Original Articles

Radiation reduces carboplatin sensitivity and enhances nedaplatin sensitivity in cervical squamous cell carcinoma *in vitro*

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Summary

Background: The study was performed to examine how the platinum anticancer drugs other than cisplatin, such as carboplatin (CBDCA) and nedaplatin (NEP) can be effectively used in chemoradiotherapy for cervical squamous cell carcinoma patients.

Materials and Methods: The radiosensitive human cervical squamous cell carcinoma cell line ME180 was examined to investigate the radiation effects on CBDCA and NEP sensitivities of the cells.

Results: Irradiation significantly reduced cellular CBDCA sensitivity. There were no significant changes in CBDCA sensitivity between the cells concurrently irradiated and those treated with CBDCA 8 h before or 8 h after irradiation. However NEP sensitivity of the cells treated 8 h before or 8 h after irradiation was significantly higher than that in cells concurrently irradiated.

Conclusions: Although CBDCA sensitivity in the concurrently irradiated cells is reduced, NEP sensitivity is enhanced by irradiation. NEP, but not CBDCA, therefore, may be a candidate anticancer drug for concurrent chemoradiotherapy for cervical cancer. For the greatest efficacy, NEP should be administered to patients several hours before or after irradiation.

Key words: Carboplatin; Cervical cancer; Chemoradiotherapy; Nedaplatin; Squamous cell carcinoma.

Introduction

Chemoradiotherapy for advanced cervical cancer is reported to have better survival results than radiotherapy alone [1-8]. Cisplatin (CDDP) is the most frequently used agent for chemoradiotherapy for cervical cancer and there are many reports showing good clinical results with CDDP chemoradiotherapy [1-5]. In previous studies of chemoradiotherapy for cervical cancer, CDDP was usually administered weekly at 40-74 mg/m^2 [1-6]. With such large doses of CDDP, patients may frequently suffer from severe digestive symptoms such as nausea, vomiting and loss of appetite. Therefore, to avoid these severe digestive symptoms, platinum anticancer drugs other than CDDP, such as carboplatin (CBDCA) and nedaplatin (NEP), have been administered to cervical cancer patients. Chemotherapy of cervical cancer with CBDCA [9, 10] or NEP [11-13] has been reported to have similar results to that with CDDP. However, there are few reports showing the clinical results of chemoradiotherapy with CBDCA [14-16] or NEP [17-18] for cervical cancer. Moreover, there is no report, to our knowledge, that shows whether weekly concurrent administration of CBDCA or NEP with radiotherapy has better results than weekly CDDP administration during radiotherapy.

Recently, we have proposed optimal chemoradiotherapy protocols for cervical cancer based on the results of in vitro studies with cultured human cervical squamous cancer cells. Cervical cancer cells can retain higher CDDP sensitivity for several months after irradiation compared with that of non-irradiated cancer cells, therefore, we proposed that CDDP should be administered to cervical cancer patients immediately after completion of radical radiotherapy [19]. In this study, we examined optimal combination protocols with radiotherapy and other platinum anticancer drugs to investigate the possibility that CBDCA or NEP can be used clinically for chemoradiotherapy of cervical cancers instead of CDDP.

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Materials and Methods

Cell line and cell culture

The human cervical squamous cancer cell line ME180 [20], which is radiosensitive [21], was obtained from the Japan Collection of Research Bioresources (JCRB) Cell Bank (Tokyo, Japan), and cultured in OPTI-MEM (GIBCO-BRL, Gaithersburg, MD, USA) containing 5% fetal calf serum (FCS; EQUITECH BIO, Ingram, TX, USA), 100 U/ml penicillin (GIBCO-BRL) and 100 μg/ml streptomycin (GIBCO-BRL). CBDCA was a kind gift from Bristol-Meyerrs Squibb Japan (Tokyo, Japan), and NEP was a kind gift from Shionogi Pharmaceutical Co. (Osaka, Japan).

