Effect of Ultraviolet Irradiation on the Antitumor Activity of Bleomycin

Hiromasa MORI

Laboratory of Theoretical and Molecular Biology, Faculty of Pharmaceutical Sciences, Kumamoto University, 5–1 Ohehonmachi, Kumamoto 862, Japan. *Received September 27, 1989*

The effect of bleomycin (BLM) on the growth of the tumor, murine B16 melanoma, implanted onto the chorioallantoic membrane of chick embryo was examined. The inhibition ratio of BLM was about 30% at 3 μg/egg. This inhibitory effect of BLM on the growth of the tumor was enhanced by an appropriate dose of ultraviolet (UV) irradiation. The inhibition ratios of BLM irradiated for 10, 30 and 60 min were in order of about 50, 30 and 30% at 3 μg/egg. However, when these BLMs pretreated with 1 mM 1,2-benzenediol (catechol) were administrated, the inhibition ratios of BLM irradiated for 0, 10, 30 and 60 min were about 30, 20, 10 and 10%, respectively. On the other hand, the direct cytotoxicity of BLM to cultured murine B16 melanoma cells was depressed with UV irradiation and its toxic activity was further decreased by treatment with catechol after irradiation. These findings show that, although the antitumor activity of BLM is enhanced by UV irradiation, the activity of UV-irradiated BLM is inhibited by catechol. Moreover, it seems to show that the present results may provide a useful manner for the *in vivo* activation of BLM.

Keywords bleomycin; ultraviolet irradiation; antitumor activity; cytotoxicity; murine B16 melanoma; chorioallantoic membrane; chick embryo

Bleomycin (BLM) is a family of glycopeptidic antibiotics isolated from *Streptomyces verticillus* which is clinically used for some cancers.[1] The BLM molecule is sensitive to ultraviolet (UV) light.[2-5] On the other hand, the activities related to deoxyribonucleic acid (DNA) of BLM, *e.g.*, breaking the DNA strand, releasing free bases from DNA and decreasing the melting temperature (T_m) of DNA, are enhanced by UV irradiation.[3,5-7] This photo-induced activation of BLM closely relates to the change of fluorescence at 350 nm.[3] Moreover, the sensitivity to deoxyribonuclease I (DNase I) of DNA and the DNA synthesis by DNA polymerase I with DNase I are enhanced by treatment with UV-irradiated BLM.[8] It is of interest to examine the effect of UV irradiation on the anticancer activity of BLM. In this study, the effects of UV irradiation on BLM's antitumor activity and direct cytotoxicity to cultured cells were investigated. The antitumor activity of BLM was examined by the reported method, which measures the growth of a tumor implanted onto the chorioallantoic membrane (CAM) of chick embryo.[9,10] It was demonstrated that the antitumor activity of BLM was also enhanced by an appropriate dose of UV irradiation. This was in harmony with the results *in vitro*.[3,7,8]

Data on the effects of anticancer drugs on tumor growth are summarized in Table I. Daunomycin (DAM), mitomycin C (MMC), methotrexate (MTX), 5-fluorouracil (5-FU), 1-(4-amino-2-methyl-5-pyrimidinyl)-1-methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU) and cyclophosphamide (CY) strongly inhibited the growth of tumor, murine B16-F10 melanoma, and vincristine sulfate (VCR) had low activity. Methotrexate and VCR were toxic to the chick embryo at doses of 30 and 1 μg/egg, respectively. The findings were approximately in accordance with the reported results[9,10] and the dose response to tumor growth of BLM showed that this method is sufficient to examine the antitumor activity of BLM. On the other hand, as shown in Table I, the mean tumor weights of the control group varied between experiments. It is considered that the difference in weight may have resulted from a change in the tumor's growing ability during the maintenance of cells.

