]	1073 ± 2169[2]	1.0 ± 2.2[2]	1.5 ± 2.3[2]
12.5	2.4 ± 1.5[1]	3001 ± 2413	3114 ± 2929[2]	4.4 ± 1.8[1]	5.6 ± 2.0
6.25	3.1 ± 1.4	5675 ± 990	6125 ± 1818	6.0 ± 1.0	5.7 ± 1.3
0[e]	3.5 ± 0.8	6534 ± 3738	8268 ± 5357	6.1 ± 1.6	6.2 ± 0.6
Treatments given 36 and 43 h after virus inoculation					
100	1.4 ± 1.2[2]	397 ± 380[2]	336 ± 383[2]	1.1 ± 1.9[2]	1.9 ± 1.8[2]
50	3.0 ± 1.1	2188 ± 2179[1]	2084 ± 2202[1]	4.1 ± 2.4[1]	4.4 ± 1.7[1]
25	3.7 ± 0.2	4894 ± 2048	4598 ± 2154	6.1 ± 1.2	6.1 ± 0.5
12.5	3.9 ± 0.3	6433 ± 2695	6600 ± 3090	6.9 ± 0.6	6.5 ± 1.0
0	3.6 ± 0.5	4310 ± 2333	4053 ± 2683	5.8 ± 1.8	5.6 ± 1.3
Normals	0.0 ± 0.0	156 ± 74	27 ± 5	0.0 ± 0.0	0.0 ± 0.0

Legends are the same as in Table 1.
[1]$P<0.05$; [2]$P<0.01$.

was performed to determine if treatments could be given even later in the infection. Treatments administered at 36 and 43 h after virus challenge were also effective, but only at the 100 mg/kg dose (Fig. 2B). The overall infection in this latter experiment was somewhat less severe as 30% of the placebo controls lived, compared to 0% in the earlier study.

The effects of 7-thia-8-oxoguanosine on other disease parameters for these therapeutic treatment regimens are presented in Table 3. The inhibition of these parameters by the nucleoside again largely correlated with survival data. Doses of 25 to 100 mg/kg (+24 and +31 h treatments) markedly suppressed all disease parameters. At 12.5 mg/kg decreases in liver score, AST values and liver virus titers were statistically significant. The dose of 100 mg/kg (+36 and +43 h treatments) significantly reduced all disease parameters. At 50 mg/kg, all parameters but liver score were significantly reduced.

Effects of divided-daily versus once-only treatments on anti-PTV activity

It was previously reported that divided-daily doses of 7-thia-8-oxoguanosine were more effective than single doses against other types of virus infections (Smee et al., 1989; Smee et al., 1990b). An experiment was conducted to determine how effective the single treatment regimen would be against PTV infections. The following mortality results (survivors/total) were achieved by single daily treatments administered 24 h after virus challenge: 100 mg/kg (6/10), 50 mg/kg (10/10), 25

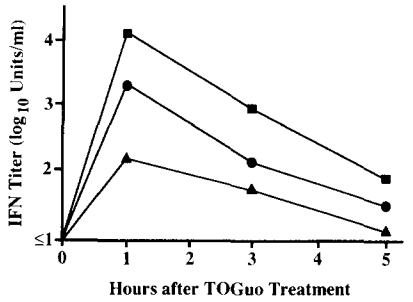

Fig. 3. IFN induction in C57BL/6 mice after single i.p. injections of 7-thia-8-oxoguanosine (TOGuo). Symbols: nucleoside at 25 (▲), 50 (●), and 100 (■) mg/kg. Five mice/group were used at each time point. Standard deviations ranged from 0.5 (lower values) to 0.9 (higher values).

mg/kg (9/10), 12.5 mg/kg (3/10) and placebo (5/20). This compares to twice daily treatments (Fig. 2A) where 90–100% survival was achieved by 12.5–100 mg/kg when all mice in the placebo control died. Significant reductions in liver scores, ALT and AST values, liver and serum virus titers were evident at 25, 50, and 100 mg/kg in the single treatment studies (data not shown). These combined results demonstrate that the divided daily treatment regimen was more effective at lower (12.5 mg/kg) and higher (100 mg/kg) doses than the single treatment regimen.

IFN induction and its role in antiviral activity

Previously we reported that 7-thia-8-oxoguanosine induced IFN in Swiss Webster mice (Smee et al., 1990c). To ascertain whether the cytokine is induced in C57BL/6 mice, animals were treated with the compound and their sera were collected for IFN analysis at varying times (Fig. 3). Doses of 25, 50 and 100 mg/kg given in single injections induced over 10^2, 10^3 and 10^4 log_{10} units of interferon per ml, 1 h after 7-thia-8-oxoguanosine administration. IFN levels declined steadily at 3 and 5 h.

