Effect of cis-diammine dichloroplatinum on vascular endothelial growth factor expression in uterine cervical carcinoma

Y. Miyahara, S. Yoshida, S. Motoyama, Y. Tateiwa, S. Hamana, T. Maruo

Department of Obstetrics and Gynecology, Kobe University Graduate School of Medicine, Kobe (Japan)

Summary

Purpose of investigation: In this study, we investigated the effects of cis-diammine dichloroplatinum (CDDP) on *VEGF* mRNA expression and VEGF production in uterine cervical carcinoma tissues obtained from patients with locally advanced disease and in CaSki cells cultured *in vitro.*

Methods: VEGF in cultured CaSki cells and in the culture media was measured using a sensitive enzyme-linked immunosorbent assay (ELISA) before and 24 h, 48 h and 72 h after 3 h exposure to CDDP. VEGF mRNA expression in CaSki cells was assessed by the semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) before and 24 h and 48 h after 3 h exposure to CDDP. We also examined the effect of CDDP on microvessel counts in uterine cervical carcinoma tissues obtained before and after high-dose CDDP intraarterial chemotherapy. Immunohistochemical staining using a monoclonal antibody against CD34 was carried out with cervical carcinoma tissue specimens, and microvessel counts were quantified by counting vessels.

Results: CDDP treatment resulted in significant increases in not only VEGF concentrations in cultured CaSki cells and culture media but also in *VEGF* mRNA expression levels in cultured CaSki cells in a time-dependent and dose-dependent manner compared to untreated controls ($p < 0.05$, $n = 5$). On the other hand, VEGF concentrations and microvessel counts in cervical carcinoma tissues were significantly lower in cases with complete response (CR) and partial response (PR), compared to those before treatment ($p < 0.05$, $n = 5$). By contrast, in cases with no change (NC) to CDDP, both VEGF concentrations and microvessel counts did not decrease and rather showed a somewhat increase compared with levels prior to the treatment.

Conclusions: These results suggest that CDDP-induced increases in VEGF production by cervical carcinoma cells may stimulate angiogenesis in the tumor lesion after CDDP treatment.

Key words: VEGF; Uterine cervical carcinoma; CaSki cells; RT-PCR; CDDP; Percutaneous pelvic perfusion extracorporeal chemofiltration (PPPEC).

Introduction

The onset and maintenance of the blood supply to solid tumors requires the formation of new blood vessel capillaries from the existing host vasculature, a process known as tumor angiogenesis [1, 2]. This process is essential for solid tumors to grow beyond microscopic sizes, and is thought to be mediated by a number of either directly or indirectly acting growth factors, many of which are produced by tumor cells themselves [1, 2]. Among the growth factors which directly affect vascular endothelial cell proliferation and motility, vascular endothelial growth factor (VEGF) has attracted the most experimental interest over the last five years [3-7].

Angiogenesis is associated with high grade cervical dysplasia and with invasive squamous cell carcinoma of the uterine cervix, and thus altered vascular patterns have proved useful in colposcopic detection of these lesions [8-12]. Recently, much attention has been focused on combination therapy, the use of anticancer agents in conjunction with angiogenesis inhibitory agents, to suppress the proliferation of cancer cells in patients [13, 14]. Several reports have demonstrated overexpression of VEGF in cervical carcinoma tissues compared to normal cervical tissues on the basis of immunohistochemical analysis [15, 16].

