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Roles of interferon and natural killer cells in the antiviral activity of 7-thia-8-oxoguanosine against Semliki Forest virus infections in mice

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Summary

7-Thia-8-oxoguanosine is a novel biological response modifier with broad-spectrum antiviral activity against many DNA and RNA viruses in vivo. Since two of its properties are to induce interferon and to activate natural killer (NK) cells, we investigated the roles of the lymphokine and NK cells in the antiviral activity of the compound against Semliki Forest virus. Antibody to interferon α/β could completely abolish the protective activity of the nucleoside against virus infection in mice, whereas antibodies to interferons β and γ could not, indicating that interferon α was of major importance to confer protection to the animals. Reduced activation of NK cells was also observed in mice treated with 7-thia-8-oxoguanosine and antibody to interferon α/β. The role of NK cells in the protective activity of the compound was directly assessed in beige mice or in Swiss Webster mice treated with asialo GM1 antibody. In both experiments, the animals were protected from lethal virus infection by treatment with nucleoside. Spleen cells primed by 7-thia-8-oxoguanosine and adoptively transferred to untreated mice could not save them from virus-induced mortality. These three results provide evidence that natural killer cells activated by 7-thia-8-oxoguanosine play a minimal role in protection from acute Semliki Forest virus infections in mice.

Nucleoside analog; Immune modulator; Guanosine analog

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Introduction

A novel nucleoside analog of guanosine, 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]-pyrimidine-2,7(3H,6H)-dione (7-thia-8-oxoguanosine), has broad-spectrum antiviral activity in vivo against many DNA and RNA viruses (Nagahara et al., 1990; Smee et al., 1989). This biological response modifier is particularly effective against Semliki Forest virus infections in mice (Smee et al., 1989). It is known to induce interferon and to activate natural killer cells (Nagahara et al., 1990). The compound is part of a class of guanosine nucleosides or heterocycles related to nucleosides that stimulate the immune system. This includes compounds such as 8-substituted (8-bromo and 8-mercapto) guanosines (Dorsch et al., 1988; Koo et al., 1988; Wicker et al., 1987), 7-methyl-8-oxoguanosine (Goodman and Hennen, 1986), and several 2-amino-pyrimidinones (Richard et al., 1987; Skulnick et al., 1985; Wicker et al., 1988). These compounds all exhibit similar properties of interferon induction and natural killer cell activation. Some of them also have been shown to activate B-cells (Wicker et al., 1987, 1988).

Interferon, when administered prophylactically to animals, is capable of mediating an antiviral effect against viruses (Stewart, 1979), and to stimulate the activation (Welsh, 1978) and proliferation (Biron et al., 1984) of natural killer cells. Natural killer cells have been shown to lyse a variety of virus-infected target cells (Welsh, 1986). In studies with Semliki Forest virus, infection leads to an activation of natural killer cell responses (Kaluza et al., 1987; MacFarlan et al., 1977). Mice depleted in natural killer cell activity by low dose tunicamycin treatment were rendered more sensitive to infections (Maheshwari et al., 1983). The interpretation of these results is questionable, however, in terms of the role of natural killer cells in resistance to Semliki Forest virus infection, since tunicamycin is a general glycosylation inhibitor that may affect many immune parameters.

In our effort to understand the antiviral mode of action of 7-thia-8-oxoguanosine, it was important to know the immunoenhancing properties of the compound that were most important to account for its antiviral activity. In this report, the roles that interferon and natural killer cells play in the protection against acute Semliki Forest virus infections were examined.

Materials and Methods

Compounds and reagents

7-Thia-8-oxoguanosine was synthesized at our institution by a published method (Nagahara et al., 1989). It was dissolved in 2% sodium bicarbonate solution (the compound was much less soluble in saline). Antibodies to interferons α/β, β, and γ were purchased from Lee Biomolecular, San Diego, CA. Asialo GM1 antibody was obtained from Wako Chemical Corp., Dallas, TX. Sodium chromate [^{51}Cr] was from ICN Radiochemicals, Irvine, CA.

Viruses and cells

Semliki Forest (Original strain) and vesicular stomatitis (VSV) (Indiana strain) viruses were obtained from the American Type Culture Collection (ATCC), Rockville, MD. African green monkey (VERO), mouse connective tissue (L929), and mouse lymphoma (YAC-1) cells were from ATCC. VERO and L929 cells were propagated in Eagle's medium supplemented with 10% fetal bovine serum (FBS), whereas YAC-1 cells were propagated in RPMI 1640 medium with 5% FBS. The viruses were passaged in VERO cells (Smee et al., 1987).

