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Geldanamycin Inhibition of 3-Methylcholanthrene-Induced Rat Embryo Cell Transformation (39830)[1]

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Ansamycins are a group of antibiotics containing an aliphatic ansa bridge. This group is represented by rifamycins, streptovaricins, tolypomycins, and geldanamycin (1, 2). Geldanamycin (Gld) (isolated from *Streptomyces hygroscropicus* var. *geldanus* var. *nova*) is the only benzoquinone among the ansamycins (3, 4) and differs from the others in that its principal activity is against protozoa rather than gram-positive bacteria (1). All of the ansamycins have antiviral properties and are inhibitors of the RNA-dependent DNA polymerase (RDDP) of type C RNA viruses (1, 2).

We have previously shown that a Fischer rat embryo cell system (F1706) sensitive to transformation by polycyclic hydrocarbons and other chemical carcinogens can be protected from transformation by treatment with two other classes of antiviral antibiotics (5, 6). Streptonigrin (5) and cordycepin (6) at levels causing minimal toxicity were shown to inhibit both the induction of the endogenous rat leukemia virus (RaLV) by 5-iodo-2′-deoxyuridine (IdU) and the *in vitro* transformation by the polycyclic hydrocarbon 3-methylcholanthrene (MCA).

In this paper we report that Gld at nontoxic levels also inhibits the induction of the endogenous RaLV by IdU and protects the cells from transformation induced by MCA.

Materials and methods. (*A*) *Toxicity testing.* Reduction in plating efficiency relative to a medium control was used to determine the toxicity of geldanamycin (Gld). Five hundred cells (F1706 p95) in 5 ml of complete medium were added to each 60-mm plastic dish (Lux). After 3 hr of incubation at 37° (to allow cell attachment), the medium was decanted and replaced with a fresh medium containing Gld. Five days later the cultures were fixed and stained (Giemsa), and macroscopic colonies were counted.

(*B*) *Transformation assay.* Gld, 0.3 or 1.0 μg/ml, was incorporated into the medium 48 hr prior to and during treatment of the cells with 0.1 μg/ml of MCA. MCA was incorporated into the medium for 1 week (one transfer) and then was removed. After MCA treatment, half of the cultures continued to receive Gld at every feed and transfer, while the other half no longer received Gld, i.e., Gld was present only during the MCA treatment. The growth medium consisted of Eagle's minimum essential medium in Earle's salts (EMEM) supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, 0.1 m*M* nonessential amino acids, 100 unit of penicillin, and 100 μg of streptomycin/ml. MCA was diluted in acetone to 1000 μg/ml and was further diluted in the growth medium to 0.1 μg/ml. Duplicate sets of mycoplasma-free Fischer rat embryo cells (F1706) were treated at subculture 95. At each subculture, one set of flasks was set aside to be held for 3 weeks without subdivision (holding series), and the other set was subdivided 1:2 weekly to provide two new sets of cultures, one for the holding series and one for subdivision. Morphological transformation was determined by the appearance of foci of cells lacking contact inhibition and orientation. Tumorigenicity was determined by subcutaneous inoculation of 1×10^6 cells into newborn Fischer rats (F344/f Mai).

(*C*) *Endogenous virus inhibition.* For inhibition of endogenous virus induction, the

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cells were planted at a concentration of 100,000 cells/ml and, 48 hr later (cultures about 90% confluent), were treated with 20 μg/ml of IdU in the dark and either 1.0 or 0.3 μg of Gld/ml. After a 48-hr incubation, the cultures were washed, and the medium was replaced with a growth medium still incorporating 0, 1.0, or 0.3 μg of Gld/ml. Twenty-four hours later the inhibition of virus induction was assayed by testing the supernatant for virus-associated RDDP (7).

Results. At a level of 0.3 μg/ml or less of Gld there was no reduction in either plating efficiency or colony size. One microgram per milliliter of Gld reduced the relative plating efficiency of F1706 p95 by approximately 38%. A Gld concentration of 5 μg/ml was toxic (Table 1).

Gld, 0.3 μg/ml (the maximum nontoxic dose) completely inhibited the induction by IdU of detectable levels of RDDP (Table 2).

When either 0.3 or 1.0 μg/ml of Gld was incorporated into the medium prior to, during MCA treatment, and continuously thereafter, the cells from duplicate cultures were still normal at the termination of the experiment, 14 subcultures after treatment. Cells protected from transformation by Gld failed to produce tumors in the inoculated rats (0/4) by 90 days postinoculation (Table 3). In contrast, cells from duplicate cultures treated with MCA in the absence of Gld showed transformed foci one subculture after treatment. Fourteen MCA-free passages later, transformed cells produced undifferentiated fibrosarcomas in six of seven rats by 60 days postinoculation. If Gld was incorporated into the medium prior to and during MCA treatment but was removed at the time of MCA removal, expression of transformation was delayed but not eliminated. Whereas MCA in the absence of Gld produced transformation within one subculture after its removal, duplicate cultures treated with 0.3 or 1.0 μg/ml of Gld during the MCA treatment period did not exhibit

TABLE 1. TOXICITY OF GELDANAMYCIN AS DETERMINED BY REDUCTION IN PLATING EFFICIENCY OF F1706 p95 RAT EMBRYO CELLS.

