proteolysis for degradation of extracellular matrices (ECM). This allows tumor cells to invade through tissue stroma and subsequently to enter and spread via the blood and lymphatic systems. Matrix metalloproteinases (MMPs), including MMP-2 and 9 (gelatinase A and B), are members of proteolytic enzymes which can degrade native collagens and other ECM components [15]. Enhanced mRNA and protein levels of MMP-2 and/or 9 have been detected in a variety of human malignant tumors. It is also noted that the urokinase-type plasminogen activator (uPA) plays an important role in the invasion process [16]. The proteolytic cascade of ECM components is triggered by the uPA-mediated conversion of plasminogen to plasmin and the subsequent activation of procollagenases [17].

The primary tumor with a high proportion of angiogenic cells is likely to give rise to metastatic implants that are already angiogenic, enabling them to grow in lymphnodes and distant organs [18]. Tumors that produce a higher level of angiogenic factors may have a more aggressive behavior than tumors negative for those factors in the process of invasion and metastasis. It may be expected that the expression of various angiogenic factors in tumor cells is closely associated with invasive phenotype of the cells. However, there have been very few reports on the co-expression patterns of the genes implicated in angiogenesis and tumor invasion [19].

In the present study, we investigated gene or protein expression levels of various angiogenic factors and proteolytic enzymes in cervical carcinoma cells and correlated them with invasive activity of the cells. Moreover, we sought to determine whether a different co-expression pattern of those genes exists between tumor cells and normal cervical tissues.

Materials and methods

Cell culture

Experiments were conducted using seven human cervical squamous cell carcinoma (SKG-I, SKG-II, SKG-IIIa, SKG-IIIb, OMC-1, YUMOTO and QG-U) and four adenocarcinoma (HOKUG, NUZ, OMC-4 and CAC-1) cell lines. The OMC-1 [20] and OMC-4 [21] cell lines were established in our laboratory. The SKG-I [22], SKG-II [23], SKG-IIIa and SKG-IIIb [24] cell lines were kindly provided by Dr. Shiro Nozawa, Keio University, Tokyo. The YUMOTO [25], QG-U [26] and NUZ [27] cell lines were kindly provided by Dr. Naotake Tanaka, Chiba University, Chiba. The HOKUG [28] and CAC-1 [29] cell lines were provided by Dr. Isamu Ishiwata, Ishiwata Hospital, Mito and Dr. Osamu Hayakawa, Sapporo Medical College, Sapporo, respectively. All of the 11 cell lines, except for the YUMOTO, QG-U and NUZ cell lines, were maintained as monolayer cultures in Ham's F-12 medium (Flow Laboratories Inc., Irvine, Scotland) supplemented with 10% fetal bovine serum (Mitsubishi Chemical Co., Tokyo) at 37°C in a humidified incubator with 5% CO_2 in air. The YUMOTO, QG-U and NUZ cell lines were cultured in RPMI-1640 medium (GIBCO BRL, Bethesda, MD) supplemented with 10% fetal bovine serum as described above. The cells were grown in 75-cm^2 tissue culture flasks (Nunc, Roskilde, Denmark), washed with phosphate-buffered saline (PBS) and then harvested after a brief treatment with 0.1% trypsin solution containing 0.02% EDTA (Flow). The cell viability was determined by trypan blue dye exclusion prior to use.

Protein solution of cervical carcinoma cells was prepared from each cell line as previously described [30]. Briefly, confluent monolayers of tumor cells grown in 10 cm plastic dishes (Corning 25010, Iwaki Glass, Tokyo) were rinsed twice with cold PBS and then lysed with modified RIPA buffer (2 mM sodium orthovanadate, 50 mM NaF, 20 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 5 mM sodium pyrophosphate, 10% glycerol, 0.2% Triton X 100, 5 mM EDTA, 1 mM PMSF, 10 mg/ml leupeptin and 10 mg/ml aprotinin). The protein concentration of each sample was determined by using a DC protein Assay Kit (BioRad Laboratories, Hercules, CA) and then stored at −80°C until use.

Tissue sample

Normal cervical tissues were obtained from 14 women who received hysterectomy under the diagnosis of uterine myoma and were used for mRNA analysis with consent. These tissues did not have any findings of cervical neoplasms and consisted of normal epithelial and stromal components, which was confirmed by pathological observation. Gene expression levels of these materials containing both components were evaluated in total as previously described by Van Trappen et al. [19]. All tissue samples were immediately frozen in liquid nitrogen and then stored at −80°C until use.

RNA isolation and cDNA preparation

RNA was extracted from cell lines and homogenized tissue samples by a combination of initial phenol/chloroform extraction according to the RNA STAT-60 protocol (Tel-Test, Inc, Friendswood, TX) and then SV-total RNA isolation kit extraction (Promega Corp, Madison, WI) according to the supplier's recommendation. Contaminating residual genomic DNA was removed by digestion with RNase free DNase (Promega). cDNAs were prepared using at least 2 μg of total RNA and SUPERSCRIPT II reverse transcriptase (GIBCO BRL Life Technologies, Gaithersburg, MD) with random hexamers as primers and were finally dissolved in diethyl pyrocarbonate-treated water and then frozen at −20°C until use.

