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Short Communication

Broad-spectrum activity of 8-chloro-7-deazaguanosine against RNA virus infections in mice and rats

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Abstract

A novel nucleoside analog, 8-chloro-7-deazaguanosine (8-Cl-7-dzGuo), was evaluated for anti-RNA virus activity in rodents in parallel with the related compound 7-deaza-7-thia-8-oxoguanosine (7-dzTOGuo). Half-daily intraperitoneal (i.p.) doses of each substance administered 24 and 18 h prior to i.p. virus challenge protected the majority of mice infected with banzi, encephalomyocarditis, San Angelo, and Semliki Forest viruses at doses of 25, 50 and 100 mg/kg/day. These compounds at 100 mg/kg/day also protected most suckling rats infected intranasally with rat coronavirus. However, no survival benefit was afforded to treated mice infected intranasally with vesicular stomatitis virus. 8-Cl-7-dzguo was orally active against Semliki Forest virus in mice at 200 and 400 mg/kg/day, whereas 7-dzTOGuo is reported to not be effective orally. In uninfected mice, the two compounds induced similar amounts of interferon following i.p. injections. Interferon was induced by oral treatments with 8-Cl-7-dzGuo but not with 7-dzTOGuo. Fifty percent acute lethal doses to uninfected mice treated i.p. in half-daily doses for one day with 7-deazaguanosine (7-dzGuo), 7-dzTOGuo, and 8-Cl-7-dzGuo were 400, 600 and > 1600 (no mortality at this dose) mg/kg/day, respectively. Daily, i.p. treatments for 14 days with these substances (100 mg/kg/day) showed 7-dzGuo as 100% lethal and the other

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two substances as not toxic. By virtue of reduced toxicity and oral bioavailability, 8-Cl-7-dzGuo appears to have the greatest clinical potential as an interferon-inducing antiviral agent.

Keywords: Antiviral;7-Deazaguanosine;Alphavirus; Bunyavirus;Flavivirus;Picornavirus;Coronavirus; Interferon inducer

Nucleoside biological response modifiers have been synthesized which inhibit many RNA and some DNA viruses in mice and rats (Smee et al., 1992). These compounds, 7- and/or 8-substituted guanosine analogues, are active by virtue of interferon induction, although other immune components such as B-cells, natural killer cells, and macrophages are also stimulated (Smee et al., 1992). Recently, we showed that the ribosyl moiety can be replaced with various alkyl side chains; many of the resulting compounds exhibited antiviral activity in mice (Michael et al., 1993). 7-deaza-7-thia-8-oxoguanosine (7-dzTOGuo) (Fig. 1) was one of the first of the interferon-inducing nucleosides to be investigated for antiviral activity (Nagahara et al., 1990; Smee et al., 1989, 1990a). It was shown to be active by intraperitoneal (i.p.) but not by oral administration. Subsequently, 7-deazaguanosine (7-dzGuo) was found to be active by both i.p. and oral routes (Smee et al., 1991a) when given for a short (one to two days) duration. However, daily dosing with 7-dzGuo for a week was fatal to mice. In a continuing search for orally active compounds with minimal toxicity, 8-chloro-7-deazaguanosine (8-Cl-7-dzGuo) (Fig. 1) was synthesized. This report describes the antiviral activity of 8-Cl-7-dzGuo compared to 7-dzTOGuo against several virus infections in vivo, and includes data of its oral activity in Semliki Forest virus-infected mice. The abilities of 8-Cl-7-dzGuo and 7-dzTOGuo to induce interferon are presented, and the toxicities of 8-Cl-7-dzGuo, 7-dzTOGuo, and 7-dzGuo are compared.

8-Cl-7-dzGuo was synthesized from 7-dzGuo (Ramasamy et al., 1988) by the following procedure. To a stirred solution of the 2′,3′,5′-tri-O-acetyl derivative of 7-dzGuo (11.8 g, 30 mmol) in dry dimethylformamide (DMF) (200 ml) was added *N*-chlorosuccinimide (4.02 g, 30 mmol) in DMF (50 ml) during a 0.5-h period. The mixture was stirred at room temperature for 15 h and evaporated to dryness. The residue was purified on a silica gel column (3 × 40 cm) using a CH_2Cl_2 to acetone gradient. The fractions containing 2′,3′,5′-tri-O-acetyl-8-chloro-7-dzGuo eluted first and were pooled, evaporated to dryness, and the residue crystallized from a mixture of CH_2Cl_2/hexane to yield 10.0 g (78.5%) of the product. A product isolated from subsequent fractions as foam was identified as 2′,3′,5′-tri-O-acetyl-7,8-dichloro-7-dzGuo in a yield