Cell viability assay

Cell proliferation was assayed using a non-RI colorimetric assay kit (XTT; Boehringer-Mannheim, Mannheim, Germany). The growth-inhibitory effects of radiation and anticancer drugs on the cells were assayed as follows. Cells in the log phase were detached with a mixture of 0.25% trypsin ad 1mM EDTA (GIBCO- BRL), and then cultured overnight in 96-well culture plates (5000 cells/well). On day 2, the cells were irradiated with various doses of γ-rays using an MBR 1520A irradiator

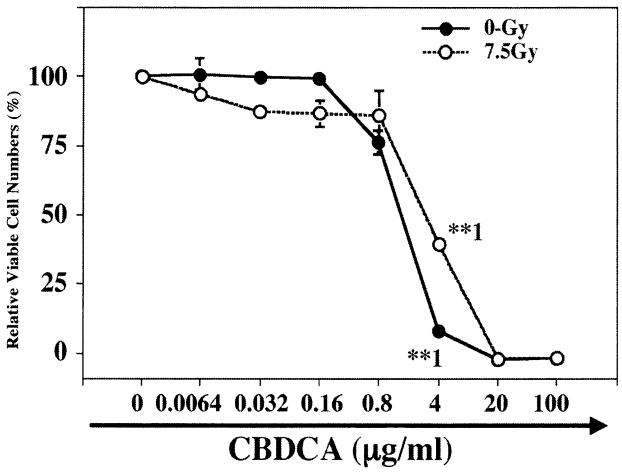

Figure 1. – Effect of irradiation on CBDCA sensitivity of ME180 cells. Within 20 min various concentrations of CBDCA were added to ME180 cells, and cells were irradiated with 7.5 Gy γ-radiation. The solid line with closed circles shows the control CBDCA sensitivity of cells cultured without irradiation. The dotted line with opern circles shows the CBDCA sensitivity of irradiated cells. Irradiation significantly reduced CBDCA sensitivity. **1: $p < 0.01$.

(Hitachi-Medico, Tokyo, Japan). On day 4, the viable cells were counted using the XTT kit. In separate experiments, cells were treated with various concentrations of CBDCA or NEP in order to examine the modulatory effects on cell induced by irradiation. The cells were irradiated with various doses of γ-ray, followed by 2-day culture. Finally, the relative viable cell numbers were calculated with the aid of the XTT kit. All the experiments were performed two or three times to verify the results. Data are shown as the mean ± SD and comparative data (n = 6) were analyzed by ANOVA.

Results

First, we examined the effect of irradiation on CBDCA sensititivy of cervical cancer cells. As shown in Figure 1, 7.5 Gy irradiation significantly reduced CBDCA sensitivity in ME180 cells. This differed from the effect of irradiation on CDDP sensitivity [19].

Second, we investigated changes in CBDCA sensitivity between cells treated for 8 h before irradiation and those treated with CBDCA 8 h after irradiation. There was no significant change in CBDCA sensitivity between the cells treated with CBDCA 8 h before irradiation and those irradiated concurrently with CBDCA (Figure 2A). Moreover, there was no significant change in CBDCA sensitivity between cells treated with CBDCA 8 h after irradiation and those irradiated concurrently with CBDCA (Figure 2B). Taken together with the results in Figure 1, these data indicate that CBDCA sensitivity may be reduced during radiotherapy.

Finally, because CDDP [19] and CBDCA sensitivity in cancer cells cannot be affected by irradiation either 8 h before or 8 h after irradiation, the effects of irradiation on NEP sensitivity were also examined. Unexpectedly, NEP sensitivity of the irradiated ME180 cells was significantly enhanced in cells treated 8 h before and 8 h after irradiation (Figure 3).

Discussion

CDDP is the most frequently used anticancer drug for chemoradiotherapy for advanced cervical cancer and there are many reports that show good clinical results with CDDP [1-5]. However, CDDP chemotherapy induces severe digestive symptoms such as nausea, vomiting and appetite loss. Moreover CDDP chemotherapy needs a lot of hydration to avoid renal damage. Therefore, other platinum anticancer drughs such as CBDCA and NEP, whose adverse digestive effects are milder than those of CDDP, and that need less hydration than CDDP, have been used for chemotherapy of cervical cancer. Several trials of chemoradiotherapy with CBDCA or NEP have been performed [14-18]. However, optimal protocols of chemoradiotherapy with CBDCA or NEP have not yet been established. The present study may be the first to propose optimal protocols for chemoradiotherapy with CBDCA or NEP for cervical cancer.

Recently, we have reported that CDDP sensitivity of ME180 cells is enhanced by irradiation and that it can not be changed from 8 h before to 8 h after irradiation [19]. The results suggest that CDDP should be administered to patients concurrently with radiotherapy and after completion of radiotherapy but not before radiotherapy. Contrary to the case of CDDP sensitivity during irradiation, CBDCA sensitivity of cells was significantly reduced by concurrent irradiation. The effect of irradiation on CBDCA sensitivity was no different 8 h before or 8 h after irradiation. These results indicate that CBDCA should not be administered concurrently with radiotherapy to cervical cancer patients.