Quantitative reverse transcription (RT)-PCR analysis

Quantitative PCR amplification was performed with a LightCycler (Roche Diagnostics, Tokyo, Japan) according to the method reported by Yamada et al. [31] with some modifications for each target gene. As an internal control, the expression of β-actin mRNA was measured. The primer pairs and hybridization probes for total VEGF-A, VEGF-A splicing variants ($VEGF_{121}$, $VEGF_{165}$ and $VEGF_{189}$), VEGF-B, C and D, bFGF, dThdPase, MMP-2, uPA and β-actin are shown in Table 1. Two microliters of cDNA aliquots was subjected to amplification in a 20 μl reaction mixture containing 2 μl LightCycler-FastStart Mix (Taq DNA polymerase, reaction buffer and deoxynucleoside triphosphate mix; Roche Molecular Biochemicals, Mannheim, Germany), 1 μl sense and antisense primers (10 pM), 1 μl hybridization probe R (8 pM), 1 μl hybridization probe F (4 pM), 3 mM $MgCl_2$ and sterile distilled water. After an initial denaturation at 95°C for 15 min, 40 cycles of denaturation at 95°C for 10 s, annealing at 62°C for 10 s and extension at 72°C for 10 s for the respective target genes were carried out on a Roche LightCycler System. A standard curve was generated using fluorescent data from the serial dilutions of the plasmid including a single PCR product for each gene. The gene expression levels were expressed as 1000 × each target gene/β-actin. Each analysis was performed in triplicate.

Sandwich enzyme-linked immunosorbent assay (sandwich ELISA) for VEGF-C and MMP-2

Protein solution of each cell line was assayed for VEGF-C expression of tumor cells according to the method reported by Weich et al. [32] with some modifications. Briefly, 10 μg/ml of anti-human VEGF-C rabbit IgG 103 (IBL, Gunma, Japan) was used for coating, and the antigen-affinity purified and horseradish-peroxidase (HRP)-conjugated antibody 408 (IBL) at 1 μg/ml was used as a detector antibody. As a standard, recombinant human VEGF-C (IBL) was used over a concentration range between 0.1 and 6.25 ng/ml. For visualization of the detector, tetra-methylbenzidine (Roche, Mannheim, Germany) was used. After stopping the reaction with 1 M H_2SO_4, the absorbance was measured at 450 nm by a microplate reader (Tosoh model MPR-A4, Tokyo). Generally, the samples were analyzed in different dilutions, measuring each dilution in triplicate.

MMP-2 expression of tumor cells was assayed according to the method reported by Fujimoto et al. [33]. A protein solution containing MMP-2 was mixed with 50 μg/l anti-MMP-2 IgG (clone 43-3F9, Fuji, Toyama, Japan)–HRP conjugate in 10 mM sodium phosphate buffer (pH 7.0) containing 10 g/l bovine serum albumin, 0.1 M NaCl and 10 mM EDTA; 0.1 ml aliquots of the mixture was transferred to each microplate well previously coated with an anti-MMP-2 IgG (clone 75-7F7, Fuji). As a standard, purified human MMP-2 (Fuji) was used. HRP activity

Table 1
Primers/probes sequences used in the real-time quantitative RT-PCR assays for 11 genes involved in angiogenesis and tumor invasion and the housekeeping gene β-actin

Genes	Sense primers	Antisense primers	Hybridization probe F	Hybridization probe R
VEGF-A total	5′-CCCTGATGAGATCGAGTACATCTT-3′	5′-ACCGCCTCGGCTTGTCAC-3′	ACTGAGGAGTCCAACATCACCATGCA	ATTATGCGGATCAAACCTCACCAAGG
121	5′-CCCTGATGAGATCGAGTACATCTT-3′	5′-GCCTCGGCTTGTCACATTTT-3′	ACTGAGGAGTCCAACATCACCATGCA	ATTATGCGGATCAAACCTCACCAAGG
165	5′-CCCTGATGAGATCGAGTACATCTT-3′	5′-AGCAAGGCCCACAGGGATTT-3′	ACTGAGGAGTCCAACATCACCATGCA	ATTATGCGGATCAAACCTCACCAAGG
189	5′-CCCTGATGAGATCGAGTACATCTT-3′	5′-AACGCTCCAGGACTTATACCG-3′	ACTGAGGAGTCCAACATCACCATGCA	ATTATGCGGATCAAACCTCACCAAGG
VEGF-B	5′-TGACATCACCCATCCCACTC-3′	5′-CACCCTGCTGAGTCTGAAAA-3′	GGGCTTAGAGCTCAACCCAGACACCT	CAGGTGCCGGAAGCTGCGAA
VEGF-C	5′-CCAGAAATCAACCCCTAAAT-3′	5′-AATATGAAGGGACACAACGA-3′	GTTCCACCACCAAACATGCAGC	GTTACAGACGGCCATGTACGAACC
VEGF-D	5′-TTCACACCAGCTAAGGAGTC-3′	5′-AGTTTTCCTCATCTGCTCTG-3′	TCCTCTCCATTCCTTGGTGCGC	GAGGCATCTGCAGCTAGAAGACATCCAT
bFGF	5′-AAGAAGAGGAAGTCACAGAA-3′	5′-TAAGGGAATGGGTAAGACAGTCTATGGTAA	CAGGGGATGGGTAAGACAGTCTATGGTAA	ACAGTCTACAGTCACAGCACATGGGAATT
dThdPase	5′-AATGTCATCCAGAGCCCAGA-3′	5′-TCCGAACTTAACGTCCACCA-3′	GAGATGTGACAGCCACCGTGGACAG	CTGCCACTCATCACAGCCTCCATTC
MMP-2	5′-GTGGATGCCGCCTTTAACTG-3′	5′-AGCAGCCTAGCCAGTCGGAT-3′	GCTTTCCAAGCTCATCGCAGATG	CTGGAATGCCATCCCCGATAACCT
uPA	5′-CTGAAGTCACCACCAAAATG-3′	5′-ATCCAGGGTAAGAAGTGTGA-3′	TTCCCTCCAAGGCCGCATGACT	TGACTGGAATTGTGAGCTGGGGC
β-actin	5′-CCAACCGCGAGAAGATGAC-3′	5′-GGAAGGAAGGCTGGAAGAGT-3′	CCTCCCCCATGCCATCCTGCGTC	GGACCTGGCTGGCCGGGACCTGA