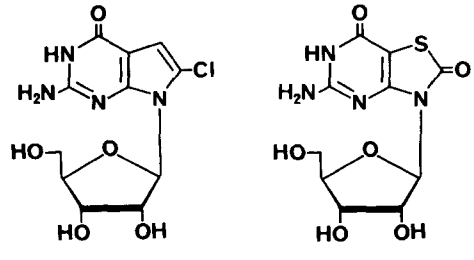

Fig. 1. Structures of 8-chloro-7-deazaguanosine (left) and 7-deaza-7-thia-8-oxoguanosine (right).

of 0.50 g (4%). To prepare 8-Cl-7-dzGuo, a solution of 2′,3′,5′-tri-O-acetyl-8-chloro-7-dzGuo (0.88 g, 2 mmol) in methanol/ammonia (100 ml, saturated at 0°C) was stirred at room temperature for 15 h in a pressure bottle. The reaction mixture was cooled to 0°C, the bottle was opened and the solvent evaporated to dryness. The residue was purified on a silica gel column (3 × 15 cm) using a CH_2Cl_2 to methanol gradient. The homogeneous fractions were pooled and evaporated to dryness. The residue was crystallized from a mixture of acetone/methanol to yield 0.60 g (95% yield). The overall yield for the entire reaction scheme was 80.5%. The purity of 8-Cl-7-dzGuo was > 99%. Nuclear magnetic resonance spectroscopy was used to identify and characterize each of the products above. 7-dzGuo and 7-dzTOGuo were synthesized using the published methods (Ramasamy et al., 1988; Nagahara et al., 1990) and were > 99% pure. The compounds were dissolved in 2% sodium bicarbonate (pH 8.6–8.9) to achieve aqueous solubility. Bicarbonate served as the placebo control for all animal experiments.

Banzi virus (H336 strain) (a flavivirus), encephalomyocarditis virus (EMC strain) (a picornavirus), San Angelo virus (a bunyavirus), Semliki Forest virus (original strain) (a togavirus), rat coronavirus (8190 strain), and vesicular stomatitis virus (Indiana strain) (a rhabdovirus) were obtained from the American Type Culture Collection, Rockville, MD. Swiss Webster female mice (18–20 g) and pregnant Lewis rats were from Charles River Labs, Wilmington, MA. These RNA viruses were chosen because 7-dzTOGuo and 7-dzGuo are active against most of these viruses (Smee et al., 1989, 1990a, 1991a). The methods for infecting the mice or suckling rats with these viruses was reported previously (Smee et al., 1989, 1990a, b). Briefly, intraperitoneal infections with banzi, encephalomyocarditis, San Angelo, and Semliki Forest viruses were made. Infections with rat coronavirus and vesicular stomatitis virus were done by intranasal route. Approximately ten 50% lethal doses (10 LD50) were given to the animals. The pre-treatment regimen employed was that found to be most effective for 7-dzTOGuo and related compounds (Smee et al., 1992). Increases in survivor numbers were evaluated by the two-tailed Fisher exact test. Mean survival time increases were statistically analyzed by the two-tailed Mann–Whitney U test. This test is not able to evaluate results when group sizes (animal that died) are fewer than three.

Interferon induction studies and toxicity determinations were conducted in uninfected mice. Serum collected from mice at 1, 3, and 5 hours after i.p. treatments with compounds was assayed for interferon on L929 cells infected 24 hours later with vesicular stomatitis virus, as described previously (Smee et al., 1991b). Four mice per group were used and their sera assayed separately for interferon content. Acute (1 day of treatment) and chronic (14 days of treatment) lethal toxicity studies were performed using 4 and 10 i.p. treated mice per group, respectively.

The results of i.p. pre-treatments with 8-Cl-7-dzGuo and 7-dzTOGuo on survival of mice or rats infected with various RNA viruses are presented in Table 1. Within the limits of biological variability, both compounds were approximately of equal potency against the different viruses. The active dosage ranges of the two compounds were similar to those reported previously for 7-dzTOGuo (Smee et al., 1989, 1990a) and 7-dzGuo (Smee et al., 1991a). 8-Cl-7-dzGuo and 7-dzTOGuo were unable to prevent mortality or delay the time to death in mice infected with vesicular stomatitis virus (data not shown), which was previously reported for 7-dzTOGuo (Smee et al., 1992).