NEP is a new platinum anticancer drug whose adverse effects such as digestive symptoms and renal damage are milder than those of CDDP. Recent reports show the clinical effectiveness of NEP in cervical cancer [11-13]. The present study revealed that NEP sensitivity of cancer cells can be significantly enhanced either 8 h before or 8 h after irradiation. The effect of irradiation on NEP sensitivity can apparently be distinguished from its effect on CDDP and CBDCA sensitivity. The present study indicates that NEP should be administered to cancer patients at least several hours before or after irradiation.

This preliminary study does not clarify which is the best anticancer drug among CDDP, CBDCA and NEP for chemoradiotherapy of cervical cancer. During chemoradiotherapy, each anticancer drug should be administered at the best time to achieve the highest synergistic effects between irradiation and the drug. Considering the results of the present study, NEP may be the best among the three drugs for concurrent chemoradiotherapy for cervical cancer because only NEP sensitivity could be enhanced both 8 h before and 8 h after irradiation. Since the enhanced CDDP sensitivity of the irradiated cancer cells can be maintained for more than several months

Fig. 2A

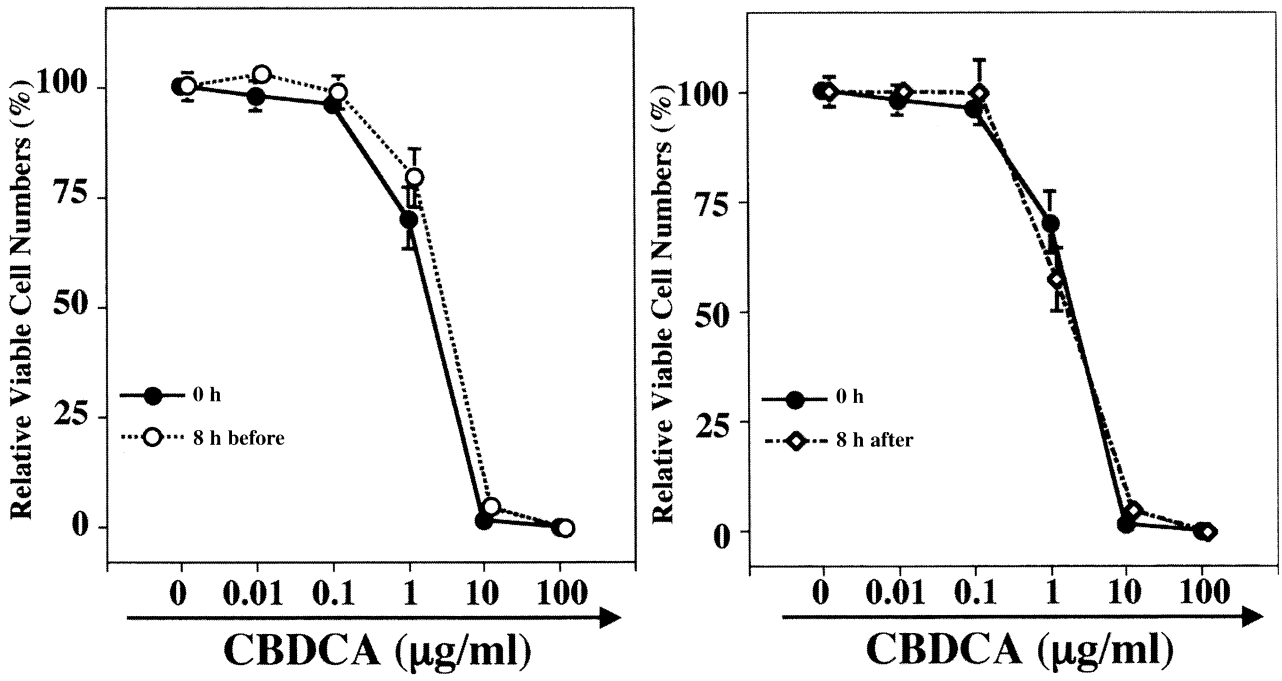

Fig.