However, little information is available regarding the effect of cis-diammine dichloroplatinum (CDDP) on VEGF expression in uterine cervical carcinoma. We have therefore evaluated VEGF mRNA expression in CaSki cells before and after exposure to CDDP by semi-quantative reverse transcription polymerase chain reaction (RT-PCR). Using a sensitive enzyme-linked immunosorbent assay (ELISA), we also determined VEGF levels in cultured cervical squamous carcinoma cell line CaSki cells before and after exposure to CDDP and in cervical carcinoma tissues collected from patients before and after high-dose CDDP intraarterial chemotherapy. In addition, we measured microvessel counts in uterine cervical carcinoma tissue specimens from patients before and after high-dose CDDP intraarterial chemotherapy. This is the first report to demonstrate the effect of CDDP on *VEGF* mRNA expression and VEGF production in uterine cervical carcinoma cells both *in vivo* and *in vitro.*

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Materials and Methods

Tissue samples

Cervical carcinoma tissue specimens were obtained from ten patients (mean age: 54.2 ± 13.1 years) with locally advanced cervical squamous carcinoma who received percutaneous pelvic perfusion with extracorporeal chomofiltration (PPPEC) in a neoadjuvant setting from April 1997 until March 2001 [17, 18]. Cis-diammine dichloroplatinum (CDDP) (140-240 mg/m^2) was infused in uterine arteries using the PPPEC system twice during a 2-week interval. The Resarch Ethics Committee of Kobe University Hospital approved the collection of patient tissue samples after informed consent was obtained from each patient.

Squamous carcinoma tissue (mean diameter: 5.1 ± 2.4 cm) was collected from the patients before and 24 h after the first course of PPPEC. The clinical stage distribution, according to the International Federation of Gynecology and Obstetrics criteria [17, 18], was seven cases of Stage IIIb and three cases of Stage IVa with bladder invasion. To evaluate tumor response, the volumetric reduction rate of cervical tumor was calculated by multiplying three spatial axes on the basis of magnetic resonance imaging (MRI) examination. Tumor response was defined as complete response (CR) if the known tumor entirely disappeared, or partial response (PR) if there was a 50% or greater reduction of the tumor; nonresponders were referred to as no change (NC).

Cell culture

The CaSki cell line is established from epidermoid carcinoma of the cervix, and is large cell non-keratinizing squamous cell carcinoma in origin (Dainippon Pharmaceutical Co. Tokyo, Japan). The cells were subcultured and grown in RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO-BRL, Grand Island, NY), penicillin (100 U/ml) and streptomycin (100 µg/ml) at a density of 5× 10^5 cells/25-sq cm plastic flask (Falcon Plastic Co., Oxnard, CA) in 5 ml medium at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Five days after subculture, the medium was replaced with fresh serum-free medium containing CDDP (either 1.5 µg/ml or 5.0 µg/ml) for 3 h. The cells were then washed three times with PBS, and incubated with serum-free medium for a subsequent period of time as follows. After 24 h, 48 h and 72 h, the cells and media were collected by trypsin-EDTA (0.05% Trypsin, 0.53 mM EDTA-4Na, GIBCO-BRL). CDDP exposure time and CDDP concentrations were calculated from the area under the concentrations curve (AUC) data generated while performing normal-dose and high-dose CDDP intraarterial chemotherapy. The AUC of 3 h exposure to 1.5 µg/ml CDDP and 5.0 µg/ml CDDP corresponded to normal-dose (75 mg/m^2) and high-dose (200 mg/m^2) CDDP chemotherapy, respectively [17-19].

Measurement of VEGF

VEGF concentrations were measured in culture media and in CaSki cell extracts before and 24 h, 48 h and 72 h after 3 h exposure to CDDP, as well as in extracts from the uterine cervical carcinoma tissues collected from patients before and 24 h after high-dose (200 mg/m^2) CDDP intraarterial chemotherapy.

