Ethanol-induced Cell Sensitization to Bleomycin Cytotoxicity and the Inhibition of Recovery from Potentially Lethal Damage

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ABSTRACT

The cytotoxic effect of bleomycin toward mouse mammary carcinoma FM3A cells in culture was markedly potentiated by ethanol exposure before drug treatment. Ethanol-induced cell sensitization to bleomycin cytotoxicity produced a decrease in cell survival which became dependent on dose and time of drug treatment. The sensitizing effect of ethanol after drug treatment was maximum when ethanol exposure immediately followed drug treatment, and it rapidly decreased as the time interval between the two treatments was increased. Ethanol did not sensitize cells to the cytotoxic effects of cis-diamminedichloroplatinum(II) and macromycin. These results are discussed in terms of the ability of ethanol to inhibit cell recovery from potentially lethal damage after bleomycin treatment.

INTRODUCTION

Exposure of various types of cultured mammalian cells to the antitumor agent bleomycin in combination with 43° hyperthermia markedly enhances the cytotoxicity over that seen at 37° (6, 9, 16). A synergistic antitumor effect of bleomycin in combination with local hyperthermia has been demonstrated in tumor-bearing animals (11, 12). These findings suggest the potential clinical usefulness of this thermochemotherapy in cancer treatment. With regard to the mechanism of hyperthermic enhancement of bleomycin cytotoxicity, there have been some reports (3, 27) which indicate that hyperthermia either exerts its biological effect by increasing membrane fluidity in mammalian cells (4, 19). We therefore examined the effect of ethanol on cell sensitivity to bleomycin cytotoxicity. In this paper, we show that ethanol induces a marked cell sensitization to bleomycin cytotoxicity when cells are exposed to ethanol before or after bleomycin treatment. The possible mechanisms of the sensitization are discussed.

MATERIALS AND METHODS

Cells. FM3A cells originally established from a spontaneous mammary carcinoma in C3H mice (18) were maintained as a suspension culture in Eagle’s minimum essential medium supplemented with 0.1% Bacto-peptone (Difco Laboratories, Detroit, Mich.) and 10% calf serum (Flow Laboratories, Rockville, Md.) in a CO₂ incubator (95% air and 5% CO₂).

Drugs. Bleomycin used in the experiments was a mixture of bleomycins (the main component is bleomycin A₂; 55 to 70% content) and was supplied by Nippon Kayaku Co., Tokyo, Japan. cis-Diamminedichloroplatinum(II) was supplied by Nippon Kayaku Co., and macromycin was supplied by Kanegafuchi Chemical Industry, Takasago, Japan. The drugs were dissolved in 0.9% NaCl solution. Ethyl alcohol (99.5%) was obtained from Showa Chemicals Co., Tokyo, Japan.

TREATMENT OF CELLS WITH BLEOMYCIN AND ETHANOL. FM3A cells were used at the exponential growth phase. The cells (1 to 1.5 x 10⁶ cells/ml) were suspended in 2 ml of fresh growth medium supplemented with 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.2) and incubated with bleomycin at 37°C for 40 min, unless otherwise indicated. 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 2°C, and then exposed to ethanol at 37°C for 40 min in fresh growth medium (pH 7.2). After ethanol exposure, the cells were washed with Hanks’ balanced salt solution and suspended in 1 ml of growth medium for survival determination. When cells were first exposed to ethanol at 37°C for 40 min, they were washed once for removal of ethanol and then treated with bleomycin at 37°C for 40 min in fresh growth medium (pH 7.2).

DETERMINATION OF CELL SURVIVAL. Cell survival was determined by following clonal growth in a soft agar medium. A 4% solution of Noble agar (Difco) was added to Eagle’s minimal essential medium supplemented with 0.1% Bacto-peptone and 15% calf serum to give an agar concentration of 0.13%. Serial 10-fold dilutions (10⁻⁶ to 10² cells/ml) 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 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 14 days in a CO₂ incubator for colony formation. Tubes containing 10 to 100 colonies were scored, and the mean cloning efficiency was determined. Each experiment was repeated twice. The cloning efficiency of untreated cells was more than 95%.

