Communications to the editor

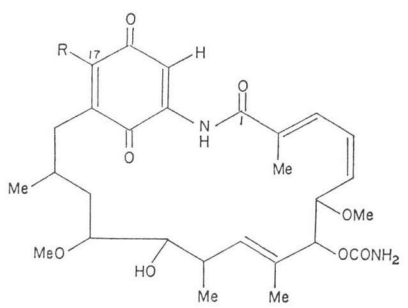
GROWTH INHIBITION OF VIRUS TRANSFORMED CELLS *IN VITRO* AND ANTITUMOR ACTIVITY *IN VIVO* OF GELDANAMYCIN AND ITS DERIVATIVES

Sir:

Geldanamycin (GLD) was discovered by DEBOER *et al.* in the culture filtrate of *Streptomyces hygroscopicus* var. *geldanus* var. nova (UC-5208) as a yellow antibiotic, especially active against *Tetrahymena pyriformis* and *Crithidia fasciculata*[1]. Beside having this antiparasitic activity, GLD has strong antitumor activity against L1210 and nasopharynx KB cells in culture[1]. In this sense the antimicrobial spectrum of GLD differs from that of the naphthoquinonoid ansamycins such as the rifamycins and streptovaricins. The chemical structure of GLD has been established by SASAKI *et al.* by spectral analysis and on the basis of its chemical characteristics[2]. GLD is the first ansamycin antibiotic shown to contain a benzoquinone nucleus, and it is of interest to note that the carbon skeleton of the structure is the same as that of the maytansines[3] and ansamitocins[4].

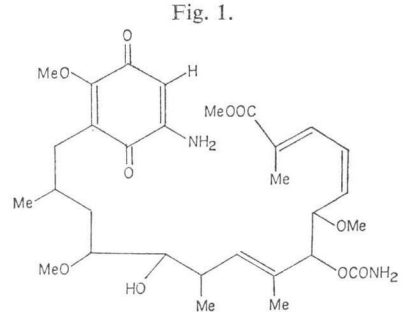
Our interest in the relationship between structure and biological activity of GLD and its derivatives comes from our work with the derivatives of damavaricin C, a degradation product of streptovaricin C. In that work we investigated their ability to kill mammalian cells including cells transformed by viruses and human leukemia cells *in vitro* and *in vivo*[5~7].

GLD was prepared in our laboratories by the fermentation of *Streptomyces hygroscopicus* subsp. *duamyceticus*, a strain 325-15, which also produces duamycin (nigericin). GLD derivatives (17-demethoxy-17-amino- or alkylamino-GLD) were synthesized by the reaction of GLD with ammonia or various alkylamines in chloroform at room temperature. Methyl geldanamycinate was prepared by the reaction of GLD



Geldanamycin (R = OMe)

Fig. 1.



Methyl geldanamycinate

Table 1. The minimum inhibitory concentration (μg/ml) of geldanamycin derivatives.

R	3Y1 cells	SV40-3Y1 cells
Methoxyl (GLD)	0.1	0.02
Hydroxyl	2.0	0.5
Amino	0.02	0.02
Methylamino	0.1	0.02
n-Propylamino	0.5	0.1
n-Butylamino	0.1	0.1
Cyclopropylamino	0.02	0.005
1′-Adamantylmethylamino	0.5	0.5
n-Dodecylamino	0.5	4.0
n-Octylamino	0.5	4.0
Dimethylamino	0.1	0.005
Methyl geldanamycinate	20.0	10.0

3Y1 or SV40-3Y1 cells were suspended in EAGLE's MEM medium containing 10% fetal calf serum at the concentration of 4×10^4 cells/ml. The suspension (0.5 ml) was poured into the wells of Linbro multidishes (4×6 wells) and incubated at 37°C for 3 hours. Then, EAGLE's MEM medium (0.5 ml) containing 10% fetal calf serum and the appropriate derivative at various concentrations, was added. Cytotoxicity was measured by microscopic observation after 3~4 days.

with potassium carbonate in boiling methanol[2] (Fig. 1).

Normal rat cells (3Y1) and the SV40 line of transformed cells were used for *in vitro* studies[8]. Sarcoma 180 cells and EHRLICH ascites carcinoma cells were used for *in vivo* studies.

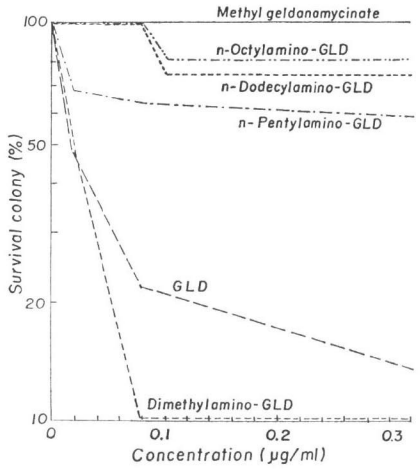
We employed two kinds of assay systems; one to observe the colony forming ability of SV40-3Y1 cells after 16 hours treatment of cells in culture medium with either GLD or one of its derivatives, and the other to determine the minimum inhibitory concentration of the agent required to show cytotoxicity.