CaSki cells and tissue specimens were lysed with lysis buffer (2 mM EDTA, 20 mM Tris: pH 7.8, 150 mM NaCl, 50 mM β-glycerol phosphate, 0.5% NP40, 1% glycerine, 1 mM sodium orthovanadate, 1 mM dithiothreitol, 5 µg/ml aprotinin, 10 mM sodium fluoride, 2 µM leupeptin, and 2 mM phenylmethyl sulphonyl fluoride). After centrifugation at 15,000 rpm for 15 min, intracellular VEGF concentrations were measured in the supernatants derived from the lysates. An ELISA kit (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN, USA) was used to measure VEGF. Samples (100 µl in duplicate) were incubated at room temperature for 2 h in microtitre plates precoated with a monoclonal antibody specific for VEGF. After washing, an enzyme-linked polyclonal antibody specific for VEGF was added, followed by streptavidin peroxidase (Sigma) and 3,3', 5', 5'-tetrametyl benzidine (Kirgaard & Perry Labs) as the substrate. After incubation at room temperature for 30 min, the intensity of color was read at 450 nm within 30 min. The results were calculated from a standard curve generated by a four parameter logistic curve-fit with recombinant human VEGF 165 (range: 31.2-2,000 pg/ml) as the standard preparation. This assay recognized both natural human VEGF and recombinant VEGF and did not cross-react with a series of cytokines and growth factors. Total protein concentrations in each supernatant was determined by colorimetric Bradford protein assay [20].

Semi-quantitative RT-PCR and Southern blot analysis

CaSki cells were seeded onto 100 mm culture plates (Falcon Plastic Co., Oxnard, CA) and cultured to subconfluency (approximately 8 × 10^4 cells/cm^2). Thereafter, the cells were stepped down to serum-free conditions by incubating in serum-free RPMI 1640 in the absence or presence of CDDP. Total RNA was isolated from cultured CaSki cells by the guanidinium thiocynate and phenol/chloroform method 24 h and 48 h after 3 h exposure to CDDP (1.5 µg/ml or 5.0 µg/ml). First strand complementary DNA (cDNA) was synthesized from 4 µg total RNA using a cDNA synthesis Kit (QIAGEN, Tokyo, Japan). PCR was performed using 1 µl cDNA as template, 6.25 pM of each primer, 2.5 mM dNTPs, 0.125 U Taq DNA polymerase (Roche-Diagnostics Inc., Germany) and 1 × reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM of KCl, 1.5 mM of $MgCl_2$, and 0.01% gelatin) in a 25 µl reaction volume.

The primers used to amplify the *VEGF* cDNA were 5'-GCTCTACCTCCACCATGCCA-3' (sense) and 5'-AGCT-CATCTCTCCTAGTGC-3' (antisense). The sense primer annealed to bases 145-164, while antisense corresponded to bases 445-464 of the *VEGF* cDNA. The length of the expected PCR product was 320 bp. PCR amplification was performed using a Gene Amp PCR System 9600-R (Perkin Elmer Corp, Netwalk, CT). The amplification procedure included an initial denaturation step (94°C, 5 min) followed by 30 cycles of denaturation (94°C, 30 sec), annealing (55°C, 30 sec) and extention (72°C, 30 sec). The sequence of the primers used to amplify the housekeeping *β-actin* gene was 5'-CTTCTACAATGAGCT-GCGTG-3' (sense) and 5'-TCATGAGGTAGTCAGTCAGG-3' (antisense). The sense primers annealed to bases 308-327, while the antisense primers corresponded to bases 593-612 of the *β-actin* gene. The length of the expected PCR product was 305 bp. The amplification procedure involved an initial denaturation step at 94°C for 5 min followed by 15 cycles of denaturation (94°C, 30 sec), annealing (55°C, 30 sec) and extention (72°C, 30 sec). The VEGF RT-PCR products were electrophoresed on 3% agarose gel, and after denaturation with alkaline solution, were transferred to a nylon membrane filter for Southern blot. After transfer, the DNA was fixed by UV irradiation; and prehybridization (65°C, 2 h) was performed, followed by hybridization (65°C, 18 h) using the 5'-^{32}P end-labeled oligonucleotide probe. The filters were then washed twice with 2 ×

saline sodium citrate (1 × SSC = 150 mM NaCl and 15 mM trisodium citrate, pH 7.0) and 0.1% sodium dodecyl sulphate (SDS) (room temperature, 20 min) and then with 0.2 × SSC and 0.1% SDS (60°C, 5 min).

