Radiation enhances cisplatin-sensitivity in human cervical squamous cancer cells *in vitro*

T. Tanaka[1], K. Yukawa[2], N. Umesaki[1]

[1]Department of Obstetrics and Gynecology, [2]Department of Physiology, Wakayama Medical University, Wakayama (Japan)

Summary

Purpose and methods of investigation: Cisplatin (CDDP) is regularly used in concurrent chemoradiotherapy in patients with advanced cervical cancer although an effective protocol of chemoradiotherapy with CDDP has not yet been established. In search of a better chemoradiotherapy protocol, we investigated both CDDP effects on radiosensitivity and irradiation effects on CDDP-sensitivity using the radiosensitive human cervical squamous cell carcinoma cell line ME180.

Results: We found that CDDP did not affect cellular radiosensitivity, and that irradiation significantly enhanced CDDP-sensitivity. Moreover, all the four post-irradiation surviving subclones obtained from repetitively irradiated ME180 cells showed significantly higher CDDP sensitivities than those of the non-irradiated parent cells.

Conclusion: These results suggest that an effective protocol would involve the concurrent administration of CDDP with radiotherapy and further administration following completion of radiotherapy in order to achieve higher CDDP-sensitivities.

Key words: Cisplatin; Chemoradiotherapy; Cervical cancer; Squamous cell carcinoma; Radiosensitivity.

Introduction

Since most cervical squamous cell carcinoma (SCC) cells are radiosensitive, patients with unresectable advanced cervical SCC are usually treated by radiotherapy as the first choice of therapy. However, standard radiotherapy of cervical cancer patients is often non-radical for locally advanced cervical cancers with either huge primary tumors, wide invasion to pelvic walls, many lymph node metastases, or possible distant micrometastases. Therefore, in order to i) eradicate cancer cells outside irradiated fields, ii) enhance the radiosensitivity of cancer cells during radiotherapy, iii) and kill surviving cancer cells after irradiation, chemoradiotherapy has sometimes been concurrently applied to patients with locally advanced cervical cancer.

Cisplatin (CDDP) is thought to be the most effective anticancer drug for cervical cancer. Therefore, CDDP has been the most frequently used worldwide in concurrent chemoradiotherapy in patients with advanced cervical SCC. Several research groups interested in concurrent chemoradiotherapy for cervical cancer patients reported significant increases in survival ratios of cancer patients treated with concurrent chemoradiotherapy using CDDP [1-5]. On the other hand, a few studies could not find any beneficial effects on survival times for patients receiving CDDP chemoradiotherapy [6]. In these studies, usual administration protocols of CDDP in concurrent chemoradiotherapy involved weekly injections of 40-75 mg/m^2 CDDP [1-6]. Recently, a few basic investigations reported the effective concurrent use of CDDP with radiotherapy. In addition, Tabata *et al.* [7] showed that cervical cancer patients pretreated with bleomycin, vincristine, mitomycin and cisplatin (BOMP) chemotherapy including CDDP before radiotherapy demonstrated a lower survival ratio compared with patients treated with radiotherapy alone. These results suggest that the use of BOMP chemotherapy before radiotherapy reduces radiosensitivity of cervical cancer cells. In the present study, we used radiosensitive human cervical squamous carcinoma cells to investigate several options of chemoradiotherapy with CDDP in order to achieve optimal treatment.

Materials and Methods

Cell line and cell culture

In this study, the radiosensitive human cervical SCC cell line ME180 with wild-type p53 genes [8] was used. The ME180 cells were obtained from Japan Resources of Cell Bank (JRCB, Tokyo, Japan). All cells used in this study were cultured in OPTI-MEM (GIBCO-BRL, Gaithersburg, MD, USA) supplemented with 5% fetal calf serum (FCS) (EQUITECH BIO Inc., Ingram, TX, USA) and a mixture of 100 U/ml penicillin/100 µg/ml streptomycin (GIBCO-BRL). CDDP used in this study was a gift from Nihon-Kayaku Co. (Tokyo, Japan).

