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ORIGINAL ARTICLE

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Etoposide enhances the lethal effect of radiation on breast cancer cells with less damage to mammary gland cells

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Abstract *Purpose*: In the present study, the cytotoxic effects of anticancer drug treatment plus radiation on breast cancer and normal mammary gland cells were investigated in vitro. *Methods*: Breast cancer and adjacent mammary gland tissue was obtained from surgically resected specimens from 13 premenopausal female patients, and was cultured. Cultured cells were treated with radiation and etoposide, and sensitivities were evaluated using an MTT assay. *Results*: In breast cancer cells, radiation alone had a cytotoxic effect. When etoposide was added, synergistic effects were achieved which were independent of the radiation dose. The cytotoxicity of etoposide against normal gland cells, however, was much the same as that against cancer cells, and no synergistic effects were observed in normal cells even when radiation was added. *Conclusions*: Radiation combined with etoposide showed lethal effects against breast cancer cells with less damage to normal mammary gland cells.

Key words Breast cancer cell · Mammary gland cell · Radiosensitivity · VP-16

Introduction

Radiation to the remaining breast tissues has been shown to be an effective treatment after breast-conserving surgery for cancer [1]. Nonetheless, local recurrence still occurs in some patients receiving this treatment [2–4], and radiation therapy has been known to induce cosmetic and functional disadvantages on the conserved breast [5, 6].

Several investigators have reported that prevention of local recurrence and prolongation of the disease-free period after breast-conserving surgery for cancer could be achieved by adjuvant, postsurgical chemoradiation therapy [7, 8]. Etoposide, which is known as a radiosensitive agent [9–11], has a distinctive mechanism as an inhibitor of topoisomerase II [12]. In addition, long-term oral administration of etoposide is known to be effective postsurgically [13]. However, the effects of chemoradiotherapy using etoposide on breast cancer and mammary gland tissue remain unknown.

In the present study, we examined the cytotoxic effects of etoposide and radiation on breast cancer cells and mammary gland cells in vitro.

Materials and methods

Cell cultures and cell treatments

From April 1994 to December 1996, 37 female patients with breast cancer underwent surgical treatment in our department. Among them, 19 were premenopausal and 18 were menopausal. Surgically resected tumor and mammary gland tissue from the 19 premenopausal patients was submitted for culture and histological examination. For culture, tumor and adjacent mammary tissue obtained from the same patient was mechanically minced, and cells were passed through a stainless steel mesh, centrifuged at 1500 rpm for 5 min, and washed with Hanks' balanced salt solution. After centrifugation, the supernatant was removed, cells were suspended in ASF301 (Ajinomoto Co., Tokyo), and cell viability was evaluated by dye exclusion with staining by 0.4% trypan blue.

The resected specimens were also utilized for histological examination. The tissues were fixed in 10% formalin and processed for embedding in parafin. The parafin-embedded specimens were sectioned at 5 μm and stained with hematoxylin and eosin. In cases with extensive spread of tumor cells into the mammary gland tissue, the cells were not entered into this study. Finally, the mammary gland cells of 13 patients were successfully cultured and were chosen for the current study. The number of cells was adjusted to 1×10^4/ml. Cells were plated in 2.0-cm^2 plastic dishes, with four dishes used for each of nine treatment groups. Eight groups of cells were irradiated with an EXS-300 irradiator (Toshiba Electronic Co., Tokyo) at a dose rate of 0.4 Gy/min. Radiation doses were 1.2, 2.4, 4.8 and 9.6 Gy in both cancer and mammary gland cell groups. Four of the eight irradiated groups of cells (both cancer and mammary gland cells) were cultured with etoposide in dim light from 3 h after irradiation [10]. Cells were exposed to etoposide for 48 h. To the remaining group of both types of cells, etoposide

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was administered without radiation. The concentration of etoposide was adjusted to 100 μg/ml which is ten times the peak plasma concentration following intravenous administration at of 80 mg/m^2 [10, 14, 15]. Control and treated cells were then incubated for 48 h at 37 °C in a humidified atmosphere containing 5% CO_2.