The blot was then exposed to X-ray film for 3 h and subsequently developed. The autoradiograph was then scanned (ScanJet 3C/ADF; Hewlett Packard, USA), and the strength of the signal was quantified (NIH Image ver 1.58). The amount of *VEGF* mRNA was expressed relative to the amounts of β-actin mRNA present in each specimen.

CD34 immunohistochemical staining on uterine cervical carcinoma tissue

Uterine cervical carcinoma tissue was collected from the patients before and 24 h after the first course of high-dose CDDP intraarterial chemotherapy, as described above. The cervical carcinoma tissue specimens were fixed in 4% buffered neutral formaldehyde, dehydrated in graded alcohol, and embedded in paraffin. Sections of 4 μm were prepared for immunohistochemical staining. Immunohistochemical staining was performed using the avidin/biotin immunoperoxidase method using a polyvalent immunoperoxidase kit (Omnitags, Lipshaw, MI) as previously described [21]. After washing with phosphate buffered saline, the sections were immersed in 3% H_2O_2 to inhibit endogenous peroxidase activity. The primary anti-CD34 protein monoclonal antibody was diluted at 1:100 in distilled water before use. The first incubation was done for 30 min with the primary antibody followed by the second incubation for 30 min with biotinylated polyvalent antibody. The third incubation was carried out with avidin-horseradish peroxidase for 30 min. Thereafter, the chromogenic reaction was developed by incubation with a freshly prepared solution of 3-amino-9-ethylcarbazole and 3% H_2O_2 for 30 min. The sections were then counterstained with Harris hematoxylin, mounted in glycerine phosphate buffer solution and examined microscopically.

To assure the specificity of the immunological reaction, control sections were subjected to the same immunoperoxidase method, except that the primary antibody was replaced by non-immune murine IgG (Miles, Erkhardt, IN) at the same dilution as the specific antibody. The replacement of the specific primary antibody with nonimmune murine IgG resulted in a lack of positive immunostaining.

Evaluation of microvessel counts

Microvessel counts were assessed by scanning tumor sections with light microscopy. As Weider *et al.* described, vessel lumens were not necessary for a structure to be defined as a vessel [3]. The highly vascular areas, containing the most capillaries and small venules at the invasive edge, were identified at low power (× 40). After ten areas with the highest neovascularization were identified, microvessel counts were performed on a × 200 field (× 20 objective and × 10 ocular). The results were then expressed as the average counts of ten fields.

Statistical analysis

The data collected are shown as the mean ± SD from at least three independent experiments. Statistical significance was evaluated using two-way ANOVA. Differences with p values of < 0.05 were considered statistically significant.

Results

VEGF levels in uterine cervical carcinoma tissue before and after high-dose CDDP intraarterial chemotherapy

The rate of CR plus PR (CR + PR) two weeks after the first course of PPPEC was 50% (5 cases). Before the high-dose CDDP intraarterial chemotherapy, VEGF levels in cervical carcinoma tissue from CR + PR cases were significantly higher than those from NC cases (mean counts ± SD, 158.3 ± 15.8 vs 110 ± 19.1 pg/mg protein, respectively: $p < 0.05$, $n = 5$) (Figure 1). After the high-dose CDDP intraarterial chemotherapy, VEGF levels in cervical carcinoma tissue from CR + PR cases were significantly lower compared with those prior to the treatment (76.1 ± 13.5 vs 158.3 ± 15.8 pg/mg protein, respectively: $p < 0.05$, $n = 5$). By contrast, VEGF levels in cervical carcinoma tissue from NC cases after high-dose CDDP intraarterial chemotherapy did not decrease and rather showed a tendency to increase compared with levels prior to the treatment (138.7 ± 18.1 vs 110 ± 19.1 pg/mg protein, respectively: $p = 0.12$, $n = 5$) (Figure 1).