It is reported that the *in vitro* activity of UV-irradiated BLM to react with DNA is inhibited by 1,2-benzenediol (catechol).[7] Table II shows the effect of UV irradiation on the antitumor activity of BLM in the presence or absence of catechol. When BLMs irradiated for the indicated times were administered at 3 μg/egg, the ability of BLM to depress tumor growth was enhanced by irradiation for 10 min and decreased with further irradiation. On the other hand, when the respective UV-irradiated BLMs were previously treated with 1 mM catechol for 60 min at 37 °C, the

TABLE I. Effects of Anticancer Drugs on Growth of Murine B16-F10 Melanoma Applied onto CAM of Chick Embryo by Intravenous Injection

Drug	Dose (μg/egg)	Tumor weight (mean ± S.D., mg)	Inhibition ratio (%)
DAM	0	62 ± 15	—
	30	49 ± 11	21
	100	19 ± 10[a]	69
MMC	0	51 ± 12	—
	10	38 ± 5	26
	30	11 ± 4[a]	78
MTX	0	86 ± 20	—
	3	49 ± 8[a]	43
	10	44 ± 8[a]	49
5-FU	0	75 ± 11	—
	100	53 ± 16[b]	29
	300	11 ± 3[a]	85
ACNU	0	78 ± 10	—
	100	13 ± 2[a]	83
CY	0	78 ± 10	—
	100	17 ± 5[a]	78
VCR	0	50 ± 7	—
	0.1	41 ± 10	18
BLM	0	100 ± 20	—
	1	98 ± 14	2
	3	69 ± 22	31
	10	65 ± 13[b]	35
	30	56 ± 8[a]	44
	100	37 ± 17[a]	63

Melanoma cells (5×10^4) were applied onto the CAM of 11-day-old embryo. Anticancer drug solutions (100 μl) were injected into a CAM vein 3 d after inoculation. After 4 d, the tumors excised from the CAM were weighed. The number of samples were 5 to 7 eggs. The experiment was repeated three times. *a*) $p < 0.01$ compared to control. *b*) $p < 0.05$ compared to control.

Table II. Effects of UV-Irradiated BLM Pretreated with or without Catechol on Growth of Murine B16-F10 Melanoma Applied onto CAM of Chick Embryo by Intravenous Injection

Irradiation time (min)	BLM (μg/egg)	Catechol (μM/egg)	Tumor weight (mean ± S.D., mg)	Inhibition ratio (%)
—	0	—	63 ± 6	—
0	3	—	50 ± 15	21
10	3	—	29 ± 3[a]	54
30	3	—	43 ± 11[a]	32
60	3	—	46 ± 13[b]	27
120	3	—	52 ± 14	17
—	0	—	98 ± 15	—
—	0	42	105 ± 16	—
0	3	42	70 ± 13[a]	33
10	3	42	85 ± 11	19
30	3	42	92 ± 14	12
60	3	42	91 ± 15	13

BLM solutions (72 μg/ml) in 0.9% NaCl of pH 7.4 were irradiated for the indicated times, stored with or without 1 mM catechol for 60 min at 37°C and diluted at the required concentration by 0.9% NaCl. The antitumor activity of BLM was assayed as described in the footnote of Table I and Experimental. The number of samples were 5 to 7 eggs. The experiment was repeated three times. *a*) $p<0.01$ compared to control. *b*) $p<0.05$ compared to control.

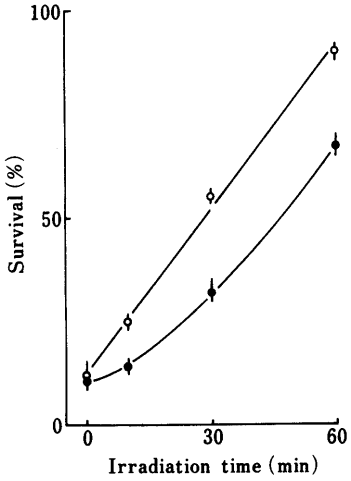

Fig. 1. Plot of Survival of Murine B16-F10 Melanoma Cells *versus* UV Irradiation Time for BLM in the Presence or Absence of Catechol