Table 1
Antiviral activities of 8-Cl-7-dzGuo and 7-dzTOGuo against several RNA virus infections in mice and rats

Compound	Dose [a] (mg/kg/day)	Survivors/ Total (%)	Mean days to death [b]
Banzi virus infection in mice			
Placebo	–	1/12 (8)	7.7 ± 1.8 [c]
8-Cl-7-dzGuo	25	11/12 (92) **	8.0 ± 0.0
	50	12/12 (100) **	> 21
	100	12/12 (100) **	> 21
7-dzTOGuo	25	7/12 (58) *	12.4 ± 3.6 **
	50	11/12 (92) **	9.0 ± 0.0 **
	100	9/12 (75) **	8.7 ± 0.6
Encephalomyocarditis virus infection in mice			
Placebo	–	1/12 (8)	4.0 ± 0.8
8-Cl-7-dzGuo	25	12/12 (100) **	> 21
	50	11/12 (92) **	4.0 ± 0.0
	100	12/12 (100) **	> 21
7-dzTOGuo	25	10/12 (83) **	8.0 ± 4.2
	50	11/12 (92) **	8.0 ± 0.0
	100	10/12 (83) **	8.0 ± 2.8
San Angelo virus infection in mice			
Placebo	–	2/11 (18)	7.2 ± 1.6
8-Cl-7-dzGuo	10	7/12 (58)	8.0 ± 1.0
	25	12/12 (100) **	> 21
	50	12/12 (100) **	> 21
7-dzTOGuo	10	7/12 (58)	7.4 ± 1.7
	25	10/12 (83) **	11.0 ± 4.2
	50	12/12 (100) **	> 21
Semliki Forest virus infection in mice			
Placebo	–	2/12 (17)	6.2 ± 1.5
8-Cl-7-dzGuo	10	9/12 (75) *	11.3 ± 3.2 **
	25	8/12 (67) *	8.8 ± 1.0 **
	50	12/12 (100) **	> 21
7-dzTOGuo	10	4/12 (33)	7.6 ± 2.7
	25	7/12 (58)	9.2 ± 2.3 **
	50	9/12 (75) *	9.3 ± 3.1 *
Rat coronavirus infection in rats			
Placebo	–	1/11 (9)	7.5 ± 2.5
8-Cl-7-dzGuo	100	11/12 (92) **	7.0 ± 0.0
7-dzTOGuo	100	10/12 (83) **	8.0 ± 0.0

[a] Half-daily intraperitoneal doses were administered 24 and 18 h before virus inoculation.
[b] Of mice dying before 21 days.
[c] Standard deviation.
* $P < 0.05$, ** $P < 0.01$.

A follow-up experiment was conducted in which Semliki Forest virus-infected mice were treated with 8-Cl-7-dzGuo in order to compare i.p. to compare i.p. to oral (by gavage) treatment regimens. 7-dzTOGuo was not evaluated since it is inactive by oral

Table 2
Antiviral activity of 8-Cl-7-dzGuo against a Semliki Forest virus infection in mice

Dose [a] (mg/kg/day)	Route	Survivors/ total (%)	Mean days to death [b]
Placebo	i.p.	1/12 (8)	6.5 ± 1.3 [c]
5	i.p.	2/12 (17)	6.2 ± 1.4
10	i.p.	4/12 (25)	7.3 ± 1.8
25	i.p.	11/12 (92) * *	6.0 ± 0.0
50	i.p.	12/12 (100) * *	> 21
100	i.p.	11/12 (92) * *	7.0 ± 0.0
200	oral	7/12 (58) *	6.2 ± 2.2
400	oral	9/12 (75) * *	6.0 ± 0.0

[a] Half-daily intraperitoneal doses were administered 24 and 18 h before virus inoculation.
[b] Of mice dying before 21 days.
[c] Standard deviation.
* $P < 0.05$, * * $P < 0.01$.

administration (Smee et al., 1989, 1990a). Significant protection was achieved with 8-Cl-7-dzGuo doses of 25, 50 and 100 mg/kg/day by i.p. route, and to a lesser extent at 200 and 400 mg/kg/day by oral administration. The degree of oral antiviral potency of 8-Cl-7-dzGuo is similar to that reported for 7-dzGuo (Smee et al., 1991a).

Only certain kinds of viruses are susceptible to treatment with interferon-inducing agents in these rodent models (Smee et al., 1992). The profile of inhibition of the RNA viruses reported here is in agreement with the former studies. 8-Cl-7-dzGuo is as active as 7-dzGuo against banzi, San Angelo, Semliki Forest, and rat coronaviruses, but was more effective than 7-dzGuo against encephalomyocarditis virus (comparing the present results with those of Smee et al., 1991a). 8-Cl-7-dzGuo and 7-dzTOGuo appeared to be very similar in terms of potency and efficacy against the RNA viruses tested when given by i.p. route.

