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SELECTIVE ENHANCEMENT OF BLEOMYCIN CYTOTOXICITY BY LOCAL ANESTHETICS [1)]

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SUMMARY: The cytotoxic effect of the antitumor antibiotic bleomycin toward cultured mouse FM3A cells was greatly enhanced by exposure of the cells to local anesthetics either before or together with treatment with bleomycin. Such local anesthetics include dibucaine, tetracaine, butacaine, lidocaine and procaine. Dibucaine-induced cell sensitization to bleomycin cytotoxicity produced a decrease in cell survival that became dependent on dose and time of bleomycin treatment. This effect of local anesthetics seems to be selective to bleomycin, since dibucaine and lidocaine do not enhance the cytotoxic effect of other antitumor agents including adriamycin, mitomycin C and cis-diamminedichloroplatinum(II).

Bleomycins are a group of glycopeptide antibiotics and currently used in the treatment of human neoplastic disease, particularly squamous cell carcinoma, lymphomas and testicular carcinoma (1). Bleomycin is unique in that myelosuppression does not occur as a consequence of its use, although pulmonary toxicity is a major side effect. The primary action of bleomycin that leads to stop of cell proliferation is thought to be breakage of cellular DNA (1,2).

We have shown previously that the cell killing of bleomycin *in vitro* is markedly potentiated by exposing cells to ethanol either before or after treatment with bleomycin (3,4). Ethanol is shown to exert its biological effect by disorganizing the

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ABBREVIATIONS: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Cisplatin, Cis-diamminedichloroplatinum(II).

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structural arrangement of lipids in cell membranes and disrupting membrane function (5,6). Our findings, therefore, suggest a role of membranes in the ethanol-induced cell sensitization to bleomycin cytotoxicity. Local anesthetics are clinically useful compounds that exert pharmacological effect by blocking nerve impulse propagation. Although molecular mechanisms of action of these drugs are not fully understood, it is generally thought that local anesthetics produce the effect by interaction with cell membranes (7,8). This type of drug-membrane interaction is not confined to neural membrane alone. Indeed, local anesthetics have been used in many studies to modify membrane-mediated cellular processes (9-12). Therefore, we have studied the effect of local anesthetics on the response of cultured mammalian cells to bleomycin cytotoxicity. In this paper, we will show that local anesthetics selectively enhance the cell killing of bleomycin.

MATERIALS AND METHODS

Cells FM3A cells originally established from a spontaneous mammary carcinoma in C3H mice (13) were maintained as a suspension culture in Eagle's minimum essential medium supplemented with 0.1% Bactopeptone (Difco) and 10% calf serum (Flow) in a CO_2 incubator (95% air and 5% CO_2).

Drugs Bleomycin used in the experiments was a mixture of bleomycins and the main component was bleomycin A_2 (55 to 70% content). Bleomycin and cisplatin were supplied by Nippon Kayaku Co., Tokyo. Adriamycin and mitomycin C were supplied by Kyowa Hakko Co., Tokyo. Procaine-HCl, lidocaine, tetracaine-HCl, dibucaine-HCl and butacaine hemisulfate were purchased from Sigma.

Treatment of Cells with Bleomycin and Local Anesthetics FM3A cells were used at the exponential growth phase. The cells (1 to 1.2 x 10^5 cells/ml) were suspended in 2 ml of fresh growth medium supplemented with 5 mM HEPES buffer (pH 7.5) and incubated with bleomycin with or without local anesthetics at 37°C for 1 hr. After incubation, the cells were chilled in an ice bath, washed once with 2 ml of ice-cold Hanks' balanced salt solution by centrifugation at 4°C and suspended in 1 ml of growth medium for survival determination.

Determination of Cell Survival Cell survival was determined by following clonal cell growth in a soft agar medium. A 4% solution of Noble agar (Difco) was added to Eagle's minimum essential medium supplemented with 0.1% Bactopeptone and 15% calf serum to

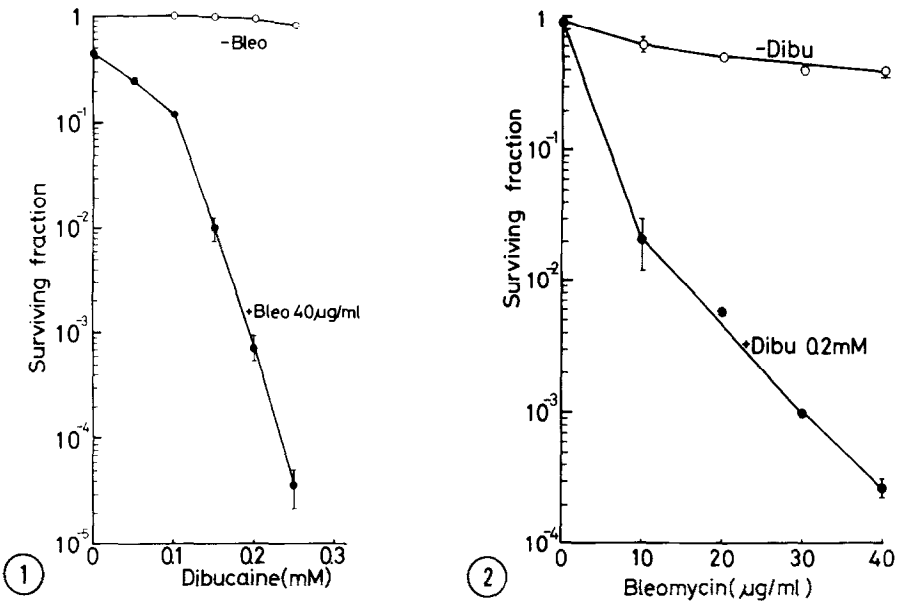

Fig. 1. Enhancement of bleomycin cytotoxicity by dibucaine. FM3A cells were incubated with or without bleomycin (40 μg/ml) in the presence of various concentrations of dibucaine at 37°C for 1 hr. Bars, S.D.

Fig. 2. Effect of dibucaine on the cell killing by graded doses of bleomycin. FM3A cells were incubated with the indicated doses of bleomycin with or without dibucaine (0.2 mM) at 37°C for 1 hr.

give an agar concentration of 0.13%. Serial 10-fold dilutions were prepared from control and experimental cell populations, 0.8 ml aliquots of the appropriate cell dilution were added to 12 x 75 mm plastic tubes (Falcon), and 3 ml of nutrient agar solution were mixed with the diluted cell suspension by inversion. Duplicate tubes were prepared for each cell dilution. The tubes were placed in an ice bath for 20 min, kept at room temperature for 30 min, and then incubated at 37°C for 12 days in a CO_2 incubator for colony formation. Colonies were counted and the mean cloning efficiency was determined. The cloning efficiency of control cells was more than 95%.

RESULTS

The effect of various doses of dibucaine on bleomycin cytotoxicity is shown in Fig. 1. The survival of cells treated with bleomycin in the presence of dibucaine at concentrations of more than 0.1 mM decreased depending on the doses of the local anesthetic Dibucaine alone showed no or only a slight cytotoxicity at these concentrations. Fig. 2 shows that the decrease in survival of cells treated with graded doses of bleomycin with a fixed concentration

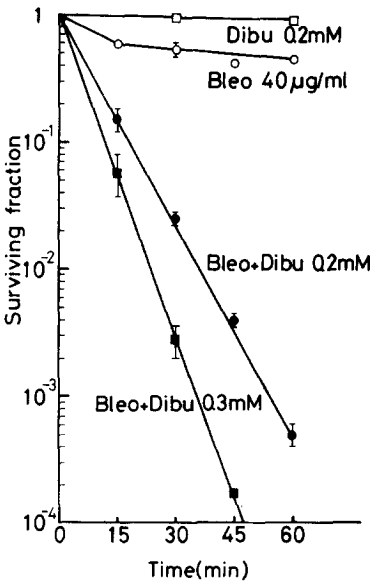

Fig. 3. Effect of time of the treatment with dibucaine and bleomycin. FM3A cells were incubated with bleomycin(40 μg/ml) with or without dibucaine (0.2 and 0.3 mM) at 37°C for 1 hr.

of dibucaine (0.2 mM) becomes dependent on the doses of bleomycin compared with the survival decrease of cells treated with bleomycin alone. Further, Fig. 3 shows that the survival of cells treated with bleomycin with dibucaine (0.2 and 0.3 mM) becomes exponentially decreased depending on the time of drug treatment, while the survival decrease of cells treated with bleomycin alone is rather biphasic. These results clearly indicate that the local anesthetic dibucaine sensitizes cells to the killing by bleomycin.

Table 1 shows that several other local anesthetics including procaine, lidocaine, butacaine and tetracaine also greatly potentiate the cell killing activity of bleomycin. Of the local anesthetics examined dibucaine showed the strongest potentiating activity, and the potency of each local anesthetic to enhance the cytotoxicity was correlated with its anesthetic potency (8).

The similar enhancement of bleomycin cytotoxicity was also observed by exposing cells to dibucaine before treatment with

Table 1. Enhancement of the cytotoxic effect of bleomycin by various local anesthetics

	Surviving fraction	
	-Bleomycin	+Bleomycin
none	1.00	0.45
Procaine 12 mM	1.00	5.0×10^{-3}
Lidocaine 10 mM	0.96	8.1×10^{-4}
Butacaine 1.5 mM	0.90	1.7×10^{-4}
Tetracaine 0.5 mM	0.85	4.4×10^{-4}
Dibucaine 0.2 mM	0.92	3.8×10^{-4}

FM3A cells were incubated with or without bleomycin (40 μg/ml) in the presence of local anesthetics at 37°C for 1 hr.

bleomycin. In this case, however, the enhancing effect of dibucaine was maximum when bleomycin treatment immediately followed dibucaine treatment, and it rapidly decreased as the time interval between the two treatments was increased. After a 2 hr interval, dibucaine did not show any sensitizing effect to bleomycin cytotoxicity (data not shown). The exposure of cells to dibucaine after tretment with bleomycin only slightly enhanced the cytotoxicity (data not shown).

Table 2 shows the effect of dibucaine on the cytotoxic effect of several other antitumor agents. Dibucaine did not induce any cell sensitization to adriamycin, mitomycin C and cisplatin. Similar results were also obtained by using lidocaine (data not shown). The results suggest that local anesthetics selectively potentiate the cell killing of bleomycin.

DISCUSSION

In the present paper, we have demonstrated that local anesthetics selectively potentiate bleomycin cytotoxicity. It is generally thought that the primary target of action of local anesthetics is cell membranes (7,8). Studies on the interaction of local anesthetics with artificial lipid membranes have shown that there

Table 2. Effect of dibucaine on the cytotoxic effect of various antitumor agents

	Surviving fraction	
	-Dibucaine	+Dibucaine
none	1.00	0.90
Adriamycin 0.2 μg/ml	0.50	0.38
Mitomycin C 1.0 μg/ml	0.34	0.44
Cisplatin 0.5 μg/ml	0.12	0.19
Bleomycin 40 μg/ml	0.39	2.7×10^{-4}

FM3A cells were incubated with the antitumor drugs with or without dibucaine (0.2 mM) at 37°C for 1 hr.

is a good correlation between the ability of local anesthetics to interact with phospholipids and their ability to modify membrane properties (14,15). Therefore, it may be thought that the mechanism of cell sensitization to bleomycin by local anesthetics is related to changes in membrane properties that result in an increased transport of bleomycin through the cell membranes or decreased efflux of bleomycin after it enters into cells. It has been shown that some polyene macrolide antibiotics which interact with sterols in cell membranes enhance the cytotoxic effect of bleomycin by increasing its cellular transport into cells (16). The mechanism of transport of bleomycin has not been established. Uehara et al. (17) recently showed that there were saturable and non-saturable components of the uptake of bleomycin by using tritium labeled pepleomycin, a new derivative of bleomycin group antibiotics. We are currently investigating the effect of local anesthetics on the transport of bleomycin using the labeled bleomycin derivative.

It has been demonstrated that bleomycin cytotoxicity is potentiated by hyperthermia (43°C) (18,19). The combination of bleomycin and hyperthermia with or without X-irradiation

has been clinically applied to patients with bladder cancer by irrigating the bladder cavity with a hot solution of bleomycin. Our recent *in vitro* studies have shown that the local anesthetic lidocaine and moderate hyperthermia (41°C) interact and synergistically induce a cell sensitization to bleomycin cytotoxicity (unpublished). It may be suggested therefore, that a warmed solution of lidocaine might be used for treating bladder cancer to sensitize the tumor cells to bleomycin cytotoxicity.

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