BLM Solutions (72 μg/ml) were irradiated for the required times, stored for 60 min at 37 °C in the presence or absence of 1 mM catechol and diluted at the required concentration by CMF-PBS. The average of the survival was obtained from three dishes. The cytotoxicity of BLM was assayed as described in Experimental. —●—; 300 ng/ml BLM and —○—; 300 ng/ml BLM treated with 1 mM catechol.

inhibitory effect of each BLM on tumor growth was further weakened with UV irradiation. As catechol hardly influenced tumor growth at 42 μM, it seems to show that the antitumor activity of UV-irradiated BLM was exclusively inhibited by catechol.

BLM was directly toxic to murine B16 melanoma cells cultured in RPMI1640 medium containing 10% fetal bovine serum. The value of an inhibitory concentration of 50% (IC_{50}; ng/ml) indicated the effect of drug was 140 ng/ml. Figure 1 shows the plot of the survival of melanoma cells *versus* irradiation time for BLM in the presence or absence of catechol. Although the survival value was about 15% when treated with non-irradiated BLM at 300 ng/ml, this value increased with UV irradiation to about 70% in treatment with BLM irradiated for 60 min. Such photo-inactivation of BLM was further strengthened by pretreatment with 1 mM catechol for 60 min at 37 °C, *i.e.*, the survival value became about 90%.

Since the method which measures the growth response of a tumor implanted onto the CAM of a chick embryo to anticancer drugs was sufficient to assay the antitumor effect of BLM, it was employed to examine the effect of UV irradiation on the antitumor activity of BLM. In harmony with the effect of UV irradiation on the interaction of DNA and BLM,[3,5-7] the antitumor activity of BLM was enhanced by irradiation for 10 min. Beyond 10 min antitumor activity decreased as shown in Table II. BLM enhances greatly sensitivity to DNase I of DNA[11] and it is further strengthened by an appropriate dose of irradiation.[8] It is suggested that the increased nuclease-sensitive sites in chromosomal DNA may also contribute to enhancement of the antitumor activity of BLM with UV irradiation.

In disagreement with enhancement of the antitumor activity, the cytotoxicity of BLM was weakened with UV irradiation (Fig. 1). It is already reported that the antibacterial activity of BLM decreases with UV irradiation and that catechol inhibits the activity of UV-irradiated BLM to react with DNA.[7] Further, the effect of catechol is due to inhibition of the UV-irradiated BLM to break the DNA strand rather than to bind to double-helical DNA.[8] Since catechol also inhibited both of the antitumor activity and the cytotoxicity of the UV-irradiated BLM, it suggests that catechol may play an important role in the photoinactivation of BLM *in vivo*. Although it is presumed that the cultured medium may inhibit selectively the UV-irradiated BLM, the metabolism of BLM in intracellular behavior remains unknown. The present findings suggest that an appropriate dose of UV irradiation may be of advantage as a means to enhance the anticancer activity of BLM.

Experimental

Materials Commercial BLM was obtained from Nippon Kayaku Co. Anticancer drugs tested here; DAM, MMC, 5-FU, ACNU, CY and VCR were commercially purchased and MTX was a gift of Lederle (Japan) Ltd. The murine B16-F10 melanoma cell line was kindly given by professor T. Sasaki of the Cancer Research Institute, Kanazawa University. The cultured medium (RPMI1640) for the maintenance of cells was from Nissui Pharmaceutical Co. and fetal bovine serum was from Gibco Co. The other reagents were from Wako Pure Chemical Industries Co. One-day-old chick eggs (dekalb) were obtained from the Poultry-Farming Cooperative Society in Kumamoto Prefecture.