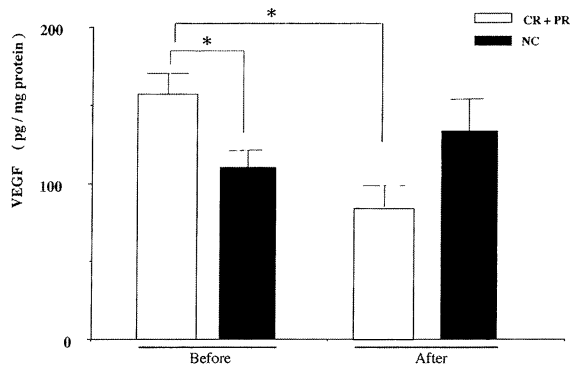

Figure 1. — VEGF levels in uterine cervical carcinoma tissues before and 24 h after the first course of high-dose CDDP (200 mg/m²) intraarterial chemotherapy. The data represent the mean ± SD. Concentrations of VEGF in cervical carcinoma tissue were measured by ELISA.
CR, complete response; PR, partial response; NC, no change.
*, $p < 0.05$

Effect of CDDP on VEGF levels in cultured CaSki cells and in culture media

The levels of VEGF in both cultured CaSki cell extracts (Figure 2) and the culture media (Figure 3) after 3 h exposure to CDDP (1.5 μg/ml or 5.0 μg/ml) significantly increased in a time- and dose-dependent manner, compared to the pre-treatment levels ($p < 0.05$, $n = 5$). The intracellular VEGF levels in CaSki cells 72 h after 3 h exposure to CDDP showed a 1.7-fold increase (128.6 ± 18.2 pg/mg protein) in 1.5 μg/ml CDDP-treated cultures and a 2.0-fold increase (145.0 ± 22.3 pg/mg protein) in

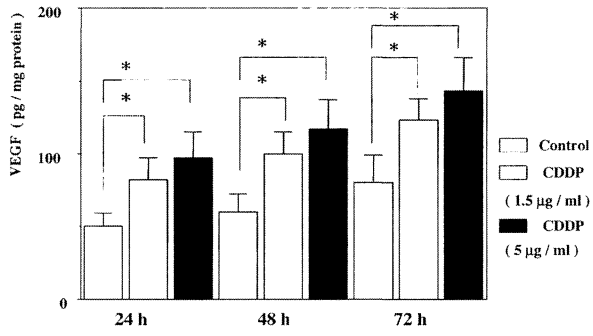

Figure 2. — Effect of 3 h exposure to CDDP (1.5 μg/ml or 5.0 μg/ml) on VEGF levels in cultured CaSki cells. The data represent the mean ± SD. Concentrations of VEGF in cultured CaSki cell extracts were measured by ELISA 24 h, 48 h and 72 h after the exposure to CDDP.
*, $p < 0.05$

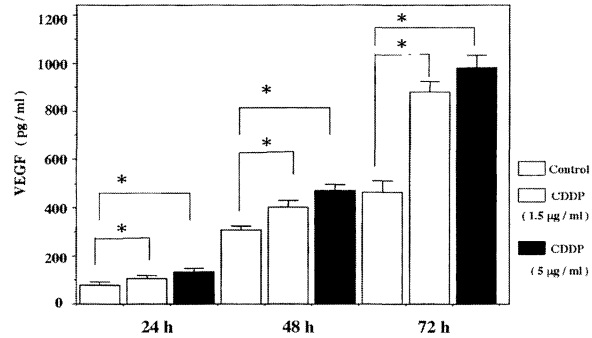

Figure 3. — Effect of 3 h exposure to CDDP (1.5 μg/ml or 5.0 μg/ml) on VEGF levels in media of cultured CaSki cells. The data represent the mean ± SD. Concentrations of VEGF in culture media were measured by ELISA 24 h, 48 h and 72 h after the exposure to CDDP.
*, $p < 0.05$

5.0 μg/ml CDDP-treated cultures compared to that in untreated control cultures (72.5 ± 19.5 pg/mg protein). On the other hand, the VEGF levels in culture media 72 h after 3 h exposure to CDDP showed a 1.8-fold increase (880.6 ± 19.2 pg/ml) in 1.5 μg/ml CDDP-treated cultures and a 2.0-fold increase (980.9 ± 20.3 pg/ml) in 5.0 μg/ml CDDP-treated cultures compared to that in untreated control cultures (472.6 ± 19.8 pg/ml).