Interferon titrations and natural killer (NK) cell assays

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 VSV 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). Natural killer cell assays were performed using a ^{51}Cr-release assay (Kiessling et al., 1975). Briefly, spleens were removed from mice and single cell suspensions prepared in Hanks' balanced salt solution (HBSS). Erythrocytes were lysed with 0.83% ammonium chloride, and the cell suspensions were washed 2 × with HBSS. The cells were resuspended in RPMI 1640 containing 20 amino acids, 25 mM Hepes buffer, 10% FBS, 500 units of penicillin/ml, 50 μg streptomycin/ml, and 50 μM 2-mercaptoethanol. These cells were the effector cells. YAC-1 cells labeled with ^{51}Cr served as the target cells (Kiessling et al., 1975). In the experiment, ^{51}Cr-labeled YAC-1 target cells were placed into triplicate wells (10^4 cells/well) of 96-well round bottom microplates. Effector cells were added to wells to give effector:target ratios of 50:1, 100:1, and 150:1. The plates were gently shaken, centrifuged at 80 × *g* for 5 min, and incubated 4 h at 37°C. Then, the plates were centrifuged at 250 × *g* for 10 min, and half of the medium (100 μl) was counted in a gamma counter. Untreated controls consisted of target cells minus effector cells (spontaneous counts released) and cells treated with 1% Triton X-100 (maximum counts released). The percent cytotoxicity for each sample was the experimental counts minus spontaneous counts divided by maximum counts minus spontaneous counts times 100. The degree that 7-thia-8-oxoguanosine activated NK cells was determined by dividing the mean cytotoxicity from spleen cells of drug-treated mice by the mean from cells of placebo-treated animals at each effector to target ratio. Cytotoxicity differences between the placebo controls and 7-thia-8-oxoguanosine treated groups were statistically analyzed using the two-tailed Student's *t*-test.

Animal experiments and models

Swiss Webster female mice weighing about 20 g each at infection were purchased from Charles River Labs, Wilmington, MA. Beige (C57BL/6J-bg) female mice (15 g each) were from Jackson Labs, Bar Harbor, ME. The virus was pretitrated in the animals to identify the 50% lethal dose (LD_{50}). Each experiment was conducted using 10 LD_{50}/mouse. This dose of virus when inoculated by intraperitoneal (i.p.) injection generally killed 90–100% of placebo-treated mice, with some variation from experiment to experiment. The Semliki Forest virus animal model has been used by us (Smee et al., 1989) and others (Skulnick et al., 1985) for evaluation of biological response modifiers.

7-Thia-8-oxoguanosine was administered 0.2 ml/mouse i.p. starting 24 h before virus inoculation. This treatment regimen has been validated in our laboratory to be optimal against Semliki Forest virus, and was initially chosen based upon work done with the interferon inducer bropirimine (Skulnick et al., 1985). For each experiment there were initially 12 mice per group that were maintained to monitor survival, and 4 extra mice per group when spleens were evaluated for natural killer cell activity.

Asialo GM1 antibody, as obtained from the manufacturer, was administered undiluted at 50 μl per mouse. This amount was determined by pre-titration in mice to be 2-fold more potent than the dose causing maximal suppression of natural killer cell activity. For the adoptive cell transfer assay, uninfected mice were inoculated with 7-thia-8-oxoguanosine (150 mg/kg), and 24 h later their spleens were removed. The spleen cells were prepared as described above for the NK cell assay and $\geq 2.5 \times 10^7$ cells were injected into different (untreated) mice by intravenous (i.v.) and/or i.p. injection at either 4 or 24 h after virus inoculation.

All animal experiments ran for 21 days, at which time the mice were considered cured from the lethal phase of the infections. Statistical evaluations compared drug-treated groups to respective placebo controls. Increases in numbers of survivors were evaluated by the two-tailed Fisher exact test. Mean survival time increases were statistically analyzed by the two-tailed Mann-Whitney *U* test.

Results

Swiss Webster mice inoculated with 7-thia-8-oxoguanosine at 100 mg/kg in a single intraperitoneal injection were induced to synthesize interferon, which was first detectable in mouse serum at 1 h (100 units/ml). Between 2 and 3 h, 1000 to 3200 units were present in sera, and by 6 h interferon titers had declined to 32 units/ml or less. Higher doses of 7-thia-8-oxoguanosine (150–200 mg/kg) induced more interferon at 3 h (up to 10000 units/ml).