Since previous work has shown that interferon induction is important in the antiviral activities of these types of nucleoside analogues (Smee et al., 1990c, 1991a, b), the abilities of 8-Cl-7-dzGuo and 7-dzTOGuo to induce interferon were assessed in parallel. The results for 7-dzTOGuo (50 mg/kg i.p.) compared favorably with those previously reported (Smee et al., 1991b), indicating $10^{3.9}$, $10^{3.5}$, and $10^{2.4}$ log10 interferon units per ml were present in serum at 1, 3, and 5 h, respectively. Similarly, serum from 8-Cl-7-dzGuo treated mice (50 mg/kg i.p.) contained $10^{3.4}$, $10^{2.7}$, and $10^{2.2}$ units of interferon at 1, 3, 5 h, respectively. Based upon the biological variation, there was no significant difference between the amounts of interferon induced by the two nucleosides. After oral treatments with 8-Cl-7-dzGuo, (200 mg/kg) interferon at $< 10^{1.0}$, $10^{2.0}$, and $10^{2.3}$ units/ml was detected in serum at 1, 3, and 5 h, respectively. No interferon was detected in serum following oral treatments with 7-dzTOGuo. This may explain why 7-dzTOGuo exhibits no oral antiviral activity in mice.

The acute and chronic toxicities of 8-Cl-7-dzGuo, 7-dzTOGuo, 7-dzGuo were compared in uninfected mice. Animals treated i.p. in half-daily doses for one day with high doses of compounds were adversely affected by 7-dzGuo and 7-dzTOGuo, with

50% lethal doses of 400 and 600 mg/kg/day, respectively. The afflicted mice died 2 to 5 days after treatment. In contrast, no mortality was observed in mice treated with $\leqslant$ 1600 mg of 8-Cl-7-dzGuo per kg/day. Animals given the high (1600 mg/kg/day) lost an average of 0.7 g within a two-day period after treatment compared to an average weight gain of 0.6 g in the placebo group, suggesting some adverse effects. Chronic 100 mg/kg/day treatments once daily for 14 days resulted in 100% mortality (7-dzGuo) compared to no mortality (7-dzTOGuo and 8-Cl-7-dzGuo). The 7-dzGuo treated mice began dying after one week of treatment. These results indicate that 8-Cl-7-dzGuo is less toxic to mice than 7-dzGuo and 7-dzTOGuo.

Evidence from unpublished studies conducted at our institution suggests that substitutions introduced at the 8-position of 7-dzGuo reduce the ability of the compound to undergo phosphorylation, and this reduces the potential for toxicity. The phosphorylated forms of nucleosides (mono-, di-, or triphosphates) generally are responsible for intracellular toxicity due to their interaction with cellular enzymes.

The mode of antiviral action of 8-Cl-7-dzGuo is likely to be through interferon induction, since interferon was induced in treated mice. By using anti-interferon serum, we previously were able to abolish the antiviral activity of 7-dzTOGuo against two unrelated RNA viruses in mice (Smee et al., 1990c, 1991b). Others have also demonstrated the importance of chemically-induced interferon in the protection of virus-infected animals (Kunder et al., 1993; Morahan et al., 1991; Sarzotti et al., 1989). Both 7-dzTOGuo and 8-Cl-7-dzGuo also markedly stimulate murine B-cell blastogenesis (Nagahara et al., 1990; manuscript submitted), but this effect probably does not contribute to antiviral activity.

Results of studies performed with 7-dzTOGuo and 7-dzGuo indicate that these types of compounds generally have to be given very early in the course of the infection in order to exert an antiviral effect (Smee et al., 1989; Smee et al., 1991a). An exception to this is Punta Toro virus infection, which could be treated as late as 36 h after virus challenge and still be curable (Smee et al., 1991b). Stringfellow et al. (1977) determined that many virus infections suppress the ability of mice to produce interferon in response to chemical inducers when the inducers are administered several hours after virus inoculation. For this reason, pre-treatments given before or very soon after infection are almost always warranted, as was done in the present studies.

Oral bioavailability is a desired property of a drug due to ease and reduced cost of administration. When first evaluated, 7-dzGuo represented an improvement over 7-dzTOGuo because it exhibited oral antiviral activity in vivo (Smee et al., 1991a), whereas 7-dzTOGuo did not (Smee et al., 1989, 1990a). The toxic effects of 7-dzGuo after repeated dosing proved to be an undesirable characteristic, however. By the substitution of the chlorine atom at the 8-position of the molecule, 8-Cl-7-dzGuo was found to be much less toxic yet equally or more active against virus infections in vivo. Recently we showed the substitution of the ribosyl portion of these molecules for alkyl groups could be made without subsequent loss of i.p. antiviral activity (Michael et al., 1993). However, these alkylated 7-deaza compounds were not orally active. In fact, out of all these related compounds synthesized and evaluated to date, only 7-dzGuo and 8-Cl-7-dzGuo are orally bioactive. 8-Cl-7-dzGuo is the more promising of the two orally-active interferon-inducing antivirals due to its decreased toxicity.

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