Irradiation reduces bleomycin sensitivity in cervical squamous cancer cells *in vitro*

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Summary

Purpose of investigation: The study was performed to examine how bleomycin (BLM) and peplomycin (PEM) should be effectively used in radiotherapy for cervical squamous cancer patients. *Methods:* The effects of BLM on radiosensitivity and the effects of radiation on the sensitivity to BLM of cancer cells were investigated using the radiosensitive human cervical squamous cell carcinoma cell line ME180. *Results:* BLM treatment did not affect radiosensitivity. However, irradiation significantly reduced cell BLM sensitivity in a dose-dependent manner. There was no significant difference in BLM sensitivity and PEM sensitivity between cells concurrently irradiated and those treated with BLM or PEM 8 h before or 8 h after irradiation. *Conclusion:* Since sensitivity to BLM is reduced during irradiation, BLM should be administered to cervical cancer patients as an adjuvant chemotherapeutic drug after completion of radiotherapy.

Key words: Bleomycin; Peplomycin; Cervical cancer; Chemoradiotherapy; Squamous cell carcinoma.

Introduction

Recent studies have shown that concurrent chemoradiotherapy for advanced cervical cancer patients has better survival rates than radiotherapy alone [1-4]. Cisplatin (CDDP) has been most frequently used in chemoradiotherapy for cervical cancer, with weekly injections of 40-75 mg/m^3. However, it has not been examined whether CDDP is the best anticancer drug for chemoradiotherapy. Because CDDP is one of the most effective chemotherapeutic drugs for cervical cancer, and because chemoradiotherapy with CDDP led to better survival than radiotherapy alone, CDDP has been used in chemoradiotherapy for cervical cancer for more than ten years. However, other drugs might show better clinical results than CDDP if used with radiotherapy, and addition of another anticancer drug to CDDP chemoradiotherapy might achieve better clinical results than CDDP chemoradiotherapy alone.

We have recently proposed optimal combination protocols of anticancer drugs and radiation for cervical cancer according to experimental results with cultured human cervical squamous cancer cells [5-7]. CDDP should be administered to cervical cancer patients after radiotherapy because of the irradiation-enhanced CDDP sensitivity of cancer cells that occurs at least several months after irradiation [5]. Pirarubicin (THP) should be administered to cervical cancer patients several hours before irradiation because the THP sensitivity of cervical cancer cells is enhanced by irradiation 8 h after THP-treatment, but is reduced by irradiation 8 h before THP treatment [6]. In the case of mitomycin (MMC), irradiation reduced the MMC sensitivity of the cancer cells, while MMC did not affect the radiosensitivity of the cells. MMC should be administered to cervical cancer patients after completion of radiotherapy, because surviving cancer cells post-irradiation show higher MMC sensitivity than non-irradiated cells [7]. In the present study, we examined optimal combination protocols of bleomycin (BLM) and irradiation for cervical cancer cells in vitro.

Materials and Methods

Cell line and cell culture

The human cervical squamous cancer cell line ME180 [8], which is radiosensitive and multi-drug sensitive, was obtained from the Japan Collection of Research Bioresources Cell Bank (Tokyo, Japan), and cultured in OPTI-MEM (GIBCO-BRL, Gaithersburg, MD, USA) containing 5% fetal calf serum (EQUITECH BIO, Ingram, TX, USA), 100 U/ml penicillin (GIBCO-BRL) and 100 μg/ml streptomycin (GIBCO-BRL). BLM and peplomycin (PEM) were kind gifts from Nippon-Kayaku Co. Ltd (Tokyo, Japan).

Cell viability assay

The growth-inhibitory effects of radiation and anticancer drugs on the cells were assayed as follows. Cells in the log phase were detached with 0.25% trypsin/1 mM EDTA, and then cultured overnight in 96-well plates (5 x 10^3 cells/well). On day 2, the cells were irradiated with various doses of γ-rays using an irradiator (MBR 1520A; Hitachi-Medico, Tokyo, Japan). On day 4, the viable cells were counted using a cell proliferation assay XTT kit (Boehringer-Mannheim, Mannheim, Germany). To examine the modulatory effects of BLM or PEM on cell death induced by irradiation, the cells were treated with various concentrations of BLM or PEM and irradiated with various doses of γ-rays, followed by 2-day culture. Finally, relative viable cell numbers (%) were calculated using the XTT kit. All of the experiments were performed two or three times to verify the results. The data are shown as the mean ± SD and comparative data (n = 6) were analyzed by ANOVA.