bound on the plate was assayed by adding 100 μl of 0.15 M citric acid–sodium phosphate buffer containing 2.0 g/l *o*-phenylenediamine and 0.02% H_2O_2. After stopping the reaction with 1 M H_2SO_4, the absorbance was measured at 492 nm by a microplate reader. Each assay was performed in triplicate.

Haptoinvasion assay

The invasive activity of tumor cells was assayed in Chemotaxicell culture chambers (Kurabo, Osaka) according to the method reported by Albini et al. [34] with some modifications as previously described [35]. Polyvinylpyrroridone-free polycarbonate filters with 8.0 μm pore size were precoated with 10 μg of fibronectin in a volume of 50 μl of PBS on the lower surface and dried overnight at room temperature under a hood. The Matrigel diluted to 500 μg/ml with cold PBS was then applied to the upper surface of the filters (5 μg/filter) and dried again. Log-phase cell cultures of tumor cells were harvested with 0.1% trypsin containing 0.02% EDTA and resuspended to a final concentration of 3.0×10^6/ml in growth medium. 200 μl of cell suspension was added to the upper compartment, and 600 μl of growth medium was immediately added to the lower compartment. The chambers were then incubated for 24 h at 37°C in a 5% CO_2 air. The filters were fixed with ethanol and stained with hematoxylin. The cells on the upper surface of the filters were removed by wiping with a cotton swab. The cells that had migrated to various areas of the lower surface were manually counted under a microscope at a magnification of 400. Each assay was performed in triplicate.

Statistical analysis

All statistical calculations were carried out using StatView statistical software. The Spearman rank correlation coefficient was used to analyze the relation between two different values. The Mann–Whitney *U* test was used to compare the gene expression levels between normal and malignant cell samples. A level of $P < 0.05$ was accepted as statistically significant.

Results

Gene co-expression patterns in cervical carcinoma cells and normal cervical tissues

Real-time quantitative RT-PCR analysis was performed on mRNA transcripts of 11 genes (total VEGF-A, $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, VEGF-B, C and D, bFGF, dThdPase, MMP-2 and uPA) involved in angiogenesis and tumor invasion. Tables 2 and 3 show the Spearman correlation matrices of gene co-expression patterns with coefficient correlation (rho) and *P* values in cervical carcinoma cells and normal cervical tissues, respectively. In malignant cervical cells, significant co-expressions were found among total VEGF-A and different VEGF-A splicing variants. VEGF-B was also co-expressed with VEGF-A. There were statistically significant correlations in gene expression levels between $VEGF_{165}$ and dThdPase or VEGF-C and MMP-2. Significant co-expressions were also observed between uPA and VEGF-A or MMP-2. There were no statistical differences in gene expression levels of various angiogenic

Table 2
Spearman correlation matrix to assess gene co-expression (with rho and *P* values) in cervical carcinoma cells

