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

Satoshi Mizuno

Department of Antibiotics, National Institute of Health, Shinagawa-ku, Tokyo 141, Japan

ABSTRACT

The cytotoxic effect of bleomycin toward mouse mammary carcinoma FM3A cells in culture was markedly potentiated by exposure of the cells to ethanol either before or after treatment with bleomycin. 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°C hyperthermia markedly enhances the cytotoxicity over that seen at 37°C (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 inhibits repair of bleomycin-induced potentially lethal damage or increases initial damage by bleomycin rather than repairing inhibition. Recently, Li et al. (10) reported that cell inactivation by hyperthermia closely resembles that by ethanol, which 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 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 (10⁻⁴ to 10⁴ 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

Received March 17, 1981; accepted June 18, 1981.

OCTOBER 1981

4111
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° 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

---

Chart 1. Effect of ethanol exposure before or after bleomycin treatment on survival of FM3A cells. Cells were exposed to various concentrations of ethanol (○), 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. ○, without bleomycin; ●, with bleomycin. Bars, S.D.

Chart 3. Survival of FM3A cells exposed to ethanol after the treatment with graded doses of bleomycin. Cells were treated with bleomycin (○) 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) (○) and then exposed to 5.9% ethanol (●). Cells were first exposed to 5.9% ethanol and then treated with bleomycin (●). The survival values were normalized with respect to the corresponding ethanol toxicities. Bars, S.D.

---

Effect of Time Interval between Bleomycin Treatment and
of cycloheximide (20 μM) during the 2-hr incubation, which inhibited protein synthesis more than 90% when estimated by [14C]leucine incorporation (data not shown).

Effect of Ethanol on the Cytotoxic Effects of Other Antitumor Agents. The effect of ethanol exposure on cell sensitivity to other antitumor agents was examined. The cytotoxic effects of cis-diaminedichloroplatinum(II) and macromomycin, a polypeptide antibiotic which induces cellular DNA strand scission (22), were not enhanced by ethanol exposure before drug treatment, whereas both cytotoxic effects were greatly potentiated by 43° hyperthermia, as reported by others (13) for the platinum compound (data not shown).

DISCUSSION

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]leucoplatinum, a new bleomycin derivative with lower pulmonary toxicity (24), the cellular uptake of the drug did not increase after ethanol exposure or 43° hyperthermic treatment (17). This indicates that the enhanced cytotoxicity is not correlated with a change in drug uptake. However, it is not clear whether the ethanol exposure prevents the efflux of the drug from the cells after it is taken up.

It has been demonstrated that cells can repair the potentially lethal damage induced by bleomycin in vitro (1, 2, 20, 26) and in vivo (7, 23, 25, 28). The repair mechanism may be related to the repair of DNA strand scission (5, 15), and/or breakage of a DNA-membrane complex (14) and of a DNA-protein complex (21). If we suppose that cell survival depends on the continuous function of this repair mechanism during and after drug treatment, the ethanol-induced cell sensitization after drug treatment can be explained by the inhibition of the repair mechanism by ethanol. The inhibition of repair converts bleomycin-induced potentially lethal damage to actually lethal damage. The rapid reduction in the sensitizing effect of ethanol (Chart 5) is probably due to the repair processes that take place during incubation at 37° before ethanol exposure (but not at 0°). Ethanol is shown to exert its biological effect by disorganizing the structural arrangement of lipids in cell membranes and disrupting membrane function (4, 19). The hypothesis of repair inhibition by ethanol implies, therefore, that some intact membrane structures (nuclear membranes) may be required for the repair processes. The present data might also be explained by an alternative hypothesis that ethanol exposure potentiates bleomycin action in the cells. Yamanaka et al. (28, 29) have shown that the action of bleomycin is strongly stimulated by the microsomal NADPH-dependent electron transport system, which produces free radicals. Ethanol might enhance the action of bleomycin by affecting the microsomal membrane system in the cells.

We recently found that the exposure of FM3A cells and HeLa S3 cells to combinations of lower concentrations of ethanol (1.5 to 4.0%) and moderate hyperthermia (39°–42°) greatly sensitized the cells to bleomycin, although the exposure to those concentrations of ethanol or the temperatures alone sensitized only weakly (8, 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-diaminedichloroplatinum(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 similar after bleomycin treatment and after X-irradiation. In addition, the mechanisms of hyperthermic enhancement may be different for bleomycin and the platinum compound or macromomycin.

ACKNOWLEDGMENTS

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

REFERENCES

S. Mizuno


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

Satoshi Mizuno


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/10/4111

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.