Concentration (μg/ml)	Average No. of colonies/ three dishes	Relative plating efficiency[a] (%)
20	0	0
10	2.3	4.6
5	4.6	9.2
1	31.3	62.6
0.3	50.3	100.6
0.03	51	102
Control	50	100

[a] Relative plating efficiency is the percentage of cells giving rise to macroscopic colonies, relative to the control, in which the absolute plating efficiency is arbitrarily set at 100%.

TABLE 2. GELDANAMYCIN INHIBITION OF 5-IODO-2'-DEOXYURIDINE-INDUCED TYPE C VIRUS EXPRESSION IN F1706 p95 RAT EMBRYO CELLS.

Treatment	[^{3}H]TMP incorporated into tissue culture fluid[a] (cpm/ml)	Inhibition of RT activity (%)
Cells + 20 μg of IdU	10191	
Cells + 20 μg of IdU + 0.3 μg of Gld	84[b]	99
Cells + 20 μg of IdU	10040	
Cells + 20 μg of IdU + 0.3 μg of Gld	0[c]	100

[a] Counts per minute incorporated minus control (no IdU treatment) counts per minute. A reading of 0 means that the count was equal to or less than the control.

[b] F1706 cells + 0.3 μg of Gld control– 316.

[c] F1706 cells + 0.3 μg of Gld control– 467.

TABLE 3. GELDANAMYCIN PROTECTION FROM TRANSFORMATION.

Treatment (per ml)	Transformation[a]	Tumors[b] (No. positive/No. innoculated)
Acetone control (1:1000)	–(P14)	0/4 (90)[c]
1 μg of Gld[d]	–(P14)	ND[e]
0.3 μg of Gld[d]	–(P14)	ND
0.1 μg of MCA control	+(P1)	6/7 (90)[f]
0.1 μg of MCA + 1 μg of Gld[d]	–(P14)	ND
0.1 μg of MCA + 0.3 μg of Gld[d]	–(P14)	0/4 (90)

[a] Transformation assay with duplicate cultures of F1706 p95 rat embryo cells. Identical results were obtained in both series.

[b] Inoculated sc at 1.5×10^6 with cells treated 14 subcultures (P14) earlier. Value in parentheses is number of days postinoculation.

[c] No tumors observed at 90 days postinoculation.

[d] Gld included in the medium at every medium change.

[e] ND, not done.

[f] 4/7 positive for tumors at 1 month, 6/7 at 2 months.

the transformed phenotype until the fifth subculture posttreatment.

Discussion. We have previously shown that our low-passage Fischer rat embryo cells require preinfection with an exogenous type C RNA virus or induction of the endogenous virus prior to treatment with a chemical carcinogen for transformation (8, 9). Beyond subculture 50, the same cells no longer require an exogenous or stimulated source of oncornavirus for chemically induced transformation (10). However, we have previously demonstrated that two antiviral antibiotics, streptonigrin (5) and cordycepin (6), which inhibit the induction of the endogenous RaLV by halogenated pyrimidines in these high-passage cells also inhibit transformation by the polycyclic hydrocarbon MCA. We report here that geldanamycin (Gld), like streptonigrin (Sn) and cordycepin (Cd) inhibits the induction of endogenous virus and protects the cells from transformation when incorporated into the medium prior to, during, and after MCA treatment. Our results with Gld however, differ from those with streptonigrin and cordycepin in that Gld was the most effective of the three in inhibiting oncornavirus induction and was the only compound having this effect at a nontoxic dose. This observation is of importance in that the possibility existed that the previously reported protective effect was due to toxicity-induced inhibition of DNA replication during the period of carcinogen treatment.

Our study with MCA complements the studies of O'Connor *et al.* (11) who reported that Gld at a dose of 1.0 μg/ml inhibited murine sarcoma virus (MSV) foci by 60% in 3T3 with a 150% increase in their cell growth. They concluded that the toxic and virus inhibitory effects of Gld are specific but different.

Summary. Geldanamycin, an ansamycin antibiotic having anti-oncornavirus properties, was shown to completely inhibit the induction of measurable endogenous rat leukemia virus by 5-iodo-2′-deoxyuridine in a cell line of Fischer rat embryo cells and to protect the same cells from transformation by the polycyclic hydrocarbon 3-methylcholanthrene. Protection was induced at a dosage that was not only non-toxic but slightly stimulatory to cell growth.

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