		$VEGF_{total}$	$VEGF_{121}$	$VEGF_{165}$	$VEGF_{189}$	VEGF-B	VEGF-C	VEGF-D	bFGF	dThdPase	MMP-2	uPA
$VEGF_{total}$	rho	1.000										
	P value											
$VEGF_{121}$	rho	0.951	1.000									
	P value	<0.0001										
$VEGF_{165}$	rho	0.972	0.909	1.000								
	P value	<0.0001	<0.0001									
$VEGF_{189}$	rho	0.965	0.916	0.909	1.000							
	P value	<0.0001	<0.0001	<0.0001								
VEGF-B	rho	0.713	0.664	0.797	0.664	1.000						
	P value	0.009	0.018	0.002	0.018							
VEGF-C	rho	0.368	0.291	0.406	0.403	0.123	1.000					
	P value	0.240	0.359	0.190	0.194	0.704						
VEGF-D	rho	−0.051	0.087	−0.094	−0.029	−0.022	−0.458	1.000				
	P value	0.876	0.788	0.771	0.929	0.946	0.135					
bFGF	rho	0.259	0.119	0.308	0.252	0.357	−0.070	0.051	1.000			
	P value	0.417	0.713	0.331	0.430	0.255	0.829	0.876				
dThdPase	rho	0.483	0.448	0.580	0.503	0.483	0.574	−0.392	0.028	1.000		
	P value	0.112	0.145	0.048	0.095	0.112	0.051	0.208	0.931			
MMP-2	rho	0.345	0.373	0.303	0.465	0.070	0.635	−0.190	−0.063	0.359	1.000	
	P value	0.272	0.232	0.339	0.128	0.828	0.027	0.555	0.845	0.252		
uPA	rho	0.644	0.683	0.588	0.764	0.518	0.311	−0.123	0.235	0.508	0.713	1.000
	P value	0.024	0.014	0.044	0.004	0.084	0.326	0.702	0.463	0.092	0.009	

Table 3
Spearman correlation matrix to assess gene co-expression (with rho and P values) in normal cervical tissues

		$VEGF_{total}$	$VEGF_{121}$	$VEGF_{165}$	$VEGF_{189}$	VEGF-B	VEGF-C	VEGF-D	bFGF	dThdPase	MMP-2	uPA
$VEGF_{total}$	rho	1.000										
	P value											
$VEGF_{121}$	rho	0.231	1.000									
	P value	0.471										
$VEGF_{165}$	rho	0.657	0.322	1.000								
	P value	0.020	0.308									
$VEGF_{189}$	rho	0.687	−0.288	0.434	1.000							
	P value	0.014	0.363	0.158								
VEGF-B	rho	0.413	0.175	0.308	0.214	1.000						
	P value	0.183	0.587	0.331	0.505							
VEGF-C	rho	−0.266	0.559	0.021	−0.363	−0.021	1.000					
	P value	0.404	0.059	0.948	0.246	0.948						
VEGF-D	rho	−0.341	−0.470	−0.433	−0.112	0.233	−0.054	1.000				
	P value	0.278	0.123	0.160	0.728	0.466	0.867					
bFGF	rho	−0.130	−0.063	−0.035	−0.337	0.522	0.112	0.458	1.000			
	P value	0.688	0.846	0.914	0.284	0.082	0.729	0.134				
dThdPase	rho	0.573	0.615	0.308	0.014	0.392	0.084	−0.387	−0.049	1.000		
	P value	0.051	0.033	0.331	0.965	0.208	0.795	0.214	0.880			
MMP-2	rho	0.294	−0.056	0.287	0.320	0.552	0.280	0.420	0.340	0.042	1.000	
	P value	0.354	0.863	0.366	0.310	0.063	0.379	0.174	0.280	0.897		
uPA	rho	−0.301	0.266	−0.308	−0.036	−0.056	0.406	−0.108	−0.102	0.063	−0.301	1.000
	P value	0.342	0.404	0.331	0.913	0.863	0.191	0.738	0.753	0.846	0.342	

factors and proteinases between squamous and adenocarcinoma cell lines. In normal cervical tissues, total VEGF-A was co-expressed with $VEGF_{165}$ or $VEGF_{189}$. Gene expression levels of $VEGF_{121}$ were also statistically correlated with those of dThdPase. However, gene co-expression was not observed among other angiogenic factors and proteinases.

Correlation between VEGF-C or MMP-2 expression and invasive phenotype in cervical carcinoma cells

We then investigated the relationship between VEGF-C or MMP-2 expression and in vitro invasive activity of tumor cells estimated by haptoinvasion assay. As shown in Figs. 1A and B, there was a statistically significant correlation between VEGF-C gene or protein expression and the number of invaded tumor cells, with a coefficient correlation of 0.917 ($P < 0.0001$) and 0.912 ($P < 0.0001$), respectively. Gene or protein expression levels of MMP-2 in 11 cultured cervical carcinoma cells were also well correlated with the number of invaded tumor cells (Figs. 1C and D), with a coefficient correlation of 0.671 ($P = 0.0238$) and 0.723 ($P = 0.0120$), respectively. However, there was no statistical correlation between mRNA expression levels of the other genes and invasive activity of the cells.

Differences in transcript levels of the genes in normal cervical tissues and malignant cervical cells

Fig. 2 illustrates the transcript levels in normal cervical tissues and malignant cervical cells for 11 genes involved in angiogenesis and tumor invasion. All normal and malignant cervical samples expressed various angiogenic factors and proteinases examined. The gene expression levels of different VEGF-A splicing variants were significantly higher in malignant compared to benign samples ($P < 0.01$). In contrast, there was no statistical difference in mRNA expression levels of VEGF-B, C and D between normal cervical tissues and cervical carcinoma cells. The levels of MMP-2 in malignant were higher than those in benign samples ($P < 0.05$), however, no statistical difference was found in bFGF, dThdPase or uPA gene expression between two groups.

