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THE COMBINED EFFECTS OF ^{60}CO GAMMA-RAYS AND CONTINUOUS LOW CONCENTRATIONS OF BLEOMYCIN ON CULTURED MAMMALIAN CELLS

HIDETOSHI KOBAYASHI and SADAYUKI SAKUMA

Department of Radiology, Nagoya University School of Medicine
Nagoya 466, Japan
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ABSTRACT

The combined effects of ^{60}Co gamma-rays and continuous, low concentrations of bleomycin on FM3A (mouse mammary adenocarcinoma cells) were examined, and the clinical application of this combination was reported in this paper. The combined effect appeared to be synergistic, and bleomycin seemed to interfere with Elkind recovery.

Keywords: FM3A, BLM, ^{60}Co, Combined Effect, Elkind Recovery

INTRODUCTION

Bleomycin (BLM) has been used with favorable results in the clinical treatment of squamous cell carcinomas of the head and neck,[1] the esophagus,[2] malignant lymphoma,[3,4] and others. Many investigaters have studied the effects of the combined use of X-rays and BLM on cultured cells,[5-11] but there has been no agreement as to whether the combined effect is synergetic[7,8,10,11] or additive.[5,6] The concentration of BLM used in in-vivo studies was higher than that of the BLM used in clinical treatment. It has been demonstrated that BLM effects a concentration-dependent mode of action producing a blockade near the S/G_2 boundary at low concentrations, and at the G_2/M boundary at high concentrations.[12,13] The combined effects of X-rays and high concentrations of BLM in in-vitro studies did not always agree with our clinically obtained results. This paper reports the results of the effect of low-concentration BLM and its combined effects with ^{60}Co irradiation.

MATERIALS AND METHODS

FM3A mouse mammary adenocarcinoma cells were used. These cells were grown in Eagles' MEM containing 10% calf serum with 0.12% $NaHCO_3$ as a buffer. The doubling time of these cultured cells at 37°C was 12.5 hr (Fig. 1). All experiments were done with these same cells during the exponential phase of growth and were repeated at least three times. Their

Author to whom request for reprints should be addressed: Hidetoshi Kobayashi, Department of Radiology, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466, Japan
小林英敏，佐久間貞行
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colony-forming ability was used as a measure of the cells survival in vitro, and Eagles' MEM containing 15% calf serum, incuvated at 37°C for 7 days, was done in order to measure the cells' multiplicity.

1) The effect of BLM on population doubling time (PDT)

BLM was dissolved in Eagles' MEM containing 10% calf serum. The concentrations of BLM added to the medium in these experiments were 0.01, 0.02, 0.05, 0.1, 0.2, 1.0, and 2.0 μg/ml.

2) The effect of BLM on cell survival

The concentrations of BLM added to the medium in these experiments were 0.1, 0.2, 0.5,

