CIRCUMVENTION OF MULTIDRUG RESISTANCE IN HUMAN CARCINOMA KB CELLS BY POLYETHER ANTIBIOTICS

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We examined the effect of various polyether antibiotics on colchicine resistance in multidrug-resistant KB-C4 cells which exhibit about 4,000-fold resistance to colchicine. As a result, 4 out of 14 polyether antibiotics were found to reverse colchicine resistance. Among them, laidlomycin was the most potent. It potentiated colchicine cytotoxicity on KB-C4 cells about 700-fold at 1 μg/ml. Degree of potentiation was calculated by dividing of the IC_{50} value of colchicine in the absence of a polyether antibiotic by the IC_{50} value of colchicine in the presence of the polyether antibiotic. Monensin, dianemycin, and leuseramycin at 3 μg/ml also potentiated the cytotoxicity, about 100-fold. We previously reported that inostamycin is a potent chemosensitizer in KB-C4 cells. Although lysocellin has a structure very similar to that of inostamycin, it didn't reverse colchicine resistance. It slightly increased [^{3}H]vinblastine accumulation in KB-C4 cells and weakly inhibited the [^{3}H]vinblastine binding to KB-C4 plasma membranes.

P-glycoprotein is considered to be a cause of multidrug resistance.[1] To circumvent drug resistance in cancer chemotherapy and to study the mechanism of P-glycoprotein action, researchers have discovered many chemosensitizers such as verapamil,[2,3] quinidine,[4] and quinacrine.[5] From such studies, it was proposed that the common structural features for chemosensitizing activity would be a basic nitrogen atom and two planar aromatic rings.[6,7]

We found earlier that inostamycin (Fig. 1), a novel polyether antibiotic, reverses multidrug resistance in human carcinoma KB-C4 cells.[8] Inostamycin inhibits binding of radioactively labeled vinblastine to the KB-C4 membranes[9] and binding of azidopine to P-glycoprotein (our unpublished results). Inostamycin does not have the common features mentioned above. Therefore, mechanistic study with polyether antibiotics may give some new insights into drug resistance research. So, in this study, we selected polyether compounds with a variety of structures, and examined their effect on drug resistance in KB-C4 cells.

Materials and Methods

Chemicals

All polyether compounds were isolated from microorganisms in each author's laboratory. [G-^{3}H]Vinblastine sulfate ([^{3}H]VBL, 11.7~16 Ci/mmol) was obtained from Amersham.

Cell Culture

Human carcinoma multidrug-resistant KB-C4[10] and parental KB-3-1 cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 5% fetal bovine serum (Cell Culture Laboratories), 100 units/ml benzylpenicillin (Sigma), and 100 μg/ml kanamycin (Sigma) at 37°C in 5% CO_2. KB-C4 cells were cultured in medium containing 4 μg/ml colchicine (Sigma) until a few days before use to maintain their drug resistance.

MTT Cell Growth Assay

To obtain the same amount of formazan formation both in KB-C4 and KB-3-1 cells, KB-C4 and KB-3-1 cells were plated at 4×10^3 and 2×10^3 cells, respectively, in 100 μl of growth medium in 96-well multiplates (Greiner), and incubated at 37°C for 24 hours. Colchicine and polyether compounds were added, and the cells were then further incubated for 3 days. Cell growth was examined by the MTT cell growth assay kit (Chemicon). 10 μl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) in phosphate-buffered saline (pH 7.4) was added to each well, and the plate was incubated at 37°C for 4 hours. Then 100 μl of isopropanol containing 0.04 N HCl was added and mixed thoroughly. Formazan formation was measured at 570 nm for the sample and at 620 nm for the reference by micro plate reader MPR-A4 (Tosoh). The blank value measured in the absence of cells was subtracted from all data.

Accumulation of [^{3}H]VBL

KB-C4 or KB-3-1 cells were plated at 3×10^5 cells/well in 12-well plates (Costar), and incubated at 37°C for 24 hours. The cells were washed with DULBECCO's phosphate buffered saline (PBS) and incubated in 0.5 ml of serum-free RPMI 1640 medium (50 mM HEPES: pH 7.4) containing 0.2 μCi/ml (17 nM) [^{3}H]VBL with or without test chemicals for 2 hours, according to the method of FOJO *et al.*[2] After incubation at 37°C, the assay medium was removed on ice, and the cells were washed twice with ice-cold Ca^{2+}, Mg^{2+}-free PBS. The trypsinized cells were dissolved in 3 ml of Atomlight (DuPont). Then, the cell-associated radioactivity was counted.