Discussion

Various kinds of genes have now been identified which are involved in both tumor neovascularization and invasion, and most of them are also expressed to some extent under normal physiologic conditions. In the present study, combined analysis of real-time quantitative RT-PCR with correlation matrices of gene expression data revealed the differences in gene co-expression patterns between malignant and normal cervical samples. In cervical carcinoma cells, significant co-expressions were found among total VEGF-A and VEGF-A splicing variants ($VEGF_{121}$, $VEGF_{165}$ or $VEGF_{189}$), which is consistent with the previous reports [19]. Moreover, VEGF-B was also co-expressed with VEGF-A. VEGF-A binds with high affinity to two tyrosine kinase receptors on the cell membrane of vascular endothelial cells; VEGF receptor (VEGFR)-1 (Flt-1) and VEGFR-2 (KDR/Flk-1). Binding of VEGF-A causes receptor dimerization followed by autophosphorylation of the receptor and signal transduction [2,3]. VEGF-

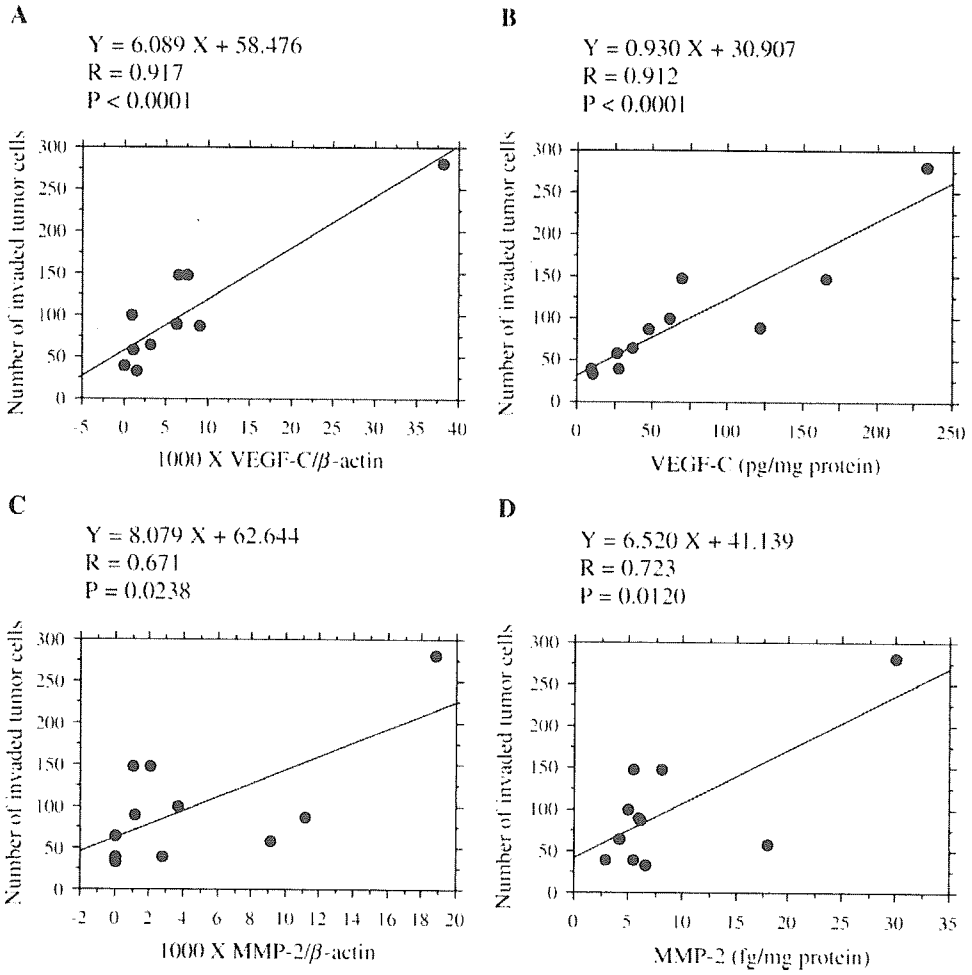

Fig. 1. Correlation between VEGF-C gene or protein expression and the number of invaded tumor cells (A or B) and between MMP-2 gene or protein expression and the number of invaded tumor cells (C or D), respectively. Each point represents the mean of triplicates for each cell line.

B, one of the new VEGF family members, also binds to Flt-1 and induces pleiotropic responses of endothelial cells [11]. The gene co-expression pattern of VEGF-A and B may suggest their cooperative role for tumor angiogenesis. Our present results further demonstrated statistical correlations in gene expression levels between $VEGF_{165}$ and dThdPase, VEGF-C and MMP-2, or uPA and VEGF-A or MMP-2. Recent studies have reported that tumors exhibiting higher expression levels of VEGF-A or VEGF-C possess a higher metastatic potential [36–38]. dThdPase expression is also considered to be strongly linked to the process of tumor invasion and metastasis [35,39]. The gene expression of angiogenic factors may be closely associated with proteolytic and invasive properties of cervical carcinoma cells. In contrast, there was no statistical correlation in gene expression levels between angiogenic factors and proteinases in normal cervical tissues. Although gene co-expression was observed among total VEGF-A, VEGF-A splicing variants and dThdPase, angiogenesis may not be related to ECM degradation by proteolytic enzymes in normal physiologic conditions.