Cell viability assay

Cell proliferation was assessed with the XTT non-RI colorimetric assay kit (Boerhinger Mannheim, Mannheim, Germany). Growth-inhibitory effects of radiation and CDDP on ME180 cells were investigated as follows. Cells in the log phase were initially dispersed with 0.25% trypsin/1 mM EDTA (GIBCO-BRL), and subsequently cultured overnight in 96-well culture plates (5,000 cells/well). On the second day, various doses of γ-rays were used to irradiate the cells using a MBR 1520A irradiator (Hitachi-Medico, Tokyo, Japan). On the fourth day, viable cells were counted with the XTT kit. In order to examine the modulatory effects of CDDP on cell death induced by irradiation, cells were treated with various concentrations of CDDP

Revised manuscript accepted for publication April 22, 2004

and immediately γ-irradiated at different doses, followed by a 2-day culture. Finally, relative viable cell numbers (expressed as a percentage) were calculated using the XTT kit. All experiments were repeated two or three times to verify the results. Data are reported as means followed by standard deviations (SD), and comparative data (n = 6) were statistically analyzed by ANOVA.

Establishment of surviving subclones following repetitive irradiations

Post-irradiation surviving subclones were established as follows. ME180 parent cells cultured in a 96-well culture plate (10,000 cells/well) were subjected to four consecutive doses of radiation (10 Gy each) once a week, and cultured for about four weeks. In a preliminary experiment, more than 90% of ME180 cells were killed after a single dose of 10 Gy γ-ray irradiation. Cells were collected from each of the four wells containing surviving cancer cell colonies and sub-cultured with a lower cell density (0.1-20 cells/well) using a limiting dilution protocol. Cloning efficiencies assessed from the limiting dilution cultures were below 10% (3.7%-9.1%). Finally, four months following the initial irradiation, four monoclonal post-irradiation surviving subclones were established.

Results

First of all, effects of CDDP on radiosensitivity of ME180 cells were examined. As illustrated in Figure 1, CDDP did not have any significant effect on ME180 radiosensitivity curves. Secondly, effects of irradiation on ME180 CDDP-sensitivity were investigated and we found that irradiation significantly enhanced CDDP-sensitivity (Figure 2). In order to determine whether post-irradiated cancer cells maintain the higher CDDP-sensitivity following irradiation, we established four post-irradiation surviving subclones according to methods described above and examined their sensitivity to CDDP. All four established subclones demonstrated significant higher CDDP-sensitivities than the parent ME180 cells as shown in Figure 3, suggesting that post-irradiation increases CDDP-sensitivity and this can be maintained for at least four months after initial irradiation.

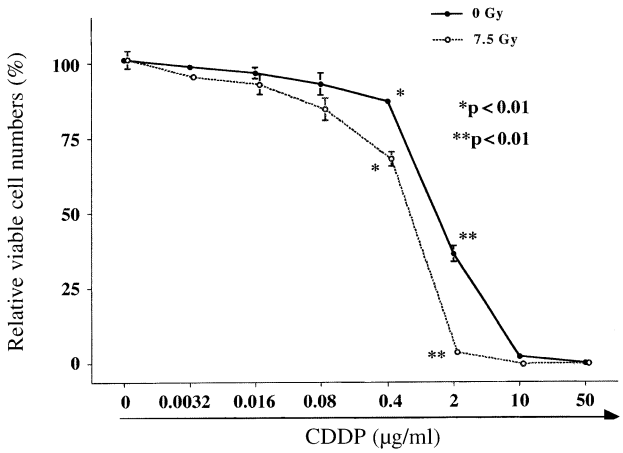

Figure 2. — *Effects of irradiation on CDDP-sensitivity of ME180 cells.*
Within 20 minutes after various concentrations of CDDP were added to ME180 cells, γ-rays were used for radiation. The solid line with closed circles shows the control CDDP-sensitivity curve of cells cultured without irradiation. γ-ray irradiation (7.5 Gy) significantly enhanced the CDDP-sensitivity (dotted line with open circles) (*p < 0.01, **p < 0.01).

