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Antiviral Activity of 3-Deazaguanine, 3-Deazaguanosine, and 3-Deazaguanylic Acid

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3-Deazaguanine (ICN 4221), 3-deazaguanosine (ICN 4793), and 3-deazaguanylic acid (ICN 5412) represent a new class of synthetic guanine analogs having antiviral activity. In vitro, nine ribonucleic acid and seven deoxyribonucleic acid viruses were inhibited, including influenza, parainfluenza, rhino-, vesicular stomatitis, adeno-, herpes-, cytomegalo-, vaccinia, pseudorabies, and myxoma viruses. They were effective orally against influenza types A and B and parainfluenza type 1 (Sendai) virus infections in mice, with a therapeutic index of 16 against the latter two viruses. The course of herpes encephalitis was altered only when the drugs were applied directly into the brain. In addition, these drugs were effective inhibitors of Friend leukemia virus-induced splenomegaly in mice; treatment also produced extensions of life in these animals.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole, ribavirin) has been reported to have broad-spectrum antiviral activity in a variety of in vitro and in vivo systems (4, 8). This antiviral activity was reversed by inosine, xanthosine, and guanosine, suggesting that ribavirin acted as a guanosine analog (10). In addition, structural similarities between guanosine and ribavirin were demonstrated by X-ray crystallography (6), and biochemical studies indicated that ribavirin inhibited enzymes, particularly inosine monophosphate (IMP) dehydrogenase, in the guanine nucleotide biosynthetic pathway (10). Since broad-spectrum antiviral activity is desirable and ribavirin is a major example for such drugs, it seemed appropriate to synthesize other guanine or guanosine analogs as possible antiviral drugs.

Deazapurines represent a class of synthetic materials having a variety of biological activity, including antiviral activity (2, 5).

Until the recent report by Cook et al. (3), 3-deazaguanine (3-DG, ICN 4221) was the only 3-deaza analog of the naturally occurring purines that had not been synthesized. This compound, whose chemical formula is 6-aminoimidazo(4,5-*c*)pyridin-4(5*H*)-one, and its corresponding nucleoside and nucleotide have been synthesized for evaluation as antiviral and antitumor drugs. The present report describes the broad-spectrum antiviral activity of this new class of synthetic guanine analogs.

MATERIALS AND METHODS

Compounds. 3-DG, 3-deazaguanosine (3-DGUO, ICN 4793), whose chemical formula is 6-amino-1-β-D-ribofuranosylimidazo(4,5-*c*)-pyridin-4(5*H*)-one, and 3-deazaguanylic acid (3-DGMP, ICN 5412), whose chemical formula is 6-amino-1-β-D-ribofuranosylimidazo(4,5-c)pyridin-4(5*H*)-one 5'-phosphate were synthesized at this institute by R. B. Meyer, Jr., and P. D. Cook by the method of Cook et al. (3). As a result of the limited supply of material, it was not possible to compare all of the above simultaneously in the various animal models. Ribavirin was tested in parallel in most in vitro experiments as a standard. It was synthesized at this institute by the method of Witkowski et al. (11).

Compound preparation. For intracerebral (i.c.) inoculation, the appropriate amounts of compounds were added to saline, solubilized by steaming, and maintained at 37°C prior to inoculation. For systemic administration, 3-DG was solubilized with 0.2 N sodium hydroxide, sterile saline was added, and the solution was neutralized with 0.2 M potassium dihydrogen phosphate. 3-DGUO and 3-DGMP were readily soluble in saline at the dosages used.

3-DG was soluble in tissue culture medium at 2 mg/ml, if the solution was warmed at 37°C prior to dilution.

Cell cultures. Monolayer cultures of secondary chicken embryo (CE), human carcinoma of the nasopharynx (KB), and rabbit kidney (RK-13) cells were grown in Eagle minimal essential medium (Grand Island Biological Co., Berkeley, Calif.) supplemented with 10% fetal bovine serum (Irvine Scientific, Fountain Valley, Calif.).

Animals. Male Swiss Webster mice were purchased from Hilltop Lab Animals, Chatsworth, Calif.