TABLE 4

Effect of antibody to interferon α/β on the anti-PTV activity of 7-thia-8-oxoguanosine in mice

7-Thia-8-oxoguanosine[a] (mg/kg/day)	Anti-IFN α/β[b] (units/mouse)	Survivors/total (%)	Mean days to death
0	0	1/10	4.2 ± 1.4
25	0	8/10 (80)[1]	9.5 ± 2.1[1]
50	0	10/10 (100)[1]	>21
0	4000	0/7 (0)	3.3 ± 0.5
25	4000	0/10 (0)	4.3 ± 0.5
50	4000	0/10 (0)	3.6 ± 0.7

[a]Treatments were given 24 and 31 h after PTV inoculation.
[b]Mice received 2000 units of antibody 30 min after each treatment with 7-thia-8-oxoguanosine.
[1]$P<0.01$.

To determine a protective role of 7-thia-8-oxoguanosine-induced IFN in PTV infections, treated mice received antibody to IFN α/β (Table 4). Mice receiving 7-thia-8-oxoguanosine at 25 and 50 mg/kg had survival rates of 80 and 100%, whereas all animals receiving the nucleoside plus anti-IFN α/β died. These results indicate a major role of IFN in anti-PTV activity.

Discussion

7-Thia-8-oxoguanosine was effective against PTV infections whether administered prophylactically or therapeutically. Its efficacy was greatest when treatments were initiated 24 h after virus challenge, but it is impressive that treatments begun as late as 36 h were also effective, albeit only at the high dose. Previous studies have indicated that the animals are showing signs of the disease by 36 h (Sidwell et al., 1988b), and some of the mice are dead by 96 h. The minimum effective dose against PTV infections was about 25 mg/kg. The maximum tolerated dose in injected mice was 300 mg/kg, indicating a therapeutic index (maximum tolerated dose divided by minimum effective dose) of approximately 12.

As an antiviral agent, 7-thia-8-oxoguanosine was found to be most effective against Semliki Forest, banzi, and encephalomyocarditis viruses when administered in divided daily doses rather than in full-daily injections (Smee et al., 1989; Smee et al., 1990b). Such was also found to be the case against PTV infections. Single treatments of 100 mg/kg were less active than lower doses, suggesting that too much of the compound led to less immune activation. By dividing the dose, 7-thia-8-oxoguanosine showed good responses in doses as low as 12.5 mg/kg against PTV.

The mode of action of the compound against PTV was investigated and IFN induction appears to be the key factor for anti-PTV activity. The level of IFN induced in C57BL/6 mice and its persistence were similar to IFN induction in Swiss Webster mice (Smee et al., 1990c). Antibodies to IFN α/β negated the protective effect of 7-thia-8-oxoguanosine. In work performed with Semliki Forest virus, Smee et al. (1990c) established that the in vivo antiviral activity of the nucleoside could also be eliminated by treatment of mice with anti-interferon antibodies. In the same report natural killer cell induction by the nucleoside did not appear to be a significant protective factor. It is important to note that the IFN inducer bropirimine (Wierenga, 1985) is also active against Punta Toro virus infections in vivo (Sidwell et al., manuscript submitted), and Pifat and Smith (1987) have shown the murine PTV infection to be highly sensitive to IFN.

Although no studies were conducted using several days of treatment, previous work with 7-thia-8-oxoguanosine against other virus infections showed that daily treatments do not increase compound efficacy (Smee et al., 1989). This most likely relates to the fact that hyporesponsiveness occurs with IFN inducers following repeated daily administration (Giese and Kirchner, 1988; Stringfellow, 1977; Stringfellow et al., 1979). In addition, certain virus infections themselves have been shown to create a state of hyporesponsiveness to IFN inducers in the host

(Stringfellow et al., 1977). Some of these effects can be overcome by treatment of the animals with prostaglandins (Stringfellow, 1980). Since 7-thia-8-oxoguanosine was active 24–36 h after virus challenge, PTV may not induce the hyporesponsiveness state in mice as do some other viruses. This point will require further investigation.

7-Thia-8-oxoguanosine was previously evaluated against San Angelo bunyavirus (Smee et al., 1989; Smee et al., 1990b), which is in the same family of viruses as PTV. Thus, some comparisons of antiviral activity in the two virus models is warranted. The activity of the nucleoside against San Angelo virus was observed at doses as low as 5 mg/kg/day (given at −24 and −18 h). Under similar conditions, it was effective at 50 mg/kg against PTV. Efficacy against PTV was achieved at 12.5 mg/kg by starting treatments at +24 h, however. In another study it was shown that 7-thia-8-oxoguanosine had only moderate antiviral activity against San Angelo virus at 200 mg/kg (the only dose evaluated) when administered as late as 24 h after virus inoculation. The differences in these results may reflect the models themselves and where the viruses replicate. San Angelo virus replicates to high titers in mice, leading to encephalitis and death (Smee et al., 1989), whereas the Adames strain of PTV is primarily hepatotropic (Sidwell et al., 1988b). Infections caused by encephalitis viruses apparently are difficult to treat once virus has entered the brain (at an early time point after virus inoculation), as was determined by Stringfellow and colleagues (1974a,b) using two different IFN inducers against encephalomyocarditis (EMC) virus in mice. The effects of IFN and associated immunomodulation apparently do not affect the brain to the degree necessary to shut down virus replication there. In related work performed with EMC virus, the ability of 7-thia-8-oxoguanosine to delay death was correlated with suppression of virus replication in visceral organs and tissues, contributing to delays in virus replication in the brain (Smee et al., 1990a).