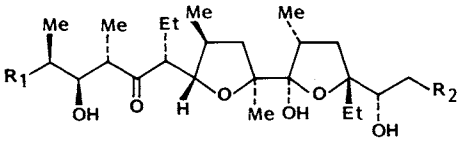
[^{3}H]VBL Binding Assay

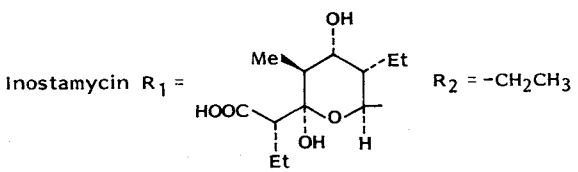
KB-C4 plasma membranes were prepared as reported previously.[9] Binding of [^{3}H]VBL to the KB-C4 membranes was measured by the method of NAITO *et al.*[11] The KB-C4 membranes (80 μg of protein) were incubated at 25°C in 50 μl of assay buffer (10 mM Tris-HCl [pH 7.5], 250 mM sucrose, 5 mM $MgCl_2$, 3 mM ATP) containing 0.17 μM [^{3}H]VBL with or without test chemicals. After 20 minutes, the reaction was stopped by addition of 4 ml of ice-cold washing buffer (10 mM Tris-HCl [pH 7.5], 250 mM sucrose, 5 mM $MgCl_2$). The membranes were collected by filtration onto a glass microfiber filter (Whatman GF/C) pretreated with 1% bovine serum albumin solution. Then, the membranes were washed with another 4 ml of ice-cold washing buffer. The filters were dried, and their radioactivity was counted. Specific binding of [^{3}H]VBL to the membranes was determined by subtraction of non-specific binding, which was assayed in the presence of excess (100 μM) cold VBL, from total binding.

Results

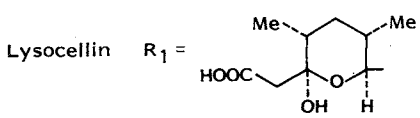
Structures of the polyether antibiotics used are shown in Fig. 1. Cytotoxicity of polyether compounds alone on KB-C4 and KB-3-1 cells and their chemosensitizing effects are summarized in Table 1. The IC_{50}'s of colchicine on KB-C4 and KB-3-1 cells were 5.9 ± 1.9 μg/ml and 0.0015 ± 0.0012 μg/ml, respectively, thus indicating KB-C4 cells to be 4,000-fold more resistant. Degree of potentiation of colchicine cytotoxicity was calculated by dividing of the IC_{50} value of colchicine in the absence of a polyether compound by the

Fig. 1. Structures of polyether compounds.

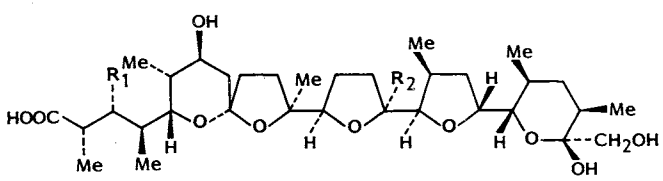




Inostamycin R_1 = $R_2 = -CH_2CH_3$

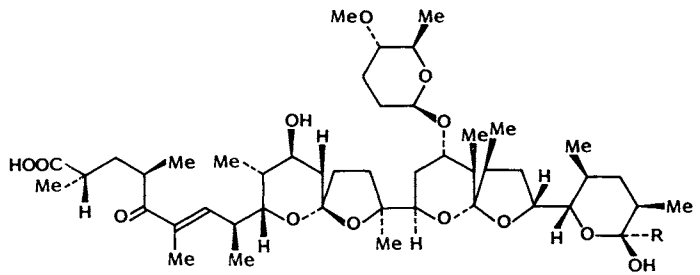


Lysocellin R_1 = $R_2 = -CH_3$



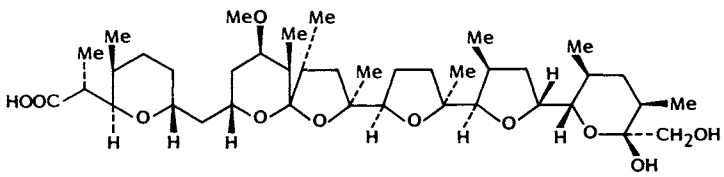
Laidlomycin $R_1 = CH_3CH_2COO-$ $R_2 = -CH_3$