Preparation of UV-Irradiated BLM for Assaying Antitumor Activity and Cytotoxicity Solutions (3 ml in a cuvette of 1 cm^2 square) of 72 μg/ml BLM in 0.9% NaCl at pH 7.4 were irradiated for 10, 30, 60 and 120 min with a mercury lamp (15 W) at a distance of 23 cm. Under these conditions, the irradiation dose was estimated to be 35 $erg/mm^2/s$ by the chemical densitometer method. After irradiation, each BLM solution was diluted to the required concentration by 0.9% NaCl. In pretreatment of UV-irradiated BLM with catechol, each BLM solution was stored with 1 mM catechol for 60 min at 37 °C and diluted at the required concentration for assaying the biological activity.

Inoculation of Cells onto CAM of Chick Embryo and Administration of Anticancer Drugs The reported method was employed for assay of the antitumor activity of BLM.[9,10] The murine B16 melanoma cells (5×10^4 cells) were implanted onto the CAM of a chick embryo incubated for 11 d at 37 °C. Anticancer drug solutions were injected into a CAM vein 3 d after inoculation of cells. After 4 d, the tumors excised from the CAM were weighed. The inhibition ratio (%) was estimated from the following equation:

$$\text{inhibition ratio (\%)} = \frac{A-B}{A}\times 100$$

where A is the mean tumor weight (mg) of the control group and B is that of the drug-treated group. The number of samples were 5 to 7 eggs. The experiment was repeated three times. The statistical significance of differences was evaluated by the Student's t-test.

Cytotoxicity of BLM For examination of the cytotoxic effect of BLM on the murine B16 melanoma cell line, 10^5 cells were placed into the dish that put RPMI1640 medium containing 10% fetal bovine serum and they were incubated at 37 °C in a humidified incubator of 5% CO_2. After 1 d, BLM solution, dissolved in 0.9% NaCl at the required concentration, was added to the cultured medium and cultivation was further continued for 3 d. Thereafter, cells were washed by calcium-magnesium free phosphate buffered saline (CMF-PBS) once, followed by trypsinization. The number of viable cells were counted using the trypan blue dye exclusion method. The average of the survival value was obtained from three dishes. The survival (%) was estimated from the following equation:

$$\text{survival (\%)} = \frac{\text{viable cell number of drug-treated group}}{\text{viable cell number of control group}} \times 100$$

Acknowledgement I am grateful to Professor T. Sasaki and his coworkers at the Cancer Research Institute, Kanazawa University for the gift of the murine B16 melanoma cell line and for their technical direction on assay of the antitumor activity of anticancer drugs.

References

1) H. Umezawa, K. Maeda, T. Takeuchi and Y. Okami, *J. Antibiot., Ser. A*, **19**, 200 (1966).
2) N. Thakrar and K. T. Douglas, *Cancer Lett.*, **13**, 265 (1981).
3) H. Mori, *Chem. Pharm. Bull.*, **33**, 4030 (1985).
4) T. Morii, T. Matsuura, I. Saito, T. Suzuki, J. Kuwahara and Y. Sugiura, *J. Am. Chem. Soc.*, **108**, 7089 (1986).
5) J. Kakinuma, M. Tanabe and H. Orii, *Photobiochem. Photobiophys.*, **7**, 183 (1984).
6) K. T. Douglas, N. Thakrar, S. J. Minter, R. W. Davies and C. Scazzochio, *Cancer Lett.*, **16**, 339 (1982).
7) H. Mori, *Chem. Pharm. Bull.*, **35**, 4503 (1987).
8) H. Mori, *Chem. Pharm. Bull.*, **37**, 680 (1989).
9) T. Sasaki, *Farumashia*, **23**, 58 (1987).
10) H. Uchida, T. Sasaki, M. Tanaka, Y. Endo, K. Nitta, K. Nishikawa, H. Chuman, H. Fukuma and K. Matsumoto, *Jpn. J. Cancer Res. (Gann)*, **78**, 729 (1987).
11) H. Yamaki, H. Suzuki, K. Nagai, N. Tanaka and H. Umezawa, *J. Antibiot.*, **24**, 178 (1971).