Effect of CDDP on VEGF mRNA expression in cultured CaSki cells

Densitometric quantification of the signals produced by hybridization in each treatment group which was normalized according to *β-actin* mRNA expression revealed that the expression of *VEGF* mRNA with a molecular basis of 320 bp in cultured CaSki cells 24 h after 3 h exposure to 1.5 μg/ml CDDP and 5.0 μg/ml CDDP showed a 2.0-fold increase and a 2.5-fold increase, respectively, compared to that in untreated control cultures (Figure 4-A).

Forty-eight hours after 3 h exposure to 1.5 μg/ml CDDP and 5.0 μg/ml CDDP, *VEGF* mRNA expression in cultured CaSki cells showed a 1.8-fold increase and 2.3-fold increase, respectively, compared to that in untreated control cultures (Figure 4-B).

Effect of CDDP on microvessel counts in uterine cervical carcinoma tissue

Before high-dose CDDP intraarterial chemotherapy, microvessel counts in cervical carcinoma tissue specimens from CR + PR cases were significantly higher compared to those from NC cases (62.3 ± 9.1 vs 40.1 ± 20.1 vessels per field, respectively: $p < 0.05$, $n = 5$) (Figure 5). After high-dose CDDP intraarterial chemotherapy, the microvessel counts in cervical carcinoma lesions significantly decreased in CR + PR cases compared to those before treatment (38.9 ± 15.4 vs 62.3 ± 9.1 vessels per field, respectively: $p < 0.05$, $n = 5$). In contrast, the microvessel counts in cervical carcinoma tissue specimens from NC cases after high-dose CDDP intraarterial chemotherapy did not decrease and rather showed a somewhat increase compared to those before treatment (40.1 ± 20.1 vs 56.4 ± 17.3 vessels per field, respectively: $p = 0.14$, $n = 5$) (Figure 5).

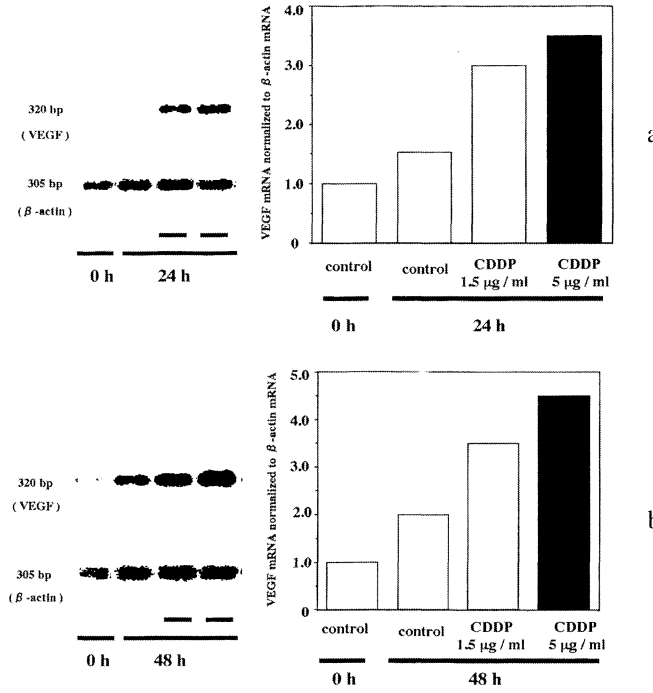

Figure 4. — Effect of 3 h exposure to CDDP (1.5 μg/ml or 5.0 μg/ml) on *VEGF* mRNA expression in cultured CaSki cells 24 h (A) and 48 h (B) after the exposure to CDDP. *VEGF* mRNA expression was analysed by semi-quantitative RT-PCR and Southern blot before exposure and 24 h, 48 h after exposure to CDDP. The intensity of the *VEGF* mRNA band was normalized to that of the β-actin band. The intensity of the transcripts was quantified by pixel densitometry.