Viruses. Type 1 herpesvirus, strains HF and 123 (HV/1); myxoma virus, strain Sanarelli (MV); vaccinia virus, strain Lederle CA (VV); human cytomegalovirus, strain Casazza (CMV); type 1A rhinorus, strain 2060 (RV/1A); type 13 rhinovirus, strain 353 (RV/13); type 3 parainfluenza virus, strain C243 (PIV/3); type B influenza virus, strain Lee (IV/B); and influenza A_2, strain Japan/305 (IV/A_2) were obtained from F. M. Schabel, Jr. (Southern Research Institute, Birmingham, Ala.). Type 2 herpesvirus, strain MS (HV/2), and vesicular stomatitis virus, strain Indiana (VSV), were purchased from the American Type Culture Collection (Rockville, Md.). Type 3 adenovirus, strain GB (AV/3), was supplied by D. A. Fuccillo (National Institute of Neurological Disease and Stroke, Bethesda, Md.). Type 2 rhinovirus, strain HGP (RV/2); type 8 rhinovirus, strain CU-MRH (RV/8); and type 56 rhinovirus, strain CH-MRH (RV/56 were furnished by C. A. Phillips (University of Vermont, Burlington). Type 1 parainfluenza virus strain Sendai (PIV/1) was obtained from E. Minuse (University of Michigan, Ann Arbor). Pseudorabies virus, the RK17C24 derivative of the Aujeszky strain (PRV), was obtained from A. S. Kaplan (Albert Einstein Medical Center, Philadelphia, Pa.). Type Ao influenza virus, strain NWS (IV/Ao), was supplied by K. W. Cochran (University of Michigan, Ann Arbor).

In vitro antiviral evaluation. In vitro evaluations were carried out in MicroTest II tissue culture plates (Falcon Plastics, Division of Bioquest, Oxnard, Calif.), with inhibition of viral cytopathic effect (CPE) observed at 3 days after virus inoculation. Compounds prepared in minimal essential medium plus 5% fetal bovine serum and 50 μg of gentamicin (Schering Corp., Kenilworth, N.J.) per ml were added to the microplates at 15 to 20 min after virus inoculation. CPE inhibition was evaluated by a virus rating (VR) procedure. After CPE reduction had been observed in certain experiments, virus titers were determined on lysates of frozen-thawed cell samples. Details of the microplate technique, VR, and virus titer determinations have been described previously by Sidwell and Huffman (7). In our experience, a VR of ≤ 0.4 represents slight or no activity, a VR of 0.5 to 0.9 represents moderate activity, and a VR ≥ 1 represents marked activity.

In vivo antiviral evaluations. In all animal experiments, infected control animals (placebo) were treated with a drug-free vehicle. In addition, uninfected, treated animals served as toxicity controls. Animal groups were observed daily for deaths for a period of 21 days unless otherwise specified.

Encephalitis experiments. Ether-anesthetized mice (~20 g) were infected i.c. with ~2 mean lethal doses of HV/1 (strain 123) as described previously (1). The drug was administered as a single i.c. treatment at 6 h after virus inoculation (1) or as multiple oral treatments.

Respiratory virus infections. Respiratory infections were initiated with approximately a 90% lethal dose of IV/A_2, IV/B, or PIV/1. Three drops of virus were instilled intranasally into lightly anesthetized mice (~20 g). These viruses were diluted in minimal essential medium (0.1% $NaHCO_3$) supplemental with 1% sorbitol to increase virus stability. Treatment routes and schedules are indicated below.

Leukemia virus infections. To evaluate these compounds against a virus-induced leukemia, ~20-g mice were infected intravenously with 0.2 ml of Friend leukemia virus (FLV), a dose known to produce an increase in spleen weights of 1.0 to 1.5 g in 21 days. Animals were treated intraperitoneally once daily for 2 weeks, starting at 30 min after virus inoculation. In one experiment, all animals were sacrificed on day 21 and differences in spleen weights were analyzed. In another experiment, the animals were observed for death patterns for a period of 9 weeks.

Statistical analysis of in vivo antiviral activity. The effectiveness of drugs in animal experiments was determined by comparing the number of survivors (Fisher exact test) and the mean day of death (t test) of drug-treated and placebo-treated animals. In addition, differences in spleen weights were also analyzed by the t test.