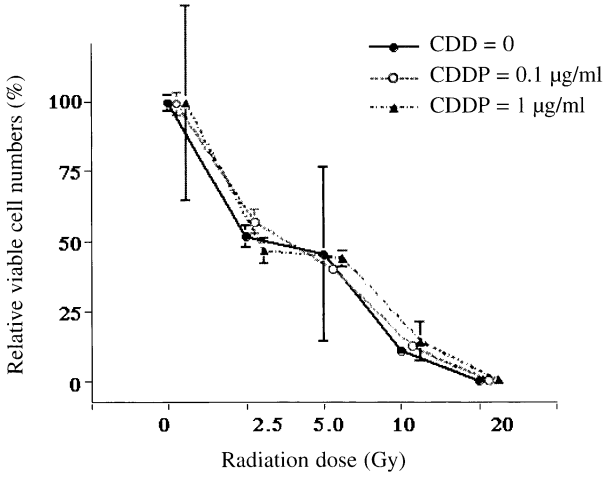

Figure 1. — *Effects of CDDP on radiosensitivity of ME180 cells.*
Within 20 minutes after initial addition of CDDP to ME180 cells, various doses of γ-rays were used for radiation. Final CDDP concentrations in culture media are 0, 0.1, and 1 μg/ml, respectively. The solid lines with closed circles show control radiosensitivity curves of cells cultured without CDDP. The dotted lines with open circles and closed triangles are those of cells cultured with CDDP. There was no significant difference between the three radiosensitivity curves.

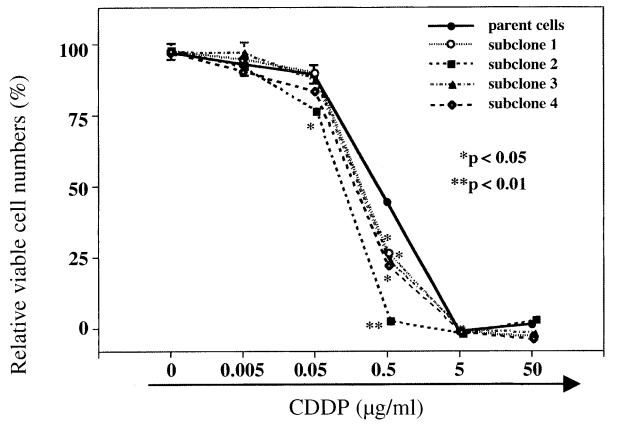

Figure 3. — *CDDP-sensitivity of post-irradiated surviving cells.*
CDDP-sensitivity of the four subclones established from surviving cells following irradiation was compared with the CDDP-sensitivity of non-irradiated ME180 parent cells. The solid line with closed circles shows the control CDDP-sensitivity curve of ME180 parent cells. All four post-irradiated surviving subclones (dotted lines) displayed significantly higher sensitivities to CDDP compared with non-irradiated parent cells (*p < 0.05, **p < 0.01).

Discussion

Radiotherapy is the most commonly used therapy for locally advanced cervical cancers and CDDP is also one of the most effective anticancer drugs used for advanced cervical cancers. Therefore, CDDP has been widely used in concurrent chemoradiotherapy for advanced cervical cancer patients [1-6]. Although many reports showed that concurrent chemoradiotherapy with CDDP resulted in significantly higher survival ratios than those of radiotherapy alone [1-5], there have been few reports on how to effectively administer CDDP during radiotherapy. Studies reporting concurrent chemoradiotherapy with CDDP used weekly injections of 40-75 mg/m^2 CDDP. When and how CDDP should be injected to cancer patients during radiotherapy has not been investigated yet. Tabata et al. reported that BOMP chemotherapy with the use of CDDP before radiotherapy in cervical cancer patients resulted in significantly lower survival ratios than those in patients treated with radiotherapy alone [7]. This report suggests that radiosensitivity of cancer cells may be reduced by BOMP chemotherapy. Additionally these results make us aware that chemoradiotherapy of cervical cancers may provide worse treatment than radiotherapy alone in cases of inadequate administration of chemotherapeutic drugs.