Along with the induction of interferon, 7-thia-8-oxoguanosine also induced the activation of natural killer (NK) cells when assayed 24 h after compound administration. In an experiment presented in Table 1, treatment of mice with the interferon inducer at 50 mg/kg activated NK cells by 2.5- to 3-fold compared to the

TABLE 1

Potentiation of natural killer cell activity[a] by 7-thia-8-oxoguanosine

Source of mouse spleen cells	Percent cytotoxicity		
	50:1[b]	100:1	150:1
Placebo-treated	4.5 ± 7.2[c]	7.7 ± 8.3	9.7 ± 8.3
50 mg/kg[d]-treated	13.4 ± 7.3	22.6 ± 10.0[e]	24.0 ± 13.8
100 mg/kg[d]-treated	26.0 ± 3.0[e]	33.4 ± 7.1[e]	43.0 ± 7.0[e]

[a]Determined by ^{51}Cr-release assays against YAC-1 target cells.
[b]Effector to target ratio.
[c]Mean ± standard deviation using 4 individual mice per group.
[d]Animals were inoculated with divided daily i.p. doses of 7-thia-8-oxoguanosine 24 and 18 h before harvesting the spleen cells.
[e]Statistically significant ($P < 0.05$), determined by two-tailed t-test.

untreated control. At 100 mg/kg the nucleoside caused a 4.4- to 5.7-fold activation. The degree of potentiation of NK cell activity was fairly consistent from experiment to experiment, although the percent cytotoxicity in the placebo controls varied from 1 to 10%.

Since interferon induction and NK cell activation are two aspects of the immunopotentiative properties of 7-thia-8-oxoguanosine, studies were conducted to determine the role of each in the anti-Semliki Forest virus activity of the nucleoside in vivo. Mice were treated with compound followed by administration of saline or antibodies to interferons (Table 2). The biological response modifier by itself or combined with anti-interferons β or γ protected the majority of mice from the otherwise lethal infection. However, many of the mice co-treated with anti-interferon α/β died. By comparison, the results indicate that protection was conferred by interferon α. In the same experiment, a group of mice was treated with

TABLE 2

Effects of antibodies to murine interferons on the activity of 7-thia-8-oxoguanosine against a lethal Semliki Forest virus infection in mice

Antiviral treatment[a]	Antibody[b] treatment	Survivors/total (%)	Mean day of death[c]
0	0	1/12 (8)	8.5 ± 2.2[d]
7-thia-8-oxoguo.	0	11/12 (92)[e]	10.0 ± 0.0
7-thia-8-oxoguo.	Anti-IFN α/β	5/12 (42)	8.9 ± 1.6
7-thia-8-oxoguo.	Anti-IFN β	10/12 (83)[e]	8.5 ± 2.1
7-thia-8-oxoguo.	Anti-IFN γ	11/12 (92)[e]	8.0 ± 0.0
Interferon α/β	0	9/12 (75)[e]	9.0 ± 2.6

[a]Half-daily intraperitoneal doses of 7-thia-8-oxoguanosine (100 mg/kg/day) or placebo were administered 24 and 18 hours before virus inoculation. Interferon α/β (10000 units per intraperitoneal injection) was given 24, 18, and 2 h pre-virus.
[b]Antibodies to interferons (1000 neutralizing units per injection) or saline were administered intraperitoneally 23, 17, and 2 h before virus challenge.
[c]Survivors lived through 21 days.
[d]Standard deviation.
[e]Statistically significant ($P < 0.005$), determined by the two-tailed Fisher exact test.

TABLE 3

Effects of antibody to interferon α/β on the activity of 7-thia-8-oxoguanosine against a lethal Semliki Forest virus infection in mice

7-Thia-8-oxoguanosine[a] dose (mg/kg)	Anti-IFN α/β Ab[b] (units)	Survivors total (%)	Mean day of death[c]
0	0	2/12 (17)	8.8 ± 1.9[d]
25	0	11/12 (92)[e]	9.0 ± 0.0
50	0	10/12 (83)[e]	9.5 ± 0.7
25	4000	0/12 (0)	7.8 ± 1.5
50	4000	0/12 (0)	8.1 ± 2.1

[a]Half-daily intraperitoneal doses of compound or placebo were administered at 24 and 18 h before virus inoculation.
[b]Half-daily intraperitoneal doses of antibody or saline were administered at 23.5 and 17.5 h prior to virus challenge.
[c]Survivors lived through 21 days.
[d]Standard deviation.
[e]Statistically significant difference ($P < 0.001$) between the 7-thia-8-oxoguanosine treated groups compared to respective groups receiving the antibody, determined by the two-tailed Fisher exact test.

interferon α/β, which protected the majority of mice from death.