A few points were clearly demonstrated from our experiments (Table 1 and Fig. 2). First, the ability to kill cells is directly related to the presence of the ansa ring structure. Fission of this ring at the NH–CO bond, as in methyl geldanamycinate, resulted in a marked reduction in ability to kill cells. Second, the selective permeability properties of the derivatives depended on the alkylamino group which was substituted for the methoxyl group at the C-17 position. Third, of the GLD derivatives, *n*-octylamino-GLD and *n*-dodecylamino-GLD were more toxic to normal cells than to SV40 transformed cells. On the other hand, dimethylamino-GLD was markedly less lethal to normal cells than to SV40 transformed cells.

The *in vivo* antitumor activity of the derivatives was examined using cyclopropylamino-GLD and β-chloroethylamino-GLD. As shown in Tables 2 and 3, the former showed an ILS greater than 62.4% at a dose level of 10 mg/kg/day and the latter an ILS greater than 116% at 2.5 mg/kg/day. The latter also displayed an IR value of 45.7% at a dose level of 0.625 mg/kg/day. β-Chloroethylamino-GLD was thus shown to be active as an antitumor agent.

Fig. 2. SV40-3Y1 cells were suspended in EAGLE's MEM medium containing 10% fetal calf serum at a concentration of 1.6×10^5 cells/ml. The suspension (1 ml) was cultivated in the Linbro multidishes at 37°C for 10 hours and 50 μl of MEM medium containing various concentrations of GLD derivatives was added and incubated for 16 hours.

Cells were measured by their colony forming ability.



Previous reports indicated that GLD inhibits transformation of rat cells by chemical carcinogens although the mechanism of action was not clear[9]. In addition, GLD has been reported to

Table 2. Effect of GLD derivatives on survival time of solid sarcoma 180.

GLD derivative	Dose level (mg/kg/day)	Tumor weight[a] (g)	IR[b] (%)	20-Day survivors / total
Cyclopropylamino-GLD	10.0	3.07±1.01	29.1*	6/6
	2.5	2.95±1.19	31.9*	6/6
	0.625	3.98±1.39	8.1	6/6
Chloroethylamino-GLD	10.0	3.17±1.16	26.8	6/6
	2.5	3.77±1.08	12.9	6/6
	0.625	2.35±1.02	45.7*	6/6
Control	—	4.33±0.01	0	6/6

Solid sarcoma 180 cells (6.9×10^6) were subcutaneously inoculated into ICR/JCL mice. GLD derivatives were dissolved in 30% gycerol in water containing 1% Tween 80 and intraperitoneally injected once daily for 7 days, starting 24 hours after tumor inoculation.

[a] Dissected tumor weight ±S.D. at day 20.

[b] Inhibitory ratio: $(1-T/C)\times100$

* $P<0.05$

Table 3. Effect of GLD derivatives on survival time of EHRLICH ascites carcinoma.

GLD derivatives	Dose level (mg/kg/day)	MST[a] (days)	ILS[b] (%)	40-Day survivors /total
Cyclopropylamino-GLD	10.0	>20.3	>62.4	1/4
	2.5	17.5	40.0	0/4
	0.625	18.5	48.0	0/4
Chloroethylamino-GLD	10.0	20.0	60.0	0/4
	2.5	>27.0	>116.0	2/4
	0.625	>18.8	>50.4	1/4
Control	—	12.5	0	0/6

EHRLICH ascites carcinoma cells (1×10^6) were intraperitoneally inoculated into ICR/JCL mice. GLD derivatives were intraperitoneally injected once daily for 7 days, starting 24 hours after tumor inoculation.
[a] Median survival time [b] Increase in life span

inhibit terminal deoxynucleotidyltransferase of leukemia cells[10]. Since this enzyme is found in large quantities in certain leukemia cells (T cell derived) and may have an important role in these cells, the preferential inhibition of this enzyme may be of chemotherapeutic value.

Since it is believed that this enzyme is specific to T cells, it is unlikely that the inhibition of growth of cells observed by us is due to the inhibition of this enzyme. The precise mode of action of GLD on mammalian cells has yet to be investigated at the molecular level.

This is the first report on the relationship between the chemical structure and the selective cell killing activity of GLD derivatives.

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(Received May 30, 1979)

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