RESULTS

Enhancement of Bleomycin Cytotoxicity by Exposure of Cells to Ethanol. When the cells were exposed to 4.8 to 5.9% ethanol and then treated with bleomycin after removal of the ethanol, the survival of cells greatly decreased depending on the concentrations of ethanol (Chart 1). This synergistic cytotoxicity indicates that ethanol induces cell sensitization to bleomycin cytotoxicity. Chart 1 also shows that cell sensitization to bleomycin is induced by ethanol exposure after drug treatment, although the sensitizing effect is decreased compared with that by ethanol exposure before drug treatment. The cell sensitization induced by the exposure to 5.9% ethanol increased progressively as the time of ethanol exposure after drug treatment was increased (Chart 2).

The survival of cells which were exposed of 6.1% ethanol after the treatment with graded doses of bleomycin decreased depending on the drug doses, instead of exhibiting the biphasic...
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Chart 1. Effect of ethanol exposure before or after bleomycin treatment on survival of FM3A cells. Cells were exposed to various concentrations of ethanol (O), and then treated with bleomycin (30 µg/ml) (●). Cells were first treated with bleomycin and then exposed to ethanol (●). Bars, S.D.

Chart 2. Effect of time of ethanol exposure after drug treatment. Cells were treated with bleomycin (30 µg/ml) and then exposed to 5.9% ethanol for the indicated times. O, without bleomycin; ●, with bleomycin. Bars, S.D.

Effect of Time Interval between Bleomycin Treatment and Ethanol Exposure. To examine the effect of time interval between drug treatment and ethanol exposure on the sensitization, the cells were first treated with bleomycin (30 µg/ml), washed free of the drug, and then either exposed immediately to 5.9% ethanol or incubated at 37° or allowed to remain at 0° in fresh growth medium for 15 min to 2 hr, and then exposed to ethanol. As shown in Chart 5, survival rapidly increased at 37° during the first 30 min incubation and then gradually increased for the next 90 min; after a 2-hr interval, it reached the level corresponding to the additive effect of the 2 treatments. This recovery of cell survival was almost completely inhibited at 0°. The recovery was not affected by the presence

response shown in the survival of the cells which were treated with bleomycin alone (Chart 3). The survival of cells treated with a fixed dose of bleomycin (30 µg/ml) for various times also showed a biphasic response. However, the exposure to ethanol either before or after drug treatment produced survival decreases which were dependent on the times of drug treatment (Chart 4).

Effect of Time Interval between Bleomycin Treatment and

Chart 3. Survival of FM3A cells exposed to ethanol after the treatment with graded doses of bleomycin. Cells were treated with bleomycin (O) and then exposed to 6.1% ethanol (●). Bars, S.D.

Chart 4. Survival of FM3A cells exposed to ethanol before or after bleomycin treatment for various times. Cells were treated with bleomycin (30 µg/ml) (O) and then exposed to 5.9% ethanol (●). Cells were first exposed to 5.7% ethanol and then treated with bleomycin (●). The survival values were normalized with respect to the corresponding ethanol toxicities. Bars, S.D.
Ethanol-induced Sensitization to Bleomycin

The data presented in this paper indicate that ethanol induces a marked cell sensitization to bleomycin cytotoxicity when cells are exposed to ethanol immediately after bleomycin treatment. The sensitizing effect of ethanol was, however, rapidly decreased by delay of the ethanol exposure after drug treatment. We showed previously that using [3H]pepleomycin, a new bleomycin derivative with lower pulmonary toxicity (24), the cellular uptake of the drug did not increase after ethanol exposure or 43° hyperthermia (17). These findings indicate a synergistic interaction between ethanol and hyperthermia in inducing a cell sensitization to bleomycin and suggest a similar mechanism of the sensitizations.

The cytotoxic effects of cis-diamminedichloroplatinum(II) and macromomycin, which induce DNA cross-linking (13) and DNA strand scission (22), respectively, were not affected by ethanol exposure but were enhanced by 43° hyperthermia. On the other hand, it is noteworthy that the cytotoxic effect of X-irradiation is enhanced both by hyperthermia and by ethanol (10). Some of the cellular lesions and repair systems may be different for bleomycin and the platinum compound (data not shown).

ACKNOWLEDGMENTS

The author wishes to thank Drs. Hanao Umesawa and Suehiko Okamoto for helpful suggestions and encouragement during the course of these studies.

REFERENCES


OCTOBER 1981

4113


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