Monensin $R_1 = CH_3O-$ $R_2 = -CH_2CH_3$

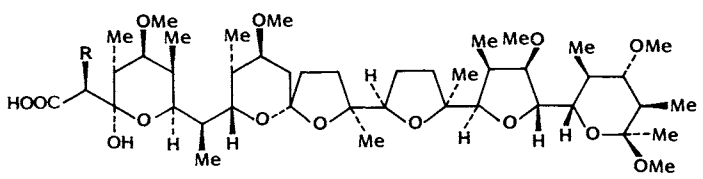


Dianemycin R = $-CH_2OH$

Leuseramycin R = $-CH_3$

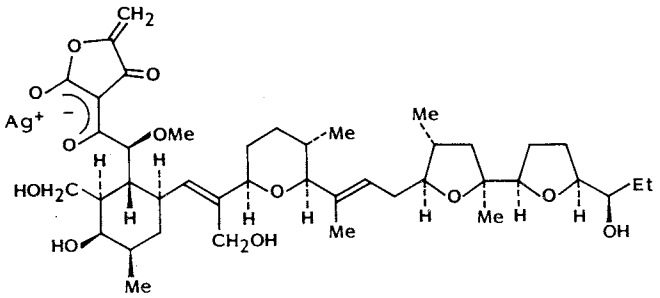


Nigericin

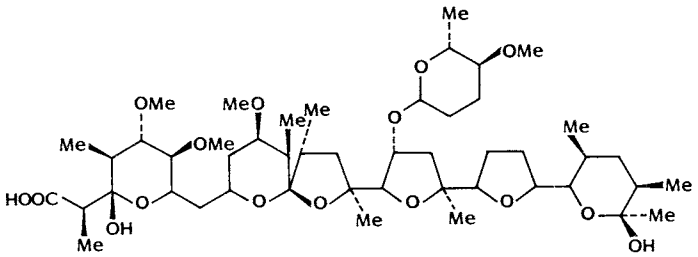


Lonomycin A R = CH_3-

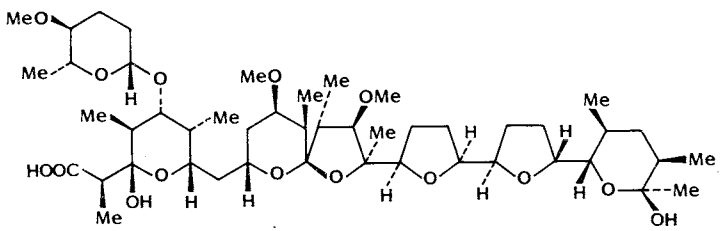
Lonomycin C R = H-



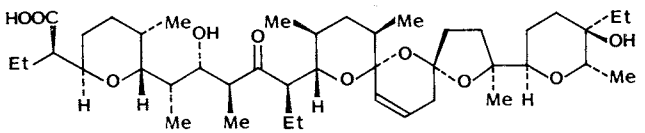
SF2487



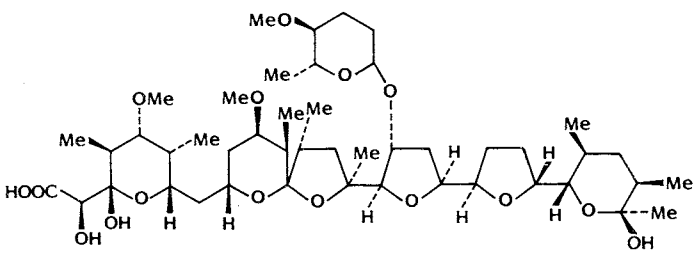
SF2361



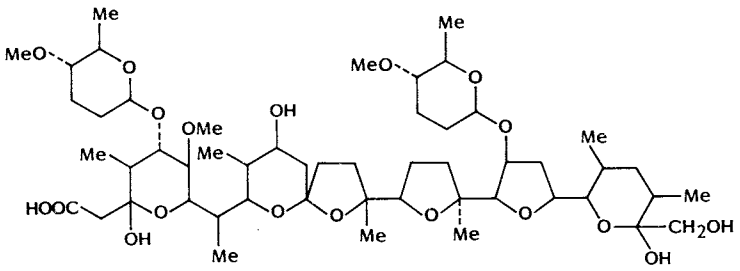
Carriomycin



Deoxy-salinomycin



Antibiotic 6016



SF2324

Table 1. Effect of polyether compounds on colchicine resistance in KB cells.

Polyether compound	KB-3-1			KB-C4		
	IC_{50}[a] (μg/ml)	Conc[b] (μg/ml)	D.P.[c] (fold)	IC_{50} (μg/ml)	Conc (μg/ml)	D.P. (fold)
Inostamycin	1.0	0.3	1.4	1.9	1	36.3
		1	1.3		3	305.3
Lysocellin	0.003	0.01	0.6	1.4	1	12.2
					3	9.7
Laidlomycin	0.0015	0.01	1.0	1.4	0.3	116.0
					1	725.0
Monensin	0.02	0.01	1.0	5.0	1	17.6
		0.03	1.2		3	103.4
Dianemycin	0.13	0.3	1.2	0.65	1	30.0
		1	0.8		3	116.1
Leuseramycin	0.12	0.3	1.1	1.0	1	2.9
		1	0.8		3	121.1
Nigericin	0.12	0.1	1.5	0.17	0.1	0.66
		0.3	0.8		0.3	1.4
Lonomycin A	0.9	1	1.0	4.5	10	17.7
Lonomycin C	27		NT[d]	50	3	13.9
					10	21.3
Carriomycin	0.36	0.3	0.4	>10	3	1.2
					10	11.8
Deoxy-salinomycin	0.65	0.3	1.7	5.0	0.3	1.7
		1	1.5		1	1.4
Antibiotic 6016	0.015	0.01	0.7	2.7	1	0.5
		0.03	0.6		3	0.7
SF2324	0.17	0.1	1.7	2.5	1	4.2
		0.3	1.7		3	16.5
SF2361	0.08	0.03	1.3	9.0	3	1.2
		0.1	0.8		10	1.4
SF2487	0.17	0.1	2.4	0.3	0.1	1.0
		0.3	1.6		3	2.5

[a] IC_{50} of polyether compound.
[b] Added concentration of polyether compound.
[c] Degree of potentiation.
[d] NT, not tested.
Values are means of duplicate determinations.

IC_{50} value of colchicine in the presence of the polyether compound. As reported before,[8] inostamycin potentiated colchicine cytotoxicity only toward KB-C4 cells but not toward KB-3-1 cells. When other polyether compounds were added at the optimal dose for chemosensitization, 4 out of 14 of them showed more than 100-fold potentiation of colchicine cytotoxicity. They were laidlomycin, monensin, dianemycin, and leuseramycin (Table 1). Among them, laidlomycin was the most effective, potentiating the cytotoxicity of colchicine toward KB-C4 cells 116 and 725 times at 0.3 and 1 μg/ml, respectively. Monensin at 3 μg/ml also enhanced it 103 times. Although dianemycin and leuseramycin at 3 μg/ml potentiated it more than 100 times, their effective concentrations were higher than their own IC_{50}'s. None of the effective polyether compounds affected the colchicine cytotoxicity toward KB-3-1 cells within the concentrations used. Interestingly, KB-C4 cells were resistant to most polyether compounds used.

Although lysocellin has a structure very similar to that of inostamycin (Fig. 1), it didn't reverse colchicine-resistance in KB-C4 cells. Furthermore, unlike inostamycin, lysocellin showed cross-resistance

Fig. 2. Effect of lysocellin and inostamycin on accumulation of [^{3}H]VBL in KB cells.

KB-C4 (closed plots) or KB-3-1 (open plots) cells were incubated with 17 nM [^{3}H]VBL with lysocellin (●, ○) or inostamycin (▲, △) for 2 hours. Values are means ± SD of triplicate determinations.

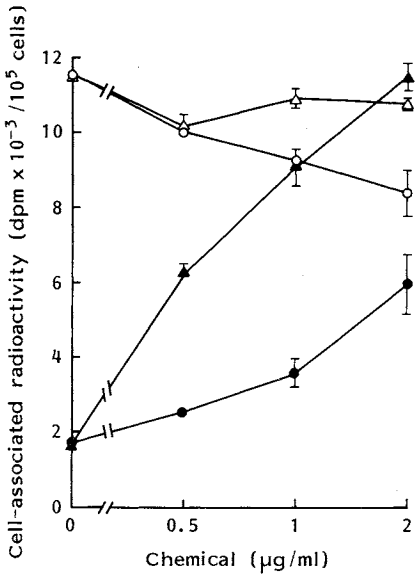
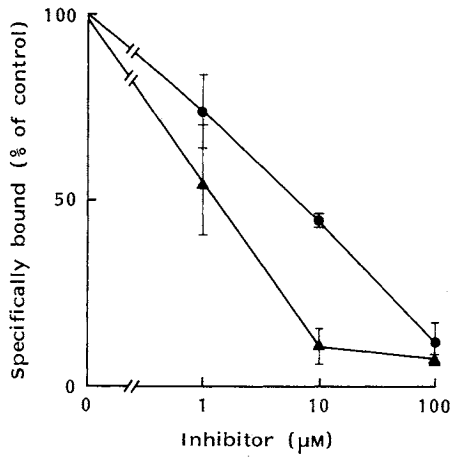


Fig. 3. Inhibition of [^{3}H]VBL binding to plasma membranes of KB-C4 cells.

KB-C4 membranes were incubated with 0.17 μM [^{3}H]VBL and lysocellin (●) or inostamycin (▲) at 25°C for 20 minutes. Values are means ± SD of triplicate determinations.



in KB-C4 cells. To study the mechanism for the lack of a reversing effect, we examined the effect of lysocellin on 2 hours accumulation of [^{3}H]VBL compared with that of inostamycin. Fig. 2 shows that in the absence of test chemicals the amount of [^{3}H]VBL accumulated in KB-C4 cells was about 6 times lower than that in KB-3-1 cells. As reported previously,[9] inostamycin increased [^{3}H]VBL accumulation only in KB-C4 cells up to the control level of KB-3-1 cells. On the other hand, lysocellin at 0.5 ~ 2 μg/ml slightly increased the amount of [^{3}H]VBL in KB-C4 cells, but not up to the level of KB-3-1 cells. Furthermore, it decreased the accumulation in KB-3-1 cells slightly. Since lysocellin at 5 μg/ml weakly inhibited the efflux of [^{3}H]VBL from KB-C4 cells (data not shown), we examined the effect of lysocellin on [^{3}H]VBL binding to KB-C4 plasma membranes. As we expected, lysocellin inhibited [^{3}H]VBL binding at higher concentrations than inostamycin, with an IC_{50} of 4.35 μg/ml (6.6 μM) (Fig. 3).

Discussion

Laidlomycin, monensin, dianemycin, and leuseramycin were found to reverse drug resistance. Although monensin and nigericin were reported to reverse doxorubicin resistance in P388 cells,[12] nigericin was not effective in our assay system. This discrepancy may be due to the difference in cell lines. Laidlomycin and monensin are structurally related compounds, in which only two side chains are different (Fig. 1). Dianemycin and leuseramycin are also related compounds, in which only one side chain is different (Fig. 1). Although lysocellin has a structure very similar to that of inostamycin (Fig. 1), it couldn't reverse drug resistance. Lysocellin inhibited the efflux of [^{3}H]VBL from KB-C4 cells and also inhibited [^{3}H]VBL binding to KB-C4 membranes at higher concentrations than inostamycin. Therefore, it couldn't increase the [^{3}H]VBL accumulation in KB-C4 cells as efficiently as inostamycin when used at the same concentration. Thus, lysocellin would essentially have a reversal effect on drug resistance; however, its effective concentration is so high that it is only cytotoxic in long-term incubation. Both inostamycin and lysocellin

inhibit phosphatidylinositol turnover.[13] Therefore, the possibility that chemosensitization by inostamycin is due to inhibition of cellular phosphatidylinositol turnover is apparently unlikely.

Acknowledgments

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