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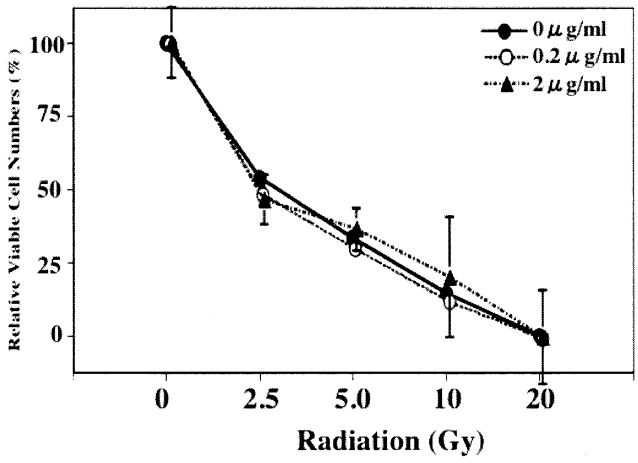
Fig. 1



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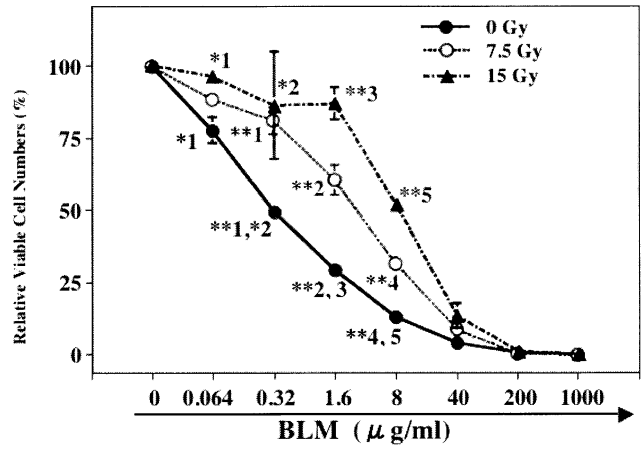

Figure 1. — Effects of BLM on the radiosensitivity of ME180 cells. Within 20 minutes after addition of BLM to ME180 cells, the cells were irradiated with various doses of γ-rays (0, 2.5, 5.0, 10.0, 20 Gy). The final BLM concentrations in the culture media were 0, 0.2 and 2 μg/ml. The solid line with closed circles shows the control radiosensitivity curve of cells cultured without BLM. The dotted lines with open circles (BLM = 0.2 μg/ml) and closed triangles (BLM = 2 μg/ml) show the radiosensitivity curves of the cells cultured with BLM. There are no significant differences among the three radiosensitivity curves.
Figure 2. — Effects of irradiation on BLM sensitivity of ME180 cells. Within 20 minutes after addition of various concentrations of BLM to ME180 cells, the cells were irradiated with 7.5 or 15 Gy of γ-rays. The solid line with closed circles shows the control BLM sensitivity curve of cells cultured without irradiation. The dotted lines with open circles (7.5 Gy) and closed triangles (15 Gy) show the BLM sensitivity curves of the irradiated cells. Irradiation reduced the BLM sensitivity in a dose-dependent manner.

Results

First, the effects of BLM on the radiosensitivity of ME180 cells were examined. As illustrated in Figure 1, BLM did not significantly affect the ME180 radiosensitivity curve. Second, the effects of irradiation on ME180 BLM sensitivity were investigated. As shown in Figure 2, concurrent irradiation significantly reduced BLM sensitivity in a dose-dependent manner.

Furthermore, to determine whether BLM sensitivity is affected immediately after or immediately before irradiation, we investigated changes in BLM sensitivity in cells treated for 8 h before irradiation and in cells treated with BLM 8 h after irradiation. There was no significant difference in BLM sensitivity between the cells treated with BLM 8 h before irradiation and those irradiated concurrently with BLM (Figure 3A). Moreover, there was no significant difference in BLM sensitivity between cells treated with BLM 8 h after irradiation and those irradiated concurrently with BLM (Figure 3B). Taken together with the results in Fig. 2, these data indicate that BLM sensitivity may be reduced during radiotherapy. Finally, we also examined the PEM sensitivity of irradiated ME180 cells to determine whether the results of the BLM experiments are common to similar derivatives. As shown in Figure 4, irradiation had no effect on the PEM sensitivity of cells treated with PEM either 8 h before or 8 h after irradiation.