In the study above, the anti-interferon α/β antibody was only partially effective to neutralize the antiviral activity of 7-thia-8-oxoguanosine. This could have occurred because the quantity of antibody was not sufficient to neutralize all of the interferon produced, or possibly because other factors besides interferon accounted for part of the antiviral effect. We hypothesized that the treatment regimen was insufficient to neutralize all of the interferon, so modified the treatment

TABLE 4

Effect of anti-interferon α/β antibody on the potentiation of natural killer cell activity[a] by 7-thia-8-oxoguanosine

7-Thia-8-oxoguanosine[b] dose (mg/kg)	Anti-IFN α/β Ab[c] (units)	Percent cytotoxicity		
		50:1[d]	100:1	150:1
0	0	2.7 ± 1.7[e]	3.6 ± 2.1	6.0 ± 2.3
0	4000	<0.1	0.5 ± 1.4	1.8 ± 0.4
50	0	13.4 ± 7.3[f]	22.6 ± 10.0[f]	24.0 ± 13.8[f]
50	4000	6.1 ± 0.2[g]	11.7 ± 2.0[g]	12.8 ± 3.5[g]

[a]Determined by ^{51}Cr release assays against YAC-1 target cells using spleen (effector) cells.
[b]Animals were inoculated with half-daily intraperitoneal doses of 7-thia-8-oxoguanosine 24 and 18 h before removal of spleens.
[c]Half-daily intraperitoneal doses of antibody or saline were administered 23.5 and 17.5 h prior to virus inoculation.
[d]Effector to target ratio.
[e]Mean ± standard deviation using 4 individual mice per group.
[f]Statistically significant difference ($P < 0.05$) between this group and the placebo control, determined by two-tailed *t*-test.
[g]Statistically significant difference ($P < 0.001$) between this group and the placebo control that received antibody to interferon α/β, determined by two-tailed *t*-test.

TABLE 5

Effects of 7-thia-8-oxoguanosine in beige (natural killer cell-deficient) mice infected with Semliki Forest virus

7-Thia-8-oxoguanosine[a] dose (mg/kg)	Survivors/total (%)	Mean day of death[b]
0	1/12 (8)	8.3 ± 4.1[c]
50	7/12 (58)[d]	10.0 ± 3.7
100	8/12 (66)[d]	12.3 ± 5.6
200	8/9[e] (89)[d]	7.0 ± 0.0

[a]Single intraperitoneal treatments with compound or placebo were given 24 h before virus inoculation.
[b]Survivors lived through 21 days.
[c]Standard deviation.
[d]Statistically significant ($P < 0.05$), determined by the two-tailed Fisher exact test.
[e]Three mice died of apparent drug toxicity one day after treatment (before virus inoculation) and were eliminated from the results.

regimen by decreasing the amount of interferon induced (by reducing the dose of 7-thia-8-oxoguanosine) and by slightly increasing the anti-interferon α/β antibody. The results in Table 3 show that 4000 units of anti-interferon α/β will completely neutralize the antiviral activity of 25–50 mg/kg of 7-thia-8-oxoguanosine.

An experiment conducted in parallel with that above evaluated the extent of NK cell activation in mice receiving both 7-thia-8-oxoguanosine and anti-interferon α/β. Table 4 shows enhanced NK cell activity with nucleoside treatment, and decreased activation in cells from mice receiving the biological response modifier and anti-interferon α/β. Antibody to interferon also decreased the NK activity of spleen cells from mice which were not treated with 7-thia-8-oxoguanosine.