Figure 2. — *Effects of CBDCA treatment and γ-irradiation on CBDCA sensitivity of ME180 cells.* A) The solid line with closed circles shows the control CBDCA sensitivity of cells irradiated with a single dose of 7.5 Gy immediately after CBDCA treatment. The dotted line with open circles shows the CBDCA sensitivity of cells treated with CBDCA 8 h before irradiation. B) The solid line with closed circles shows the control CBDCA sensitivity of cells irradiated with a single dose of 7.5 Gy immediately after CBDCA treatment. The dotted line with open circles shows the CBDCA sensitivity curve of cells treated with CBDCA 8 h after irradiation. No significant changes in the CBDCA sensitivity curves were observed.

Fig. 3A

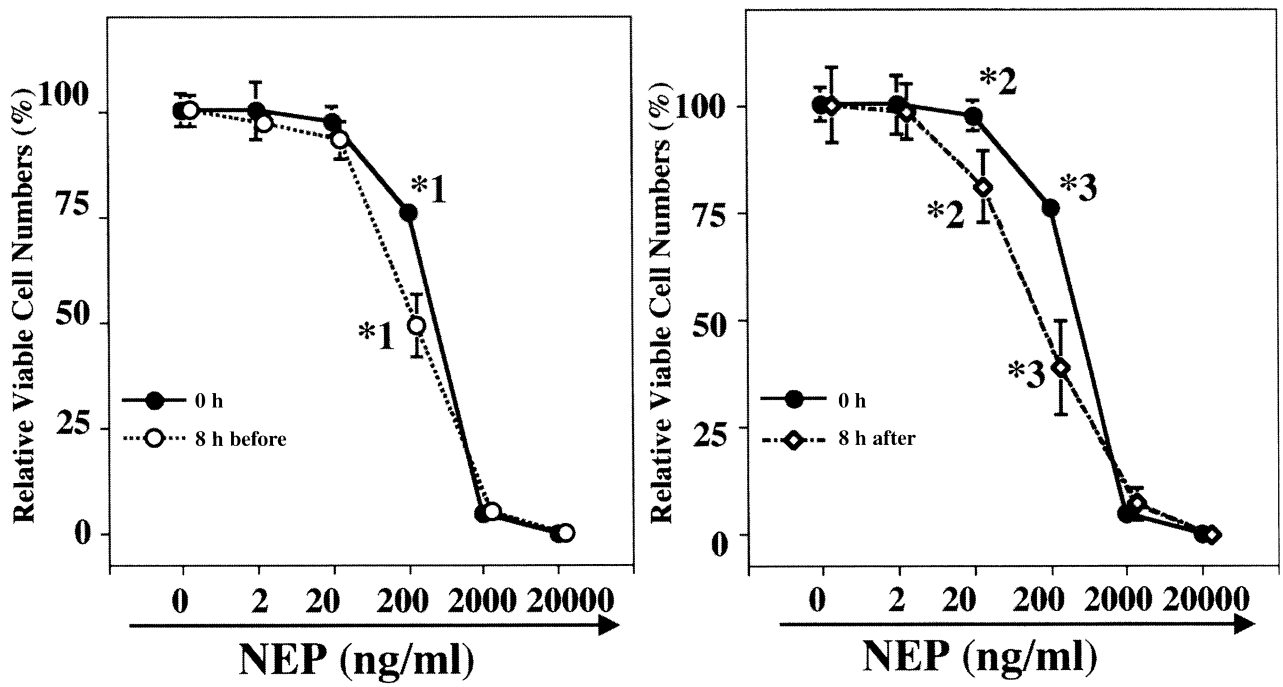

Fig.

Figure 3. — *Effects of NEP treatment and γ-irradiation on NEP sensitivity of ME180 cells.* A) The solid line with closed circles shows the control NEP sensitivity of cells irradiated with a single dose of 7.5 Gy immediately after NEP treatment. The dotted line with open circles shows the NEP sensitivity of cells treated with NEP 8 h before irradiation. B) The solid line with closed circles shows the control NEP sensitivity of cells irradiated with a single dose of 7.5 Gy immediately after NEP treatment. The dotted line with open circles shows the NEP sensitivity of cells treated with NEP 8 h after irradiation. NEP sensitivity was significantly enhanced when the irradiated cells were treated with NEP both 8 h before and 8 h after irradiation. *1, *2, *3: $p < 0.05$.

[20], CDDP should be administered to patients after completion of radiotherapy rather than as concurrent chemoradiotherapy. CBDCA cannot be recommended for chemoradiotherapy of cervical cancer.

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