The results indicate that 7-thia-8-oxoguanosine is effective in the treatment of PTV infections in mice. Because of these results, the compound is a possible candidate for follow-up studies in models of related viruses such as Rift Valley fever virus. The role of this and other IFN inducing agents to treat serious bunyavirus infections deserves further exploration.

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References

Cash, P., Vezza, A.C., Gentsch, J.R. and Bishop, D.H.L. (1979) Genome complexities of the three mRNA species of snowshoe hare bunyavirus and in vivo translation of SmRNA to viral N polypeptide. J. Virol. 31, 685–694.

Giese, M. and Kirchner, H. (1988) Blocking of interferon synthesis in murine macrophages by pretreatment with interferon. Immunol. Lett. 18, 109–114.

Meegan, J.M., Watten, R.H. and Laughlin, L.W. (1981) Clinical experience with Rift Valley fever in humans during the 1977 Egyptian epizootic. Conf. Epidemiol. Biostat. 3,114–123.

Pifat, D.Y. and Smith, J.F. (1987) Punta Toro virus infection of C57BL/6J mice: a model for phlebovirus-induced disease. Microb. Pathog. 3, 409–422.

Sabin, A.B. (1948) Phlebotomus fever. In: T.M. Rivers (Ed.), Viral and Rickettsial Infections of Man, pp. 454–461. Lippincott Publishing Co., Philadelphia.

Sather, G.E. and Hammon, W.M. (1967) Antigenic patterns within the California-encephalitis-virus group. Am. J. Trop. Med. Hyg. 16, 548–557.

Sidwell, R.W. and Huffman, J.H. (1971) Use of disposable micro tissue culture plates for antiviral and interferon induction studies. Appl. Microbiol. 22, 797–801.

Sidwell, R.W., Huffman, J.H., Barnard, D.L. and Pifat, D.Y. (1988a) Effects of ribamidine, a 3-carboxamidine derivative of ribavirin, on experimentally induced *Phlebovirus* infections. Antiviral Res. 10, 193–208.

Sidwell, R.W., Huffman, J.H., Barnett, B.B. and Pifat, D.Y. (1988b) In vitro and in vivo *Phlebovirus* inhibition by ribavirin. Antimicrob. Agents Chemother. 32, 331–336.

Smee, D.F., Alaghamandan, H.A., Bartlett, M.L. and Robins, R.K. (1990a) Intranasal treatment of picornavirus and coronavirus infections in rodents using 7-thia-8-oxoguanosine. Antiviral Chem. Chemother. 1, 47–52.

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. (1990b) Antiviral activity of the novel immune modulator 7-thia-8-oxoguanosine. J. Biol. Response Mod. 13, 91–102.

Smee, D.F., Alaghamandan, H.A., Jin, A., Sharma, B.S. and Jolley, W.B. (1990c) 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. 13, 91–102.

Stringfellow, D.A. (1977) Comparative interferon-inducing and antiviral properties of 2-amino-5-bromo-6-methyl-4-pyrimidinol (U-25,166), tilorone hydrochloride, and polyinosinic-polycytidylic acid. Antimicrob. Agents Chemother. 11, 984–992.

Stringfellow, D.A. (1980) Antiviral and interferon-inducing properties of interferon inducers administered with prostaglandins. Antimicrob. Agents Chemother. 17, 455–460.

Stringfellow, D.A., Kern, E.R., Kelsey, D.K. and Glasgow, L.A. (1977) Suppressed response to interferon induction in mice infected with encephalomyocarditis virus, Semliki Forest virus, influenza A2 virus, *Herpesvirus hominis* type 2, or murine cytomegalovirus. J. Infect. Dis. 135, 540–551.

Stringfellow, D.A., Overall, J.C., Jr. and Glasgow, L.A. (1974a) Interferon inducers in therapy of infection with encephalomyocarditis virus in mice. I. Effect of single doses of polyriboinosinic-polyribocytidylic acid and tilorone hydrochloride on viral pathogenesis. J. Infect. Dis. 130, 470–480.

Stringfellow, D.A., Overall, J.C., Jr. and Glasgow, L.A. (1974b) Interferon inducers in therapy of infection with encephalomyocarditis virus in mice. II. Effect of multiple doses of polyriboinosinic-polyribocytidylic acid on viral pathogenesis. J. Infect. Dis. 130, 481–488.

Stringfellow, D.A., Weed, S.D. and Underwood, G.E. (1979) Antiviral and interferon-inducing properties of 1,5-diamino anthraquinones. Antimicrob. Agents Chemother. 15, 111–118.

Travassos da Rosa, A.P.A., Tesh, R.B., Pinheiro, F.P., Travassos da Rosa, J.F.S. and Peterson, N.E. (1983) Characterization of eight new phlebotomus fever serogroup arboviruses (Bunyaviridae: *Phlebovirus*) from the Amazon region of Brazil. Am. J. Trop. Med. Hyg. 2, 1164–1171.

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