In a preliminary study, cells were treated with etoposide at 100, 10 and 5 μg/ml. Dose dependency of cytotoxicity at these concentrations of etoposide was not observed.

Cytotoxicity assays

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co., St Louis, Mo.) assays based on succinate dehydrogenase (SD) inhibition were utilized [14, 15]. Briefly, 0.1 mol/l sodium succinate solution and 0.4% MTT solution were administered to control and treated cells after 48 h incubation, and then incubation was continued for 3 h. The supernatant was removed, and formazan was dissolved in 2 *N* KOH solution containing 50% dimethyl sulfoxide. SD activity was taken as the absorbance of formazan measured at 565 nm. Cytotoxicity against both tumor and mammary gland cells is presented as the SD activity in treated cells expressed as a percentage of that in control cells.

Statistical Analysis

Data are presented as means ± standard deviation. Cytotoxisity induced by treatments with radiation and/or etoposide were statistically analyzed by two-way factorial ANOVA.

Results

Cytotoxicity

The SD activities in tumor cells and mammary gland cells treated with etoposide and/or radiation are shown in Table 1. Etoposide exposure had a lethal effect on both tumor and mammary gland cells. There were no significant differences in SD activities between tumor and mammary gland cells treated with etoposide. There were no differences in viability between groups irradiated with 1.2 Gy and those irradiated with 9.6 Gy in either cell type. Combined treatment with radiation and etoposide caused significantly lower survival in tumor cells compared with mammary gland cells treated with a corresponding dose of radiation. Intragroup analyses in both tumor and mammary gland cells showed no significant differences.

Table 1 Comparison in succinate dehydrogenase (SD) activities in tumor cells and mammary gland cells treated with etoposide and/or radiation

Treatment	SD activity (%)	
	Tumor cells ($n = 13$)	Mammary gland cells ($n = 13$)
Nil (control)	100	100
Etoposide (100 μg/ml)	70 ± 4	73 ± 3
Radiation (Gy)		
1.2	88 ± 7	93 ± 10
2.4	88 ± 6	90 ± 7
4.8	90 ± 5	90 ± 7
9.6	91 ± 3	92 ± 2
Radiation (Gy) + etoposide (100 μg/ml)		
1.2	53 ± 7*	71 ± 5
2.4	51 ± 6*	72 ± 5
4.8	54 ± 4*	70 ± 8
9.6	48 ± 5*	84 ± 4

*$P < 0.001$

Discusssion

Etoposide is known to be a radiosensitizer in some cancer cell lines [9–11]. As shown by Minehan and Bonner [10], etoposide causes radiosensitization even if it is applied 3 h after irradiation. However, sublethal damage repair is a rapid process, and thus the inhibition of repair interactions induced by etoposide would not be an entire mechanism for synergistic interaction between etoposide and radiation [11].

DNA synthesis has been shown to be inhibited by radiation [16]. Intrinsic radiosensitivity has been established as $G_2M > G_1 > S$, and cells in late S phase are most resistant [11]. Conversely, S phase cells are most susceptible to the lethal action of etoposide [17, 18]. Moreover, etoposide might be able to impair topoisomerase IIα which is involved in DNA repair even within the radiation-induced G_2 block [11]. Therefore, repair interaction and cytokinetic combination appear to be involved as mechanisms in the synergistic effect of etoposide and radiation [11].

In cultured mammary gland cells, including secretory cells and teat cells, the percentage of nondividing (G_0G_1) cells has been shown to increase as the cells reach confluency [19]. Moreover, it has been found that radiation causes an prolongation of the generation cycle of tumor cells while shortening the cell cycle of normal cells [20].

In the present study, the different distribution of cells sensitive to radiation and etoposide in the cell cycle between tumor and mammary gland cells in each patient might have caused differences in the lethal effect in both cell types when treated with etoposide following radiation. In conclusion, the differences in the lethal effect between tumor cells and mammary gland cells indicates that postsurgical treatment with radiation and etoposide might cause lethal effects on breast cancer cells without substantially damaging mammary gland cells.

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