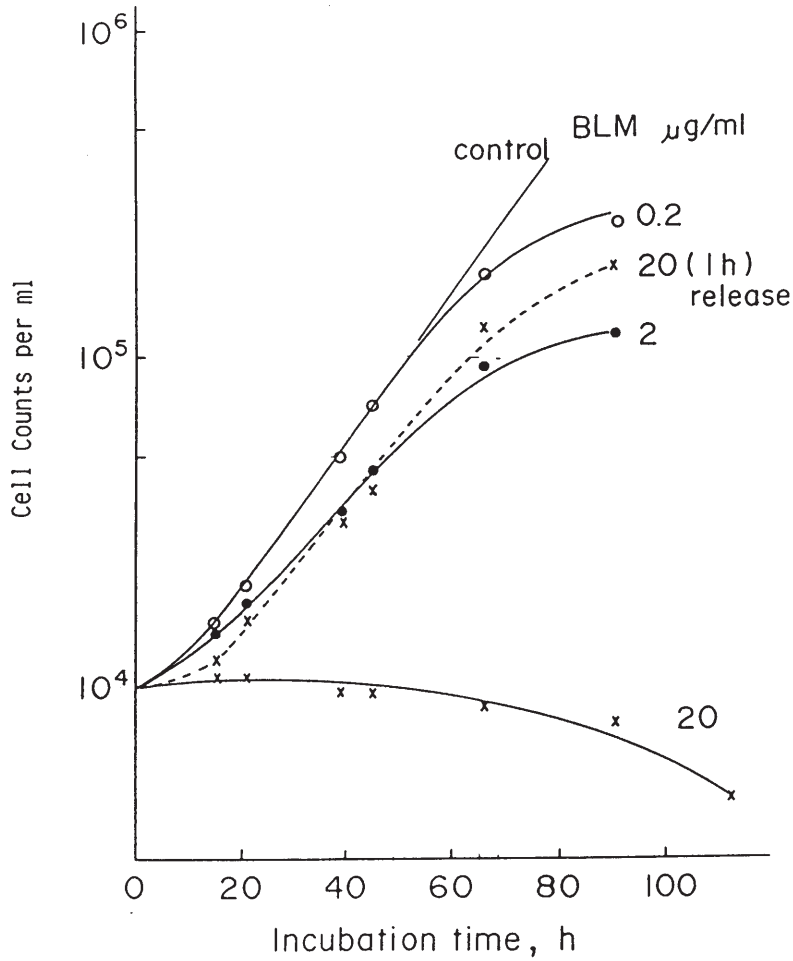

Fig. 1 Growth curve of FM3A cells treated with BLM at various concentrations.
Dark circle: Growth curve of cells treated with BLM at 2.0 μg/ml. Prolongation of PDT is evident.
Open circle: Growth curve of cells treated with BLM at 0.2 μg/ml. Prolongation of PDT is insignificant.
Broken line: Growth curve of cells after treated with BLM at 20 μg/ml for 1 hour. Prolongation of plateau phase is evident.
The increase of cells was not investigated when cells were treated with a medium containing a concentration of 20 μg/ml BLM.

1.0, 5.0, 25, 50, 75, 100, 150, 200, 250, and 300 μg/ml.

The growth medium in the test tube was replaced with a medium cntaining BLM, and the cells received this treatment for 3 hours. When the process terminated, the medium containing BLM was removed and the cells were rinsed in a fresh medium. The cells were then incubated to observe colony formation.

3) The effects of the combination BLM and radiation

Irradiation was performed with ^{60}Co at a dose rate of 1 Gy/min. The cells were planted in a test tube for 1 hour before irradiation in mediums containing 15% calf serum with 2 μg/ml BLM and without BLM. After treatment the test tube was maintained at a temperature of 37°C and its colony formation was observed for 7 days. The radiosensitivity of cells in a culture was expressed by the slope of the cells survival curve and was expressed in terms of D_0, that is, the single dose of radiation nessessary to kill 63% of the cells using the straight part of the curve. The magnitude of intracellular repair was measured by the broadness of the shoulder denoted by D_q.

RESULTS

The PDT of FM3A cells grown in Eagles' MEM containing 10% calf serum was 12.5 hr (Fig. 1). The colony-forming efficiency of FM3A cells was from 95 to 100%.

1) The effect of BLM on PDT

Prolongation of the PDT containing 0.2 μg/ml BLM was insignificant when compared with the control. Prolongation of the PDT containing 2.0 μg/ml BLM was evident when compared with the control, and the maximum number of cells was decreased at this concentration. A cellular increase was not observed when 20 μg/ml BLM was applied. The broken line in Fig. 1 shows the growth curve after one hour's exposure to 20 μg/ml of BLM. Prolongation of the plateau phase was evident (Fig. 1).

Fig. 2 shows the changes in the PDT resulting from the use of BLM concentrations ranging

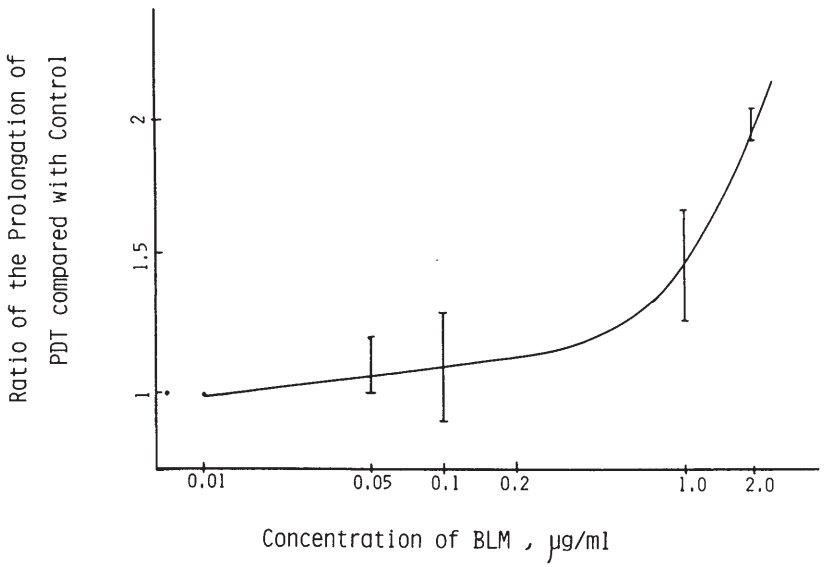

Fig. 2 The result of changes in PDT in concentrations of BLM from 0.01 to 2.0 μg/ml. There is a prolongation of PDT beyond a concentration of 0.2 μg/ml BLM.