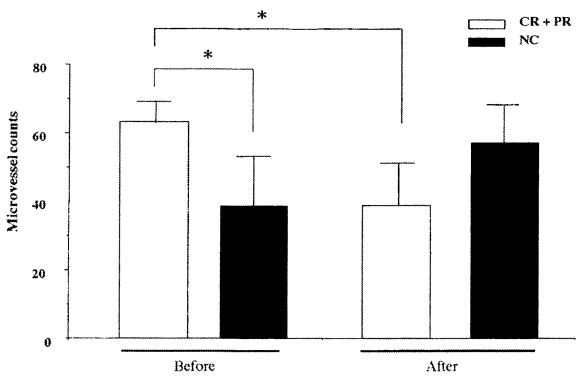

Figure 5. — Evaluation of microvessel counts in uterine cervical carcinoma tissue before and 24 h after high-dose CDDP (200 mg/m^2) intraarterial chemotherapy. The microscopic vessel densities were examined by immunohistochemical staining for CD34. The data represent the mean ± SD. CR, complete response; PR, partial response; NC, no change.
*, $p < 0.05$.

Discussion

Angiogenesis is critical for tumor growth beyond a minimal size, and to date, numerous angiogenic factors have been identified [1, 2]. VEGF is one of the most important regulators of tumor angiogenesis. This multifunctional protein is well known to affect vascular permeability, cell proliferation and migration via its receptors on endothelial cells, all of which are required for angiogenesis [3-7]. Several reports have shown that angiogenesisis is associated with high-grade squamous intraepithelial lesions and invasive squamous cell carcinoma of the uterine cervix [8-12]. Neoplastic epithelial cells abundantly express VEGF in the majority of cases of invasive cervical carcinoma and in nearly half of the cases of high-grade intraepithelial lesions [8-12]. Furthermore, abundant expression of VEGF has been associated with significantly increased microvessel counts in cervical stroma and with a higher incidence of vascular cuffing at the epithelial-stroma interface [22-24]. In contrast, only focal low-levels of VEGF are expressed in benign epithelia and low-grade squamous intraepithelial lesions of the uterine cervix [22-24]. Thus, increased expression of VEGF seems to be associated with angiogenesis in uterine cervical carcinomas.

Until now, however, there have been few reports which examined the effects of anticancer agents on VEGF expression in gynecological tumors. Thus, the present study was conducted to investigate the effect of CDDP on VEGF expression in cervical squamous carcinomas. Our *in vivo* results demonstrate that VEGF levels in cervical carinoma tissue before high-dose CDDP treatment were significantly higher in the sum of patients showing complete response and partial response compared with those in non-responders. Consistent with this, the microvessel counts in uterine cervical carcinoma tissue before high-dose CDDP treatment were significantly higher in CR + PR cases than those in NC cases. To add, VEGF levels in cervical carcinoma tissue after high-dose CDDP treatment significantly decreased in CR + PR cases compared with those prior to the treatment. In contrast, in NC cases, VEGF levels somewhat increased after high-dose CDDP treatment compared with those before treatment. As angiogenesis is closely associated with increased VEGF expression in carcinoma tissue [1-7], our data suggest that microvessel-rich and VEGF-rich cervical carcinomas might respond better to the treatment with high-dose CDDP, and that VEGF might be a useful marker to predict the efficacy of CDDP treatment.