RESULTS

In vitro antiviral activity. (i) Spectrum of activity. 3-DG, 3-DGUO, and 3-DGMP were tested in parallel with ribavirin against nine ribonucleic acid and seven deoxyribonucleic acid viruses, and the results of a single experiment with each virus are shown in Table 1. In these systems the deaza derivatives of guanine and guanosine had activity equal to or greater than that of ribavirin, although only the two nucleosides ribavirin and 3-DGUO were active against IV/Ao.

(ii) Effect on CPE and virus production. The effects of 3-DG and ribavirin on CPE and virus produced by RV/13 were compared (Fig. 1). The lowest dosages at which 100% CPE reduction occurred were 10 μg/ml for 3-DG and 100 μg/ml for ribavirin. 3-DG reduced the amount of virus below detectable levels at 10 μg/ml, whereas >100 μg of ribavirin per ml was required to accomplish the same degree of reduction.

(iii) Comparison of CPE inhibition and cytotoxicity. Although 3-DG appeared to be inhibitory at lower dosages than ribavirin, cytotoxicity was also observed at lower dosages. This increased cytotoxicity prompted an experiment to compare the antivaccinia activity and cytotoxicity of 3-DG and ribavirin in two cell lines. These effects are illustrated for KB cells in Fig. 2 and for RK-13 cells in Fig. 3. When doses closest to the 50% inhibitory (vaccinia CPE) and cytotoxic concentrations for the calculation of therapeutic index (TI) were used, 3-DG had a TI of 3.2 KB cells, whereas that of the less toxic ribavirin was 30. In RK-13 cells the 3-DG TI increased to 10, whereas that of ribavirin was ≥ 10.

TABLE 1. *In vitro effect of 3-DG, 3-DGUO, 3-DGMP, and ribavirin on deoxyribonucleic acid and ribonucleic acid viruses*

Virus	Cell line	VR[a]			
		3-DG	3-DGUO	3-DGMP	Ribavirin
RNA					
IV/A_0	CE	0.2	0.6	0.1	0.8
PIV/1	KB	1.1	0.8	0.8	0.8
PIV/3	KB	1.2	0.9	0.8	0.8
RV/1A	KB	≥1.3	0.9	1.1	1.1
RV/2	KB	1.1	0.9	1.0	0.9
RV/8	KB	≥1.3	1.1	ND[b]	0.7
RV/13	KB	1.2	1.0	1.1	0.7
RV/56	KB	1.2	0.9	ND	0.7
VSV	KB	1.3	1.1	ND	1.0
DNA					
AV/3	KB	1.1	0.8	0.8	0.6
HV/1	KB	1.2	0.8	0.9	0.8
HV/2	KB	1.1	0.9	0.8	0.6
CMV	WI-38	1.0	0.7	ND	ND
VV	RK-13	1.3	1.1	1.0	0.9
PRV	RK-13	0.7	0.6	0.3	0.3
MV	RK-13	0.6	0.7	0.8	1.0

[a] The VR was determined by comparing CPE development in drug-treated cells (T) and virus control cells (C). The CPE value (0 to 4) assigned to T for each drug level was substracted from that of C, and the differences (C − T) were totaled. If any toxicity was evident at any drug level, the C − T of that level was divided by 2. The sum of all C − T values was then divided by 10 times the number of test cups used per drug level.

[b] ND, Not determined.

In vivo antiviral activity. (i) Herpes encephalitis. In experiment 1, the maximum tolerated doses of 3-DG and 3-DGUO were administered i.c. Both compounds produced slight, but significant, increases in survivor numbers, whereas only 3-DG significantly prolonged the life of dying animals (Table 2). This study was followed by two experiments in which 3-DG, 3-DGUO, and 3-DGMP were administered orally either two or five times per day, beginning at 4 h after virus inoculation, in daily dosages of 40, 10, 2.5, or 0.625 mg/kg. The maximum tolerated (nonlethal) dose was ~40 mg/kg per day for each drug. No efficacy was observed in these systemic treatment experiments.