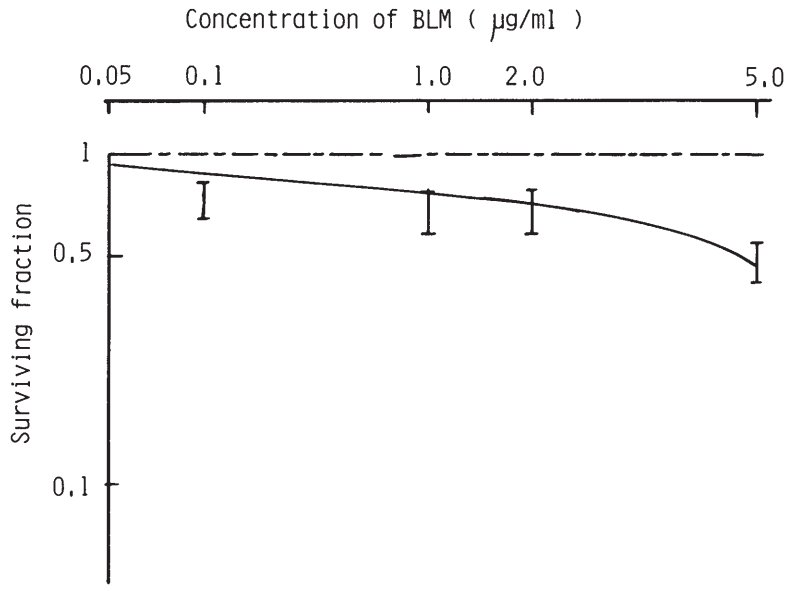

Fig. 3 Dose-survival curve of FM3A cells treated with low concentrations of BLM (from 0.05 to 5.0 μg/ml).

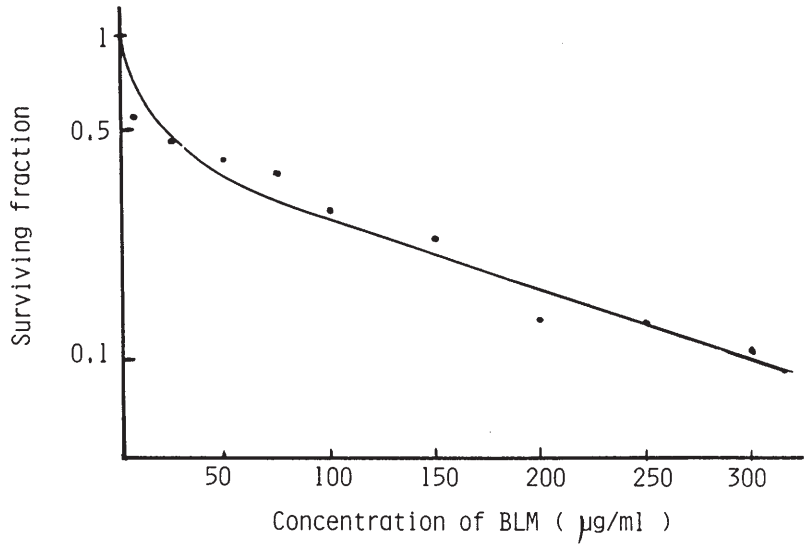

Fig. 4 Dose-survival curve of FM3A cells treated with high concentrations of BLM (from 5.0 to 300 μg/ml).

from 0.01 to 2.0 μg/ml. There was a prolongation of PDT beyond a concentration of 0.2 μg/ml BLM (Fig. 2).

2) The effect of BLM on cell survival

Fig. 3 represents the results of experiments in which FM3A cells were treated with lower concentrations of BLM for 3 hours, and the dose-response threshold curve along with an inflection point at the concentration less than 5.0 μg/ml is shown in this chart. The dose-survival curve from 5.0 μg/ml to 300 μg/ml demonstrated the exponentially inactivating initial portion followed by the less sensitive final portion (Fig. 4). This high dose-survival curve was previously reported.[8,17,18]

3) The combined effect of BLM at 2.0 μg/ml and radiation

The results are shown in Fig. 5. The effects of combined treatment were apparent in the D_0 value and the D_q value. The D_0 value of 2.13 Gy for ^{60}Co gamma-irradiation alone was decreased to 1.67 Gy with BLM treatment. The D_q value of 2.33 Gy for ^{60}Co gamma-irradiation alone was decreased to 1.0 Gy with BLM treatment (Fig. 5).