Interestingly, Lissoni *et al.* reported that the mean values of VEGF in serum of breast cancer patients significantly decreased during paclitaxel therapy in patients with partial response or stable disease, whereas this was not observed in cases with progressive disease [25]. Kohno *et al.* reported that in patients with Stage IIa-IVa invasive squamous cell carcinoma of the cervix, the degree of tumor cell necrosis following intraarterial chemotherapy was proportional to the tumor vascularity [26]. These observations are consistent with our *in vivo* data, which suggest that the efficacy of CDDP treatment in cervical carcinoma is closely related to the neovascular density in tumor lesions. On the other hand, our *in vitro* data demonstrate that CaSki cells constitutively express measurable amounts of *VEGF* mRNA and that *VEGF* mRNA expression in cultured CaSki cells and the intracellular VEGF levels significantly increased in survived CaSki cells 24 h and 48 h after 3 h exposure to CDDP, in either concentrations of 1.5 μg/ml or 5.0 μg/ml. This suggests that CDDP treatment induces the production of VEGF in surviving CaSki cells in culture. Consistent with the present *in vitro* findings, VEGF levels in remaining cervical carcinoma tissue after high-dose CDDP intraarterial chemotherapy showed a tendency to increase compared to those prior to the treatment. In view of our *in vivo* and *in vitro* data, we can assume that CDDP may induce VEGF production in surviving uterine cervical carcinoma cells after treatment with CDDP.

Kato *et al.* reported that VEGF inhibited apoptoic cell death in hematopoietic cells induced by various stresses including ionizing radiation and chemotherapeutic drugs [27]. They also showed that MCL1, an antiapoptotic factor and member of the bcl-2 family, was induced in hematopoietic cells by VEGF [27]. Fernandes *et al.* reported that bcl-2 contributes to tumor progression at multiple levels through both the inhibition of apoptosis and the induction of angiogenesis [28]. Taking these into account, it seems likely that increased levels of VEGF and the subsequent suppression of apoptosis may play an important role in the regrowth of surviving carcinoma cells following chemotherapy with CDDP. Foekens *et al.* reported that the tumor VEGF level was an independent marker that predicts the efficacy of chemotherapy in advanced breast cancer, and that the determination of the tumor levels of VEGF might be helpful in selecting patients who may benefit from treatment with antiangiogensis agents combined with conventionally used chemotherapeutic drugs [29]. In this regard, much atten-

tion has recently been paid to antiangiogenesis agents, especially TNP470 [30-33]. Antitumor and antimetastatic activities of an antiangiogenesis agent, TNP-470, O-(Chloroacetyl-carbamoyl) fumagillol, an analog of fumagillin derived from Aspergillus fumigatus, have been reported in various human malignant cell lines [30-33]. TNP470 inhibits both *in vivo* and *in vitro* angiogenesis even in the presence of angiogenesis factors [30-33]. Kato *et al.* reported significant combinative effects of TNP-470 with mitomycin-C, adriamycin, CDDP and 5-fluorouracil in mouse models [34]. Yamaoka *et al.* demonstrated the enhanced effect of TNP-470 on prostate carcinomas when combined with CDDP [35]. On the basis of the present *in vitro* and *in vivo* data, TNP-470 combined with CDDP may also lead to improved efficacy of the treatment of uterine cervical carcinoma with increased microvessels.

Conclusion

We have demonstrated that CDDP treatment increased VEGF expression both in cultured cervical carcinoma cell line CaSki cells and in uterine cervical carcinoma tissue obtained from patients. This is the first report to demonstrate the effect of CDDP on *VEGF* mRNA expression and VEGF production in uterine cervical carcinoma cells. Since increased expression of VEGF in cervical carcinoma treated with CDDP may induce angiogenesis and subsequent regrowth of the remaining tumor after CDDP treatment, it should be emphasized that treatment with CDDP in invasive uterine cervical carcinoma should be followed by the main therapy such as radical surgery or irradiation with intervals as short as possible unless a complete response has been achieved.

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Address reprint requests to:
T. MARUO, M.D., Ph.D.
Department of Obstetrics and Gynecology
Kobe University Graduate School of Medicine
7-5-1 Kusunoki-cho, Chuo-ku,
Kobe 650-0017 (Japan)