We next examined the correlation between gene expression levels of angiogenic factors and proteinases and in vitro invasive activity of cervical carcinoma cells. Interestingly, VEGF-C and MMP-2 gene expression was closely associated with the number of invaded tumor cells. Moreover, their protein expression was also well correlated with invasive activity of the cells. VEGF-C is a ligand for VEGFR-3 (Flt-4), a tyrosine kinase receptor which is predominantly expressed in the endothelium of lymphatic vessels [10]. Experimental results with the VEGF-C-transgenic mouse have shown that VEGF-C expression is associated with hyperplasia of lymphatic vessels [40]. Thus, VEGF-C is ranked first as a lymphoangiogenic factor, which induces lymphatic proliferation and spread of solid tumors. Recently, some investigators have demonstrated the close correlation between VEGF-C expression and lymphatic invasion and lymphnode metastasis in a variety of human malignant tumors [41–43]. Moreover, it is generally accepted that tumor cell invasion into the lymphatic vessels is established by the destruction of stroma around the lymphatic vessels via activation of matrix-digesting

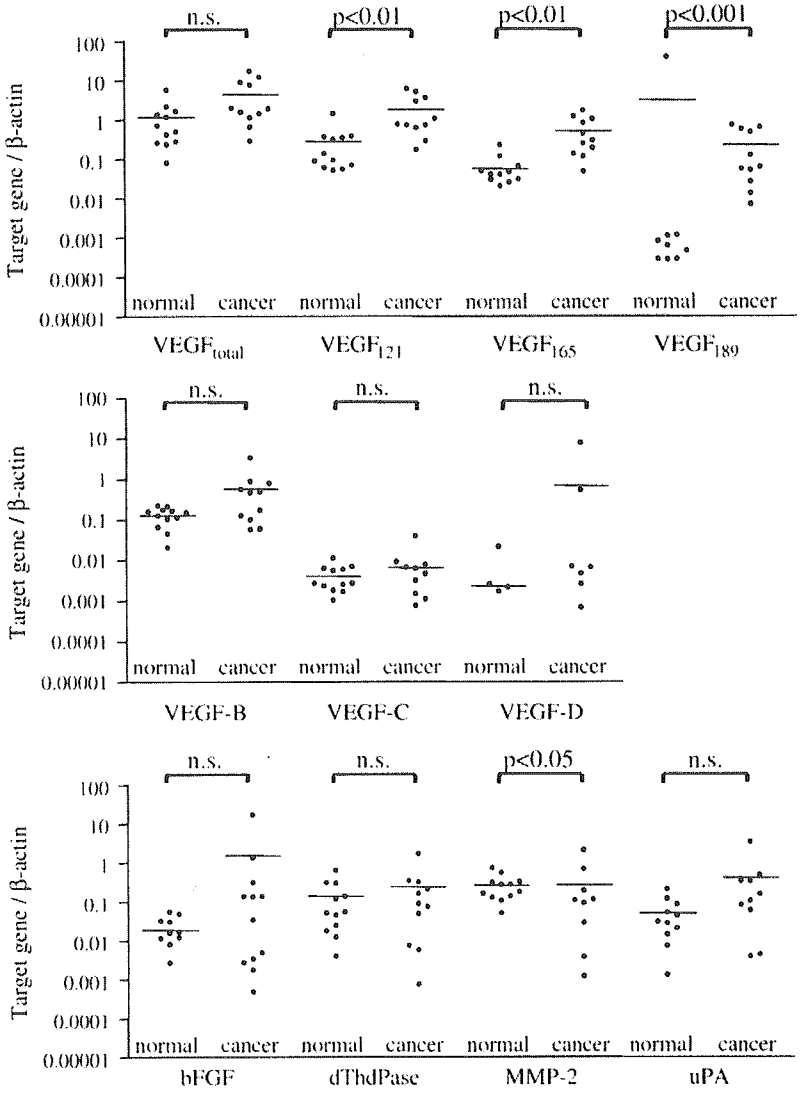

Fig. 2. Transcript levels (target gene/β-actin) of the genes implicated in angiogenesis and tumor invasion in normal cervical tissues and cultured cervical carcinoma cells. Each point represents the mean of triplicates for each tissue sample or cell line. Bars, median values.

enzymes, which are produced by tumor cells or fibroblasts [44]. Therefore, it could be expected that VEGF-C expression in tumor cells is linked to invasive phenotype of the cells. Our experimental results on the correlation among VEGF-C, MMP-2 and invasiveness suggest that cervical carcinoma cells producing VEGF-C may have a higher invasive and metastatic potential because of their capacity to pass through tissue barriers by degrading ECM with MMP-2.