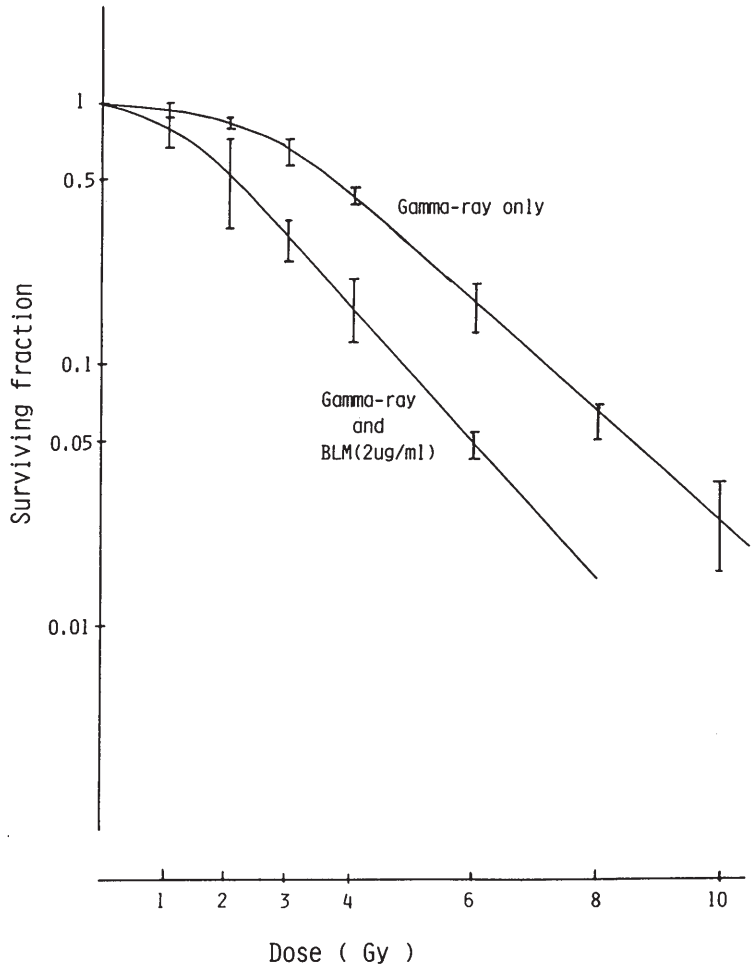

Fig. 5 Survival of FM3A cells after combined treatment of ^{60}Co gamma-rays and continuous treatment of BLM at 2.0 μg/ml.

DISCUSSION

BLM is and anti-tumor drug that was isolated by Umezawa from cultured Streptomyces verticillus.[14,15] This drug has been used in the clinical treatment of a wide range of squamous cell carcinoma,[1,2] malignant lymphoma[3,4] and others. The combined use of X-rays and BLM was initially proposed by Jorgensen,[16] but there is presently no agreement as to whether the combined effect is synergetic[7,9,10,11] or only additive.[5,6] There are many factors which have great influence on the combined effect. The concentration of BLM, the used cell-lines, the phase of cultured cells, and exposure time are but a few examples of the more important factors.

Many previous experiments have reported that the dose-survival curve shows an exponential phase in low concentrations of BLM.[8,17,18] However, in our experiment with lower concentrations of BLM, the dose-survival curve elicited a shoulder portion. Elkind and Sakamoto suggested that interaction between radiation and drugs was likely when the drug demonstrated a threshold dose-response curve.[8] The survival curve of FM3A cells exposed to low concentrations of BLM was of the threshold type, so interaction with radiation was expected. The combined effect is different depending on whether the cells are treated with BLM before irradiation or after irradiation[7,8,9,17]. In our experiment, FM3A cells were continuously treated. This continuous treatment of BLM is a new experimental method.

In the combined treatment of BLM with irradiation, the decrease of D_0 and D_q values was recognized; therefore, the combined effect seems to be synergetic in action. Many investigators previously thought that BLM did not interfere with Elkind recovery[8] because BLM did not have any effect on the D_q vlue. However, our experiments, since the D_q value was decreased, it would seem that BLM does interfere not only with potentially lethal damage, but also with Elkind recovery. The results of the combined effect were different in our experiments from those given in other reports. We feel that this is due to the concentration of BLM in previous reports being too high and the treatment time of BLM being too short.

BLM is known to inhibit DNA synthesis ant to produce DNA strand breaks[12,13,19,20] at the molecular level. Since Elkind recovery is the type of rapid repair of sublethal damage and is closely connected with DNA, it is suspected that BLM interferes with Elkind recovery. The effct of BLM on PDT shows an inflection point at the concentration region less than 0.5 μg/ml. The PDT was compared twice with the control at the region of 2.0 μg/ml. These results provide even more evidence that BLM effects DNA, even when its concentration is low.

CONCLUSION

1) The dose-survival curve showed the threshold type in low concentrations of BLM.
2) The effect of BLM on cell growth showed an inflection point at the concentration region less than 0.5 μg/ml.
3) The combined effect of ^{60}Co -irradiation with continuous treatment of BLM at 2 μg/ml concentration showed a synergetic effect, and BLM seems to interfere with Elkind recovery.

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