In our study, we used radiosensitive human cervical SCC cells to report optimal treatment conditions for combined chemoradiotherapy with CDDP. Although CDDP does not affect radiosensitivity of cancer cells, irradiation significantly enhances CDDP-sensitivity. Moreover, we found that surviving cancer cells following irradiation have a higher CDDP-sensitivity. These results suggest that CDDP should be administered to cervical cancer patients not before but after irradiation. Our results coincide with clinical results reported by Tabata *et al.* [7]. In conclusion, post-irradiation CDDP injection may be a better treatment than concurrent chemoradiotherapy alone because irradiated cancer cells may retain higher CDDP-sensitivity for several months after irradiation. Moreover, our proposed investigative procedures can be applied to other studies aimed to optimize other combinations of anticancer drugs with radiotherapy in cervical cancer patients.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and a Grant-in-Aid for Scientific Research from the Ministry of Welfare and Labor of Japan. We would like to thank Nihon-Kayaku Co. for donating CDDP.

References

[1] Whitney C.W., Sause W., Bundy B.N., Malfetano J.H., Hannigan E.V., Fowler W.C. Jr. *et al.*: "Randomized comparison of fluorouracil plus cisplatin versus hydroxyurea as an adjunct to radiation therapy in stage IIB-IVA carcinoma of the cervix with negative para-aortic lymph nodes: a Gynecologic Oncology Group and Southeast Oncology Group Study". *J. Clin. Oncol.,* 1999, *17,* 1339.

[2] Morris M., Eifel P.J., Lu J., Grigsby P.W., Levenback C., Stevens R.E. *et al.*: "Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer". *New Eng. J. Med.,* 1999, *340,* 1137.

[3] Rose P.G., Bundy B.N., Watkins E.B., Thigpen J.T., Deppe G., Maiman M.A. *et al.*: "Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer". *New Eng. J. Med.,* 1999, *340,* 1144.

[4] Keys H.M., Bundy B.N., Stehman F.B., Muderspach L.I., Chafe W.E., Suggs C.L. 3rd *et al.*: "Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma". *New Eng. J. Med.,* 1999, *340,* 1154.

[5] Peters W.A. 3rd, Liu P.Y., Barrett R.J. 2nd, Stock RJ, Monk B.J., Berek J.S. *et al.*: "Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix". *J. Clin. Oncol.,* 2000, *18,* 1606.

[6] Pearcey R., Brundage M., Drouin P., Jeffrey J., Johnston D., Lukka H. *et al.*: "Phase III trial comparing radical radiotherapy with and without cisplatin chemotherapy in patients with advanced squamous cell cancer of the cervix". *J. Clin. Oncol.,* 2002, *20,* 966.

[7] Tabata T., Takeshima N., Nishida H., Hirai Y., Hasumi K.: "A randomized study of primary bleomycin, vincristine, mitomycin and cisplatin (BOMP) chemotherapy followed by radiotherapy versus radiotherapy alone in stage IIIB and IVA squamous cell carcinoma of the cervix". *Anticancer Res.,* 2003, *23,* 2885.

[8] Lancillotti F., Giandomenico V., Affabris E., Fiorucci G., Romeo G., Rossi G.B.: "Interferon alpha-2b and retinoic acid combined treatment affects proliferation and gene expression of human cervical carcinoma cells". *Cancer Res.,* 1995, *55,* 3158.

Address reprint requests to:
T. TANAKA, M.D., Ph.D.
Department of Obstetrics and Gynecology
Wakayama Medical University
811-1 Kimi-idera,
Wakayama 641-0012 (Japan)