(ii) Respiratory virus experiments. Initially, 3-DGUO and 3-DGMP were evaluated orally against IV/B-induced pneumonia in mice. Drugs were administered twice daily for 9 days, starting at 2 h before virus inoculation, at dosages of 40, 10, 2.5, or 0.625 mg/kg per day. 3-DGUO was significantly effective, increasing survivor numbers at 2.5 mg/kg and the mean day of death at 0.625 mg/kg (Table 3). Neither was effective at 10 or 40 mg/kg per day.

This experiment was repeated with all three drugs, but using PIV/1 as the challenge virus. Again, only 3-DGUO, at a dosage of 2.5 mg/kg per day, was significantly effective in increasing the number of surviving animals (Table 4). Only a prolongation of life of the animals was seen with the lowest dose of 3-DG.

Earlier work with ribavirin and several challenge viruses indicated that a single high dose (200 to 400 mg/kg) was sufficient to increase survivors and prolong life. Therefore, comparable nonlethal doses of all three drugs were administered orally at 15 min after IV/A_2 inoculation. Significant survivor increases were seen by all at the highest doses (200 mg/kg), but only 3-DG prolonged life at the lower dose (Table 5).

(iii) Leukemia virus experiments. Two FLV experiments were carried out, with single daily doses of 3-DG, 3-DGUO, and 3-DGMP administered intraperitoneally for 14 days starting at 30 min after intravenous infection. Only the highest doses of compounds significantly prevented spleen weight increases (Table 6). In experiment 2, only the high doses of compounds were given on the same schedule and animals were observed for death patterns over a 9-week period. At the time of sacrifice, 3-DG and 3-DGUO had increased numbers of survivors (Table 6), but the animals showed signs of serious illness when sacrificed.

DISCUSSION

The antiviral activities of a new guanine analog, 3-DG, and its corresponding nucleoside and nucleotide have been presented. In vitro

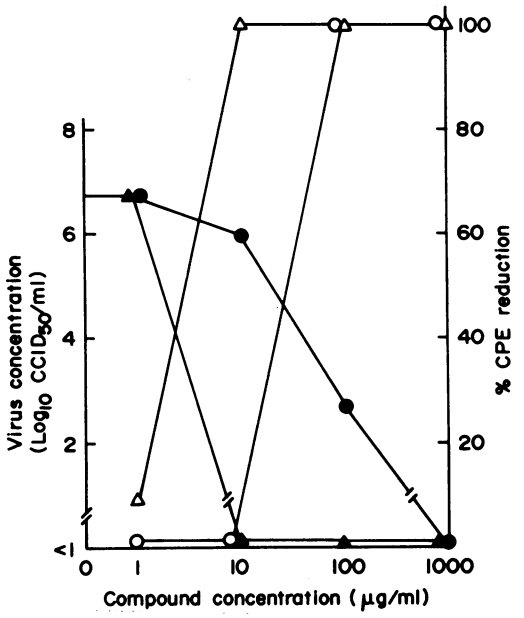

FIG. 1. *Effect of 3-DG and ribavirin on RV113 in KB cells. Virus concentration: (▲) 3-DG, (●) ribavirin. CPE reduction: (△) 3-DG, (○) ribavirin. $CCID_{50}$, Mean cell culture 50% infective dose.*

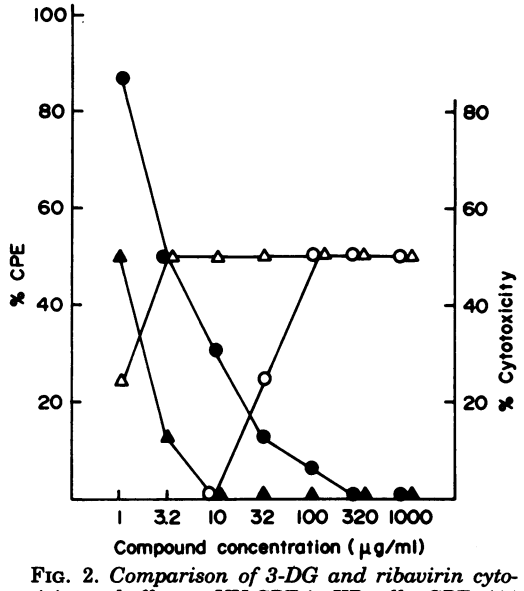

FIG. 2. *Comparison of 3-DG and ribavirin cytotoxicity and effect on VV CPE in KB cells. CPE: (▲) 3-DG, (●) ribavirin. Cytotoxicity; (△) 3-DG, (○) ribavirin.*

these compounds exhibited a wide spectrum of activity similar to that of the previously described nucleoside, ribavirin.