TABLE 6

Effects of 7-thia-8-oxoguanosine on a Semliki Forest virus infection in normal Swiss Webster mice and in mice treated with Asialo GM1 (natural killer cell-destroying) antibody

7-Thia-8-oxoguanosine[a] dose (mg/kg)	Antibody[b] treatment	Survivors/total (%)	Mean day of death[c]
0	−	2/12 (17)	6.6 ± 1.3[d]
0	+	4/12 (25)	7.0 ± 2.5
150	−	10/12 (83)[e]	8.0 ± 0.0
150	+	11/12 (92)[f]	8.0 ± 0.0

[a]Single intraperitoneal treatments with compound or placebo were administered 24 h prior to virus inoculation.
[b]Asialo GM1 antibody or saline were administered by intraperitoneal injection 22 h before virus challenge.
[c]Survivors lived through 21 days.
[d]Standard deviation.
[e]Statistically significant difference ($P < 0.01$) between this group and the placebo control, determined by the two-tailed Fisher exact test.
[f]Statistically significant difference ($P < 0.05$) between this group and the placebo control that received asialo GM1 antibody, determined by the two-tailed Fisher exact test.

The previous results showed that interferon α played a major role in the antiviral activity of 7-thia-8-oxoguanosine. Since NK cell activation is linked to interferon induction (Welsh, 1978), we wanted to determine if these cells by themselves exerted any protective effect against the virus infection. The first experiment to help demonstrate this was to determine the antiviral activity of 7-thia-8-oxoguanosine in beige (NK cell-deficient) (Shellam et al., 1981) mice (Table 5). The compound was administered in 3 doses given as single intraperitoneal injections. Each dose provided substantial protection to the animals.

A second experiment was conducted using normal Swiss Webster mice treated with asialo GM1 (NK cell-destroying; Habu et al., 1981) antibody. The amount of antibody administered was sufficient to reduce NK cell activity in mice treated with 100–200 mg 7-thia-8-oxoguanosine per kg to <1% cytotoxicity. Mice receiving the nucleoside were protected to nearly the same extent whether or not they received the antibody (Table 6).

In a final experiment, infected mice received spleen cells (the source of NK cells) which had been activated 24 h earlier in vivo by 7-thia-8-oxoguanosine. Mice receiving 50 million spleen cells i.p. and 25 million cells i.v. 4 h after virus challenge were not protected from a lethal Semliki Forest virus infection. Other mice receiving 50 million spleen cells i.p. 24 h after virus inoculation also died from infection. Mean survival times of the treated mice were not extended relative to placebo control animals. The results of this experiment and the two above it indicate that natural killer cells do not exert much of a protective effect in Semliki Forest virus-infected mice.

Discussion

It was evident from these studies that interferon α induced by 7-thia-8-oxoguanosine was the major, if not the sole factor responsible for prophylactic antiviral activity against acute Semliki Forest virus infections in mice. Since the antibody used to neutralize interferon activity was a mixture of anti-α and anti-β, we cannot completely rule out the possibility that some interferon β was induced by the nucleoside. The results in Table 2 suggest that interferon β was not involved in disease prevention, although we did not attempt to give higher doses of anti-interferon β to further substantiate this point. It is very unlikely that interferon γ was induced to any extent because 7-thia-8-oxoguanosine has no direct T-cell activating properties, and only causes a moderate activation of adjuvant-stimulated T-cells (B.S. Sharma et al., unpublished data). As a positive control, exogenously administered interferon α/β was shown to protect mice from the lethal virus infection.

Interferon is induced in mice infected with Semliki Forest virus, and some authors feel it may provide an early defense resulting in a delay to onset of death (Kaluza et al., 1987). Thus, it is possible that treatment with antibody to interferon in the placebo control groups (Tables 2 and 3) may have worsened the infection in these animals. We did not run placebo controls treated with interferon

antibody to investigate this possibility. Even though Table 3 shows that mice treated with 7-thia-8-oxoguanosine and anti-interferon α/β died somewhat earlier than placebo-treated animals, these differences were not statistically significant and cannot support the hypothesis of disease enhancement. Also, in none of the animal experiments were the mean survival times significantly extended in dying mice treated with 7-thia-8-oxoguanosine. This suggests to us that interferon induction ultimately provides an all or none type of protection in this model. The mean survival time results compare favorably with our initial published studies of the compound against Semliki Forest virus in mice (Smee et al., 1989). The same report shows other virus infection models where treatment with this interferon inducer did delay time to death. Separate detailed studies will therefore be necessary to determine if antibodies to interferon exacerbate Semliki Forest virus infections in mice.

Several experiments demonstrated that natural killer cells by themselves had a minimal, if any, role to modify the course of the disease. The most convincing experiment was that done in normal Swiss Webster mice treated with NK cell destroying asialo GM1 antibody (Table 6), where the activity of 7-thia-8-oxoguanosine was unchanged relative to its effects in NK-competent mice. The antibody is not completely selective for NK cells, however, since the ganglioside asialo GM1 is also a cell surface marker on macrophages and polymorphonuclear leukocytes (Herberman and Ortaldo, 1981). This fact does not negate the results because the protection elicited by 7-thia-8-oxoguanosine was still evident even though more than one type of immune cell may have been killed by antibody treatment. As a side issue, it may be important to mention unpublished results from our laboratory which showed that the nucleoside was also effective against Semliki Forest virus infections in mice pretreated with fumed silica (which inactivates macrophages; Allison et al., 1966).

The data of the beige mice experiment are interesting but by themselves are not conclusive to negate the role of NK cells in resistance to infection, since NK cell activity in these mice is not completely deficient. For example, Shellam et al. (1981) showed that NK cell activity in beige mice infected with murine cytomegalovirus was enhanced within 2–3 days after virus inoculation.

Results of the adoptive transfer assay are suggestive but not definitive that NK cells were minimally involved in protection from the acute virus infection. Although no apparent benefits were achieved under the treatment conditions employed, perhaps more positive results would have been obtained by inoculating larger amounts of activated spleen cells or by using different treatment regimens. It is significant that the adoptive transfer regimens employed here were very effective to block tumor metastasis in a B16 melanoma model in mice (B.S. Sharma et al., unpublished data), which is consistent with the anti-tumor properties of NK cells (Herberman and Ortaldo, 1981).

Natural killer cells are known to be induced following infection of mice with Semliki Forest virus (MacFarlan et al., 1977). Their role in infection has not been well studied, however (Welsh, 1986). The present experiments shed some light on this issue. These results also extend to observations made by Bukowski et al. (1987)

who showed that natural killer cells are not required for interferon-mediated prophylaxis against vaccinia virus. But in the same article the authors established that NK cells play a role in resistance to murine cytomegalovirus, by acting additively or possibly synergistically with interferon to reduce virus replication in vivo. In an extensive literature review covering most groups of animal viruses, Welsh (1986) points out that NK cells play a defensive role against some but not all virus infections.

It is not certain whether 7-thia-8-oxoguanosine caused some degree of direct activation of NK cells or whether all of the observed activation was achieved indirectly via interferon induction. The results in Table 4 show that part of the NK cell activation came from interferon, since the antibody to interferon caused a decrease in the extent of NK activation. But some NK cell activity above baseline was still present in these cultures. Was this because enough interferon escaped neutralization by the antibody to cause NK cell activation, or did 7-thia-8-oxoguanosine by itself activate these cells? In unpublished studies, we were unable to demonstrate a blockage of NK cell activation in vitro with interferon antibodies in 7-thia-8-oxoguanosine treated cultures, suggesting that the nucleoside can directly activate the cells. The induction of interferon would help to further augment chemical-induced NK cell activation in vivo. Koo et al. (1988) showed results similar to these using 8-bromoguanosine.

Regarding the toxicity of 7-thia-8-oxoguanosine in mice, single i.p. treatments sporadically kill mice at 200 mg/kg, such as occurred in Table 5. These mice usually are found dead 1 day after treatment at the time of virus inoculation, or sometimes a day later. We are inclined to think, that death occurs due to an overstimulation of the immune system. The problem can be circumvented by treating mice with half-daily doses of compound (Smee et al., 1989). Unfortunately some of the studies in this report were conducted before this toxicity problem and its solution were fully recognized.

From what is known about the use of 7-thia-8-oxoguanosine in the treatment of virus infections, prophylaxis or very early therapy (within 4 to 24 hours after virus challenge, depending upon the virus) is important to achieve efficacy (Smee et al., 1989). This is consistent with the behavior of interferon in animal infection models (Stewart, 1979). Whatever benefit is derived from such treatment generally cannot be improved upon by repeated administration of the nucleoside, most likely due to the hyporesponsiveness phenomenon of interferon induction by chemical inducers (Giese and Kirchner, 1988; Wierenga, 1985).

The present studies helped to elucidate the role of interferon in the antiviral activity of 7-thia-8-oxoguanosine against Semliki Forest virus, but these results cannot be generalized to other unrelated viruses which are also inhibited by in vivo treatment (Smee et al., 1989). In these cases we may need to investigate other immune functions besides interferon induction to understand the mode of action of this novel biological response modifier.

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