Discussion

Concurrent chemoradiotherapy has been reported to lead to better survival in cervical cancer patients than radiotherapy alone [1-4]. CDDP has been most frequently used in concurrent chemoradiotherapy for cervical cancer. Although BLM has been widely used for chemotherapy in cervical cancer patients, almost all previous reports showed results for BLM combined with CDDP-based chemotherapy. However, lung toxicity, a BLM-specific adverse effect, has been indicated. PEM could be used in chemotherapy for cervical cancer instead of BLM, because PEM is considered to have lower lung toxicity than BLM. Since PEM was developed mainly in Japan, most reports on PEM-combined chemotherapy have come from Japanese groups [9, 10]. At first, neoadjuvant BLM-combined chemotherapy before radiotherapy was reported to achieve better survival rates in advanced cervical cancer patients [11]. Thereafter, many studies reported that neoadjuvant BLM-combined chemotherapy before radiotherapy did not have any clinical benefits [12-14]. Preoperative neoadjuvant chemotherapy with BLM or PEM was reported to have clinical benefits [10, 15], while several reports demonstrated that preoperative neoadjuvant chemotherapy did not have any clinical benefits [16, 17]. Postoperative adjuvant chemotherapy with BLM or PEM for advanced cervical cancer patients was reported to show better clinical results than surgery alone [9, 18]. Although there are many reports that neoadjuvant chemotherapy before radiotherapy for cervical cancer had lower survival rates, concurrent chemoradiotherapy with BLM has been reported to show better clinical results, [19, 20] while several reports have shown that BLM-combined concurrent chemoradiotherapy did not show any clinical benefits [21, 22]. These previous reports were therefore

Fig. 3

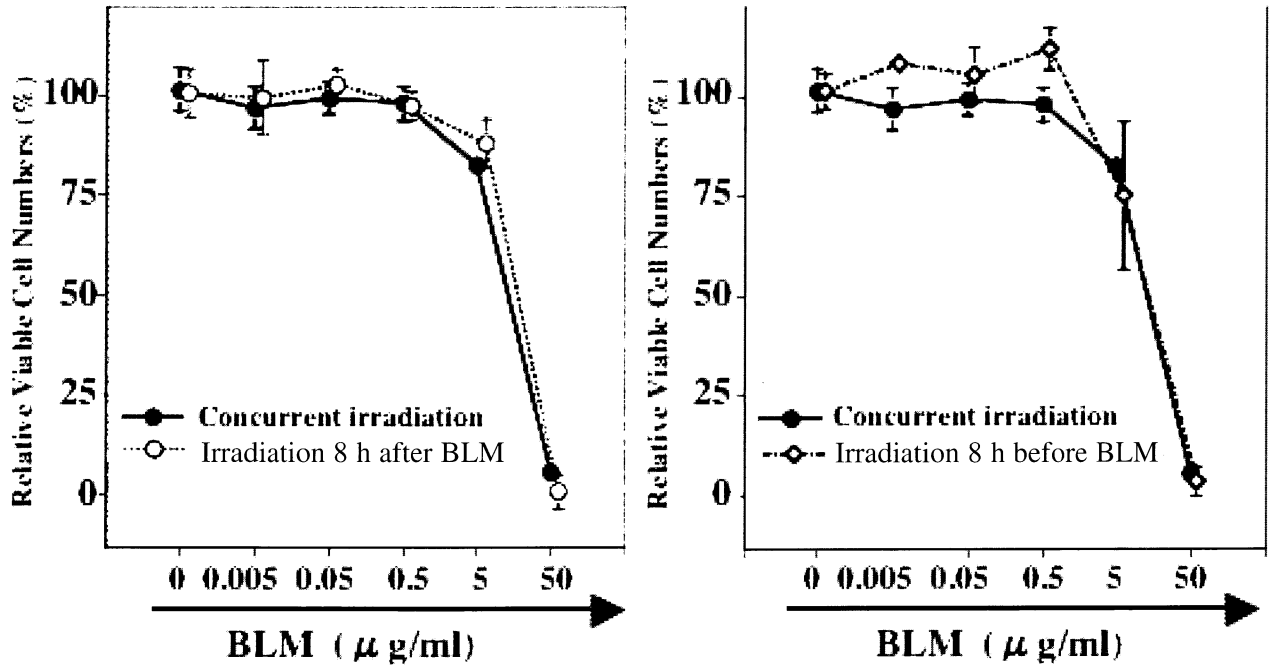

Fig. 4

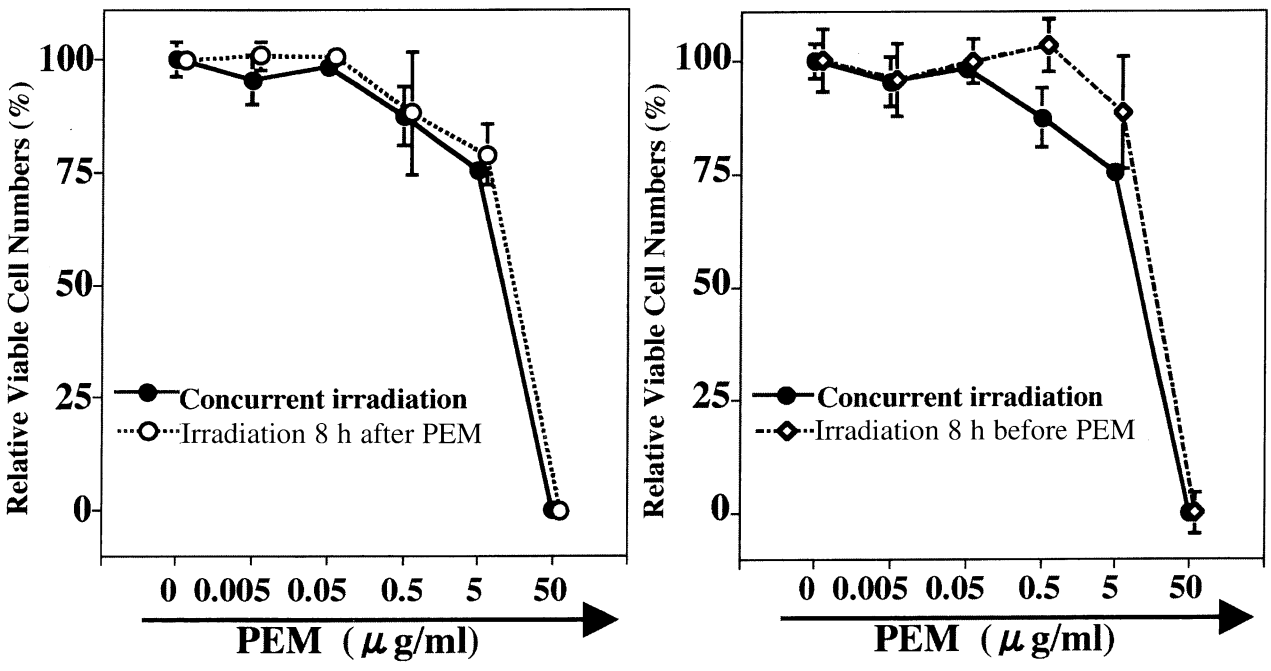

Figure 3. — Effects of BLM treatment and γ-ray irradiation on the BLM sensitivity of ME180 cells.
A. The solid line with closed circles shows the control BLM sensitivity curve of cells irradiated with a single dose of 15 Gy immediately after addition of BLM. The dotted line with open circles shows the BLM sensitivity curve of cells treated with BLM 8 h before irradiation. B. The solid line with closed circles shows the control BLM sensitivity curve of cells irradiated with a single dose of 15 Gy immediately after addition of BLM. The dotted line with open circles shows the BLM sensitivity curve of cells treated with BLM 8 h after irradiation. No significant differences in the BLM sensitivity curves can be observed.
Figure 4. — Effects of PEM treatment and γ-ray irradiation on the PEM sensitivity of ME180 cells.
A. The solid line with closed circles shows the control PEM sensitivity curve of cells irradiated with a single dose of 15 Gy immediately after addition of PEM. The dotted line with open circles shows the PEM sensitivity curve of cells treated with PEM 8 h before irradiation. B. The solid line with closed circles shows the control PEM sensitivity curve of cells irradiated with a single dose of 15 Gy immediately after addition of PEM. The dotted line with open circles shows the PEM sensitivity curve of cells treated with PEM 8 h after irradiation. No significant differences in the PEM sensitivity curves can be observed.

classified into two groups: reports that BLM-combined chemotherapy was effective in cervical cancer when BLM was administered before, during or after surgery or radiotherapy; and reports that BLM-combined chemotherapy did not show any clinical benefits.

In most previous reports, BLM and PEM were used in combination chemotherapy with CDDP or combination chemoradiotherapy with CDDP for advanced cervical cancer. CDDP is considered to be the most effective anticancer drug for cervical cancer. Therefore, even when BLM/PEM combined chemotherapy showed clinical efficacy, we cannot determine whether addition of BLM or PEM had greater clinical efficacy than CDDP combined chemotherapy without BLM or PEM. Indeed, there are several reports that addition of BLM to concurrent chemoradiotherapy did not show any clinical benefits for cervical cancer patients [23, 24]. Addition of CDDP to BLM-combined chemotherapy was reported to have no additional benefits [25]. In the present study, therefore, we have investigated *in vitro,* using a human cervical squamous cancer cell line, whether addition of BLM or PEM to radiotherapy can improve the cytotoxic effects of chemoradiotherapy.

In this study, we showed that irradiation reduces the BLM sensitivity of cervical cancer cells, and that BLM does not affect the radiosensitivity of the cells. Moreover, BLM sensitivity and PEM sensitivity were not affected from 8 h before irradiation to 8 h after irradiation. These results indicate that a concurrent combination of BLM or PEM with radiotherapy does not have any clinical benefits in cervical cancer. Since there are many reports that combination chemotherapy with BLM or PEM improved survival rates of cervical cancer patients and that neoadjuvant chemotherapy followed by radiotherapy showed lower survival rates of cervical cancer patients than radiotherapy alone [12-14], BLM and PEM should be administered to cancer patients as an anticancer drug in adjuvant chemotherapy after completion of radiotherapy.

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