In contrast to ribavirin, 3-DG exhibited dif-

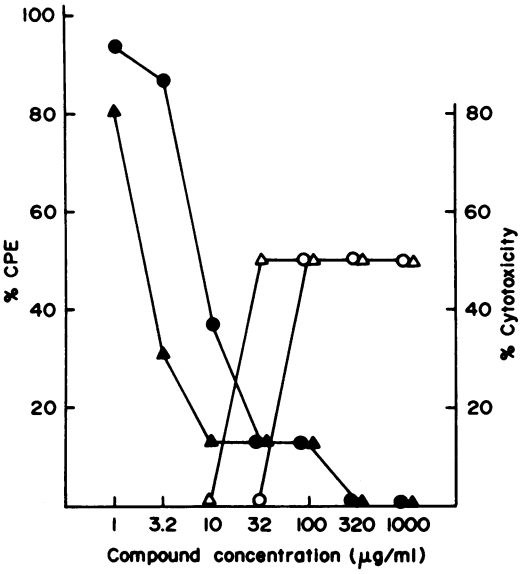

FIG. 3. *Comparison of 3-DG and ribavirin cytotoxicity and effect on VV CPE in RK-13 cells. CPE: (▲) 3-DG, (●) ribavirin. Cytotoxicity: (△) 3-DG, (○) ribavirin.*

fering degrees of cytotoxicity in KB and RK-13 cells. The inspection of other data revealed that it was much more toxic in HeLa and HEp-2 cells than in CE and Vero cells and was of intermediate toxicity in WI-38 cells. Since the most sensitive cells were derived from human cancer tissues, this cytotoxicity may reflect a species specificity (human cells) or a possible toxicity against cancer cells.

When the compounds were tested in animals infected with FLV, some activity was seen in preventing spleen enlargement and delaying virus-induced deaths. This activity was seen only at the maximum tolerated dose when it was administered as a single daily dose. In later experiments, when that dose was divided into two or five treatments per day, practically no activity was seen. This would suggest that very high tissue and blood levels were necessary for only a short period of time to produce activity. 3-DG has recently been shown to possess significant activity against solid tumors in animals, but only slight activity against leukemias (T. Khwaja, L. Kigwana, R. Meyer, Jr., and R. Robins, Proc. Am. Assoc. Cancer Res. 16:162, 1975.

In ribonucleic acid respiratory virus experiments, the nucleoside 3-DGUO appeared to be the most consistently effective. In contrast to the FLV experiments, very low dosages of 3-DGUO (2.5 mg/kg per day, twice a day) were significantly effective in increasing survivors

TABLE 2. *Effect of 3-DG or 3-DGUO[a] on the development of HV/1-induced encephalitis in mice*

Compound	Dose (mg/kg per day)	No. of survivors/total	Mean day of death
Saline	0.03 ml	0/20	6.5
3-DG	15[b]	3/9 ($P < 0.05$)	9.5 ($P < 0.001$)
3-DGUO	20[b]	3/10 ($P < 0.05$)	7.7

[a] Compound administered i.c. at 6 h after i.c. inoculation of virus.
[b] MTD, No toxic deaths.

TABLE 3. *Effect of orally administered[a] 3-DGUO and 3-DGMP on IV/B infections in mice*

Compound	Dose (mg/kg per day)	No. of survivors/total	Mean day of death
Saline		2/30	7.2
3-DGUO	2.5[b]	5/10 ($P < 0.01$)	7.6
	0.625	2/10	8.1 ($P < 0.05$)
3-DGMP	2.5[b]	3/10	7.0
	0.625	1/10	6.6

[a] Twice a day for 9 days, starting at 2 h before virus inoculation.
[b] MTD, ~40 mg/kg per day; no toxicity deaths at these dosages.

TABLE 4. *Effect of orally administered[a] 3-DG, 3-DGUO, and 3-DGMP on PIV/1 (Sendai)-induced infections in mice*

Compound	Dose (mg/kg per day)	No. of survivors/total	Mean day of death
Saline	ND[b]	4/20	8.0
3-DG	2.5	3/10	8.4
	0.625	2/10	10.0 ($P < 0.05$)
3-DGUO	2.5	8/10 ($P < 0.01$)	9.0
	0.625	2/10	8.3
3-DGMP	2.5	2/10	8.1
	0.625	4/10	7.2

[a] Twice a day for 9 days, starting at 2 h before virus inoculation.

TABLE 5. *Effect of a single oral treatment[a] with 3-DG, 3-DGUO, and 3-DGMP on IV/A_T-induced infections in mice*

Compound	Dose (mg/kg)	No. of survivors/total	Mean day of death
Saline		1/19	7.0
3-DG	200[b]	4/10 ($P < 0.03$)	7.5
	100	1/9	10.4 ($P < 0.01$)
3-DGUO	200[b]	5/10 ($P < 0.01$)	9.0
	100	1/10	6.7
3-DGMP	200[b]	4/9 ($P < 0.05$)	6.2
	100	0/10	7.4

[a] At 15 min after virus inoculation.
[b] A total of 5/5 survivors in toxicity control groups.

and extending life, whereas higher dosages were ineffective. To explain these differences, it is possible that the metabolism in general, and particularly of the drug, may vary in different infected tissues. When 40 is used as the maximum tolerated dose (MTD) and 2.5 is used as the minimum effective dose (MED), the TI (MTD/MED) in the PIV/1 and IV/B models is 16.

It is interesting that in animals 3-DG and 3-DGUO were only effective against herpes encephalitis when the drug was applied directly to the infected organ. This may suggest that after systemic administration, the drugs are metabolically inactivated or altered such that the effective material does not cross the blood brain barrier.

Streeter and Koyama (9) have investigated the inhibitory effects of these compounds on purine nucleotide biosynthesis in Ehrlich as-

TABLE 6. *Effect of 3-DG and 3-DGUO on FLV-induced spleen enlargement and deaths*

Drug	Expt 1		Expt 2	
	Dosage (mg/kg per day)[a]	Splenomegaly inhibition[b]	No. of survivors/total[c]	Mean day of death
Saline		0	4/20	46.3
3-DG	40	−62.5 ($P < 0.001$)	6/10 ($P < 0.05$)	38.5
	10	−26.6		
	2.5	0		
	0.625	0		
3-DGUO	40	−67.8 ($P < 0.001$)	4/10	49.3
	10	0		
	2.5	0		
	0.625	0		

[a] Drug administered once per day (0 to 13).
[b] A total of 10 animals per group.
[c] Observations on day 63.

cites tumor cells. In their system, the effect of the compounds on eight enzymes involved in purine nucleotide biosynthesis was investigated. The enzymes most affected by these compounds were hypoxanthine guanine phosphoribosyltransferase and IMP dehydrogenase. 3-DG, 3-DGUO, and 3-DGMP show inhibitory patterns for hypoxanthine guanine phosphoribosyltransferase similar to those for ribavirin (9). All also inhibited IMP dehydrogenase, but 3-DGMP (60% inhibition) most closely approached the activity of ribavirin (70% inhibition). In contrast to ribavirin, this group of compounds did not appreciably inhibit guanosine diphosphate kinase.

These authors felt that the most likely inhibitor of IMP dehydrogenase would be 3-DGMP, since both guanylic acid and ribavirin 5'-phosphate are inhibitors of an IMP dehydrogenase isolated from *Escherichia coli* cells. It was assumed that 3-DGMP and 3-DGUO would be converted down to 3-DG and then phosphoribosylated back up to 3-DGMP, the proposed active compound.

Both the antitumor and antiviral activities of this group of compounds suggest that further work should be done in these areas. In light of the antitumor work of T. Khwaja, C. Potter, and A. Mittelman (personal communication), we believe the greatest potential for practical application of 3-DG will probably be in the treatment of solid tumors.

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