Finally, we compared the transcript levels of angiogenic factors and proteinases between normal cervical tissues and cultured cervical carcinoma cells. The gene expression levels of different VEGF-A splicing variants and MMP-2 were higher in malignant compared to benign samples, however, no statistical difference was found in other genes examined. Van Trappen et al. [19] reported that VEGF-A isoforms ($VEGF_{121}$ and $VEGF_{165}$), VEGF-C and MMP-9 gene expression were significantly increased in malignant compared to normal cervical tissues and that there was no significant difference in mRNA expression levels of $VEGF_{189}$, bFGF and MMP-2 between two groups. They used malignant cervical tissue samples surgically obtained from the patients. The discrepancy might be due to the differences of materials and culture conditions. It has been demonstrated that VEGF-A is essential for the initial but not for continued growth of human breast carcinomas and that other angiogenic factors can substitute for VEGF-A during disease progression [45]. In the present study, transcript levels of VEGF-A splicing variants were increased in cervical carcinoma cells compared to normal cervical tissues but not associated with the invasive activity of tumor cells as described above. VEGF-A isoforms might be involved in the process leading to the transformation of normal cervical cells rather than in the development of cervical carcinomas.

In conclusion, gene co-expression analysis revealed the significant correlation between angiogenic factors and proteinases in cervical carcinoma cells but not in normal cervical tissues. Among the genes examined, VEGF-C and MMP-2 were closely related to invasive phenotype of tumor cells, whereas VEGF-A isoforms were considered to be involved in cervical carcinogenesis. Further studies are needed to clarify the molecular events that coregulate the genes implicated in angiogenesis and tumor invasion.

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References

[1] Folkman J. Clinical applications of research on angiogenesis. N Engl J Med 1995;333:1757–63.
[2] Connolly DT. Vascular permeability factor: a unique regulator of blood vessel function. J Cell Biochem 1991;47:219–23.
[3] Shweiki D, Neeman M, Itin A, Keshet E. Induction of vascular endothelial growth factor expression by hypoxia and by glucose deficiency in multiple spheroids: implications for tumor angiogenesis. Proc Natl Acad Sci U S A 1995;92:768–72.
[4] Kandel J, Bassy-Wetzel E, Radvanyi F, Klagsburn M, Folkman J, Hanahan D. Neovascularization is associated with a switch to the export of b-FGF in the multistep development of fibrosarcoma. Cell 1991;66:1095–104.
[5] Ohtani H, Nakamura S, Watanabe Y, Mizoi T, Saku T, Nagura H. Immunocytochemical localization of basic fibroblast growth factor in carcinomas and inflammatory lesions of the human digestive tract. Lab Invest 1993;68:520–7.
[6] Furukawa T, Yoshimura A, Sumizawa T, Haraguchi M, Akiyama S. Angiogenic factor. Nature (London) 1992;356:668.
[7] Haraguchi M, Miyadera K, Uemura K, Sumizawa T, Furukawa T, Yamada K, et al. Angiogenic activity of enzymes. Nature (London) 1994;368:198.
[8] Moghaddam A, Zhang HT, Fan TPD, Hu DE, Lees VC, Turley H, et al. Thymidine phosphorylase is angiogenic and promotes tumor growth. Proc Natl Acad Sci U S A 1995;92:998–1002.
[9] Veikkola T, Alitalo K. VEGFs, receptors and angiogenesis. Semin Cancer Biol 1999;9:211–20.
[10] Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. Nature 2000;407:242–8.
[11] Olofsson B, Pajusola K, Kaipainen A, von Euler G, Joukov V, Saksela O, et al. Vascular endothelial growth factor B, a novel growth factor for endothelial cells. Proc Natl Acad Sci U S A 1996;93:2576–81.
[12] Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinase. EMBO J 1996;15:290–8.
[13] Achen MG, Jeltsch M, Kukk E, Makinen T, Vitali A, Wilks AF, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). Proc Natl Acad Sci U S A 1998;95:548–53.
[14] Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1991;64:327–36.
[15] Woessner JFJ. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991;5:2145–54.
[16] Liotta LA, Rao CV, Barsky SH. Tumor invasion and the extracellular matrix. Lab Invest 1983;49:636–49.
[17] Dano K, Andreasen PA, Grondahl-Hansen J, Kristensen P, Nielsen LS, Skriver L. Plasminogen activators, tissue degradation and cancer. Adv Cancer Res 1985;44:139–266.
[18] Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 1991;324:1–8.
[19] Van Trappen PO, Ryan A, Carroll M, Lecoeur C, Goff L, Gyselman VG, et al. A model for co-expression pattern analysis of genes implicated in angiogenesis and tumour cell invasion in cervical cancer. Brit J Cancer 2002;87:537–44.
[20] Ueda M, Ueki M, Yamada T, Okamoto Y, Maeda T, Sugimoto O, et al. Scatchard analysis of EGF receptor and effects of EGF on growth and TA-4 production of newly established uterine cervical cancer cell line (OMC-1). Hum Cell 1989;2:401–10.
[21] Ueda M, Ueki M, Sugimoto O. Characterization of epidermal growth factor (EGF) receptor and biological effect of EGF on human uterine cervical adenocarcinoma cell line OMC-4. Hum Cell 1993;6:218–25.
[22] Taguchi S. Establishment and characterization of the human uterine cervical epidermoid cancer cell line. Acta Obstet Gynecol Jpn 1981; 33:1180–8.
[23] Ishiwata I, Nozawa S, Kiguchi K, Kurihara S, Okumura H. Establishment of human uterine cervical cancer cell line and comparative studies between normal and malignant uterine cervical cells in vitro. Acta Obstet Gynaecol Jpn 1978;30:731–8.
[24] Nozawa S, Udagawa Y, Ohta H, Kurihara S, Fishman WH. Newly established uterine cervical cancer cell line (SKG-III) with Regan isoenzyme, human chorionic gonadotropin β-subunit, and pregnancy-specific β1-glycoprotein phenotypes. Cancer Res 1983;43:1748–60.
[25] Mitsuhashi A, Tanaka H, Tanaka N, Sugita M, Shirasawa H, Tokita H, et al. Establishment and characterization of a new HPV-negative squamous cell carcinoma cell line (Yumoto) from the human uterine cervix. Gynecol Oncol 1998;70:339–47.
[26] Shirasawa H, Tomita Y, Fuse A, Yamamoto T, Tanzawa H, Sekiya S, et al. Structure and expression of an integrated human papillomavirus type 16 genome amplified in a cervical carcinoma cell line. J Gen Virol 1989;70:1913–9.
[27] Suzumori K, Yasui Y. NUZ-1. Hum Cell 1988;1:454.
[28] Ishiwata I, Ishiwata C, Soma M, Ono I, Nakaguchi T, Nozawa S, et al. Differences between cell lines of uterine cervical glassy cell carcinoma and large cell nonkeratinizing squamous cell carcinoma. Anal Quant Cytol Histol 1990;12:290–8.
[29] Koizumi M, Uede T, Shijubo N, Kudo R, Hashimoto M, Kikuchi K. New monoclonal antibody, 1C5, reactive with human cervical adenocarcinoma of the uterus, with immunodiagnostic potential. Cancer Res 1988;48:6565–72.
[30] Terai Y, Abe M, Miyamoto K, Koike M, Yamasaki M, Ueda M, et al. Vascular smooth muscle cell growth-promoting factor/F-spondin inhibits angiogenesis via the blockade of integrin αVβ3 on vascular endothelial cells. J Cell Physiol 2001;188:394–402.
[31] Yamada Y, Kuroiwa T, Nakagawa T, Kajimoto Y, Dohi T, Azuma H, et al. Transcriptional expression of survivin and its splice variants in brain tumors in humans. J Neurosurg 2003;99:738–45.
[32] Weich HA, Bando H, Brokelmann M, Baumann P, Toi M, Barleon B, et al. Quantification of vascular endothelial growth factor-C (VEGF-C) by a novel ELISA. J Immunol Methods 2004;285:145–55.
[33] Fujimoto N, Mouri N, Iwata K, Ohuchi E, Okada Y, Hayakawa T. A one-step sandwich enzyme immunoassay for human matrix metal-

loproteinase 2 (72-kDa gelatinase/type IV collagenase) using monoclonal antibodies. Clin Chim Acta 1993;221:91–103.
[34] Albini A, Iwamoto Y, Kleinman HK, Martin GR, Aaronson SA, Kozlowski JM, et al. A rapid in vitro assay for quantitating the invasive potential of tumor cells. Cancer Res 1987;47:3239–45.
[35] Ueda M, Terai Y, Kumagai K, Ueki K, Kanemura M, Ueki M. Correlation between thymidine phosphorylase expression and invasion phenotype in cervical carcinoma cells. Int J Cancer 2001;91:778–82.
[36] Potgens AJ, van Altena MC, Lubsen NH, Ruiter DJ, de Waal RM. Analysis of the tumor vasculature and metastatic behavior of xenografts of human melanoma cell lines transfected with vascular permeability factor. Am J Pathol 1996;148:1203–17.
[37] Ohta Y, Watanabe Y, Murakami S, Oda M, Hayashi Y, Nonomura A, et al. Vascular endothelial growth factor and lymph node metastasis in primary lung cancer. Br J Cancer 1997;76:1041–5.
[38] Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med 2001;7:192–8.
[39] Ueda M, Terai Y, Kumagai K, Ueki K, Okamoto Y, Ueki M. Correlation between tumor angiogenesis and expression of thymidine phosphorylase, and patient outcome in uterine cervical carcinoma. Hum Pathol 1999;30:1389–94.
[40] Jetsch M, Kaipainen A, Joukov V, Meng XM, Lakso M, Rauvala H, et al. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science (Washington, DC) 1997;276:1423–5.
[41] Yonemura Y, Endo Y, Fujita H, Fushida S, Ninomiya I, Bandou E, et al. Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer. Clin Cancer Res 1999;5:1823–9.
[42] Valtola R, Salven P, Heikkila P, Taipale J, Joensuu H, Rehn M, et al. VEGFR-3 and its ligand VEGF-C are associated with angiogenesis in breast cancer. Am J Pathol 1999;154:1381–90.
[43] Bunone G, Vigneri P, Mariani L, Buto S, Collini P, Pilotti S, et al. Expression of angiogenesis stