Potentiation of Bleomycin by an Antifungal Polyene, Pentamycin, in Transformed Animal Cells

Tadashi Nakashima, Michihiko Kuwano, Katsuko Matsui, Sohtaro Komiyama, Ikuichiro Hiroto, and Hideya Endo

Cancer Research Institute [T. N., M. K., K. M., H. E.] and Department of Otorhinolaryngology [S. K., I. H.], Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

SUMMARY

Bleomycin, a potent antitumor agent against human squamous cell carcinoma, was examined in vitro to determine whether its action is increased under synergistic conditions with a polyene antibiotic, pentamycin. The inhibition of DNA, RNA, and protein synthesis by bleomycin was greatly enhanced by combination with pentamycin in tissue culture. However, in comparison with bleomycin or α-amanitin, there was less enhancement of action of both 5-fluorouracil and fusidic acid by pentamycin.

INTRODUCTION

The synergistic use of various chemical agents with polyene antibiotics (5, 7), which alter membrane permeability, provides a valuable approach to cancer chemotherapy. Amphotericin B, a heptane polyene, was shown to increase greatly the action of fusidic acid, 5-fluorouracil, chromomycin A₃, or α-amanitin when each agent alone had little or no effect in tissue culture (9, 11–13). Independently, rifampicin, rifampicin analogs, and 1,3-bis(2-chloroethyl)-1-nitrosourea each showed strengthened action by amphotericin B in leukemic and L-cells (4, 14–16).

In vitro synergism showed that the action of bleomycin, a potent anticancer agent (6, 21), alone or in combination with amphotericin B, was inert against our transformed fibroblastic cells, but bleomycin could be enhanced when combined with a high dose of polymyxin B, which is not a polyene compound (12). The loss of bleomycin action in the absence of polymyxin B might be due to its inability to penetrate the membrane. Therefore, the experiment was designed to determine whether other polyene antibiotics could enhance its action in vitro, and in the present paper we report that pentamycin, an antifungal macrolide antibiotic (20), can increase the action of bleomycin possibly through increasing its permeability.

MATERIALS AND METHODS

Isotopes and Chemical Compounds. Leucine-³H (32 Ci/m mole; Daiichi Pure Chemical Co., Tokyo, Japan), uridine⁻¹⁴C (50 μCi/230 μg; New England Nuclear, Boston, Mass.), and thymidine⁻³H (20 Ci/m mole; New England Nuclear) were used as isotopes. 5-Fluorouracil (Kyowa Hakko Ind. Co., Tokyo, Japan), fusidic acid (Sankyo Med. Co., Tokyo, Japan), and α-amanitin (Lot A 32778; Boehringer/Mannheim, Mannheim, Germany) were used for the study of synergism. Bleomycin A₂ was a gift from H. Umezawa. Amphotericin B was purchased from Grand Island Biological Co., Grand Island, N. Y., and pentamycin was a gift from Nikken Chemical Co., Tokyo, Japan. The antifungal activity of pentamycin was stable over weeks in various solvents (20); in this case, pentamycin was dissolved in dimethyl sulfoxide.

Cells and the Study of Synergism. Transformed rat fibroblastic cells, RFL-T, were used throughout this experiment; characteristics of this cell line have been described previously (10). Some 1 to 2 × 10⁵ cells/ml of RFL-T cells cultured in 3 ml Eagle's essential medium in Petri dishes were exposed to leucine⁻³H (1 μCi/ml), uridine⁻¹⁴C (0.05 μCi/ml), or thymidine⁻³H (1 μCi/ml) for 18 hr under the various conditions described in the text, and 10% trichloroacetic acid insoluble fraction was counted on glass filter paper as described earlier (9).

RESULTS

Enhancement of Action of Bleomycin by Pentamycin in Vitro. Pentamycin alone inhibited DNA or RNA synthesis of RFL-T cells only slightly (less than 20% of control) within the concentration of 2 μg/ml, but almost complete blockage was observed at 5 μg/ml (Chart 2). We attempted to determine whether this pentane polyene could enhance the action of bleomycin in tissue culture using transformed fibroblastic RFL-T cells. Since bleomycin was known to inhibit DNA synthesis in HeLa cells (19), the effect under synergistic conditions was assayed by measuring cellular DNA and RNA formation (Chart 1). The dose-response curve of bleomycin shows that the inhibition of both DNA and RNA formation by bleomycin was enhanced greatly only when it was combined with pentamycin (0.5 and 1.5 μg/ml). The extent of inhibition of DNA synthesis increased with increasing doses of pentamycin, as clearly seen in Chart 1A (see, for example, the activity at bleomycin (50 or 75 μg/ml) with/without polyene). Then, in presence of bleomycin (50 μg/ml), the dose response of pentamycin to RNA synthesis was examined (Chart 2). The inhibition of RNA synthesis by the antitumor agent could apparently be magnified when the concentration of pentamycin...
cin was increased, although a high dose of polyene (5 μg/ml) alone was enough to inhibit RNA synthesis completely. These data strongly suggest that the pentane polyene enhances the action of bleomycin possibly as a function of the increase of intracellular concentration in transformed cells in vitro.

Morphological examination of the synergistic combination of bleomycin (50 μg/ml) and pentamycin (1.5 μg/ml) was carried out by microscope. As shown in Fig. 1, almost all of the cells were detached, and fibroblastic cells transformed into round ones only when both agents were present together. Little change in cell morphology was observed when each agent was present alone (Fig. 1).

**Potentiation of Various Chemical Agents by Pentamycin.** In

![Chart 1](image1)

**Chart 1.** Dose response of bleomycin in absence or presence of pentamycin on DNA (A) and RNA (B) synthesis. RFL-T cells were exposed to various concentrations of bleomycin A2 alone (○) or in combination with pentemycin [0.5 μg/ml (■) or 1.5 μg/ml (●)] for 18 hr, and thymidine-3 H (A) or uridine-4 C (B) incorporation into acid-insoluble fraction was measured as described in "Materials and Methods." The normalized activity is presented when 100% corresponds to cpm in absence of bleomycin A2.

![Chart 2](image2)

**Chart 2.** Dose response of pentamycin in absence or presence of bleomycin on RNA synthesis. RFL-T cells were exposed to various doses of pentamycin in absence (○) or presence of bleomycin A3 (50 μg/ml) (●) with uridine-14 C (0.5 μCi/ml) for 18 hr. Normalized activity of RNA synthesis is shown when 100% corresponds to 1200 cpm in absence of drug and 1000 cpm in presence of bleomycin alone, respectively, when background (50 cpm) is subtracted.

![Fig. 1](image3)

**Fig. 1.** RFL-T cells (1 × 10^4 cells/ml) in Petri dish treated for 18 hr under synergistic conditions. B, with bleomycin (50 μg/ml) alone; C, with pentamycin (1.5 μg/ml) alone; D, with bleomycin (50 μg/ml) and pentamycin (1.5 μg/ml) together. A, untreated control cells.
our system using RFL-T cells, previous study showed that the action of fusidic acid, 5-fluorouracil, and α-amanitin, but not bleomycin, was increased markedly in each case by amphotericin B (9, 11–13). Therefore, the membrane-activating action of pentamycin was analyzed to determine whether it enhanced these various chemical agents. As shown in Table 1, pentamycin, unlike amphotericin B, increased only slightly the action of fusidic acid or 5-fluorouracil, although the action of α-amanitin and of bleomycin was enhanced by pentamycin. Treatment with pentamycin did not alter the specificity of 2nd agents because α-amanitin preferentially inhibited RNA synthesis without markedly affecting protein synthesis, and bleomycin also rather specifically inhibited DNA or RNA synthesis (Table 1; Chart 1). The dosage of 5-fluorouracil or fusidic acid used here might have been too low to show increasing effect by pentamycin, but amphotericin B was previously shown to increase the penetration when combined with the same dose of these agents (12, 13).

### DISCUSSION

Bleomycin showed a much higher sensitivity to HeLa cells than to fibroblastic RFL-T cells (12), which might be attributed in part to the differences in the abilities of the two cell lines to permeate the agent. When the membrane barrier to bleomycin could be overcome by polymyxin B (12) or pentamycin, fibroblastic cells were sensitive against the antitumor agent. The mechanism involved here might be similar to that in an Escherichia coli mutant sensitive to bleomycin, which exhibited greater permeability to the agent (22). Since amphotericin B increased the action of 5-fluorouracil or fusidic acid more effectively than did pentamycin (9, 12, 13), these 2 polyenes seemed to control the permeability differently. The differential abilities of polyene antibiotics to enhance membrane permeability could be closely correlated with the affinity of polyenes to sterols (2, 5, 7).

On the other hand, filipin III and lucensomycin, polyene antibiotics, are shown to have rather preferential affinity for the membrane of malignant cells (18). The differential sensitivity to amphotericin B among established cell lines in vitro suggested a positive correlation between levels of density-dependent inhibition of cell growth and the sensitivity (1). In addition, amphotericin B increased the therapeutic action of 1,3-bis(2-chloroethyl)-1-nitrosourea against a transplanted AKR leukemia (17). These data could give us a clue to finding a specific control of tumor chemotherapy through modifying the permeation of antitumor agents. Synergistic usage of antitumor agents with less toxic membrane-active agents such as vitamin A was partly successful in vivo as well as in vitro (8, 16). In particular, therapeutic effects of 1,3-bis(2-chloroethyl)-1-nitrosourea and cyclophosphamide were enhanced by vitamin A in mice bearing leukemia (3). During our application over 1.5 years of the synergism of 5-fluorouracil and vitamin A in combination with 60Co (8) to clinical chemotherapy of laryngeal carcinoma, we have obtained satisfactory results thus far (manuscript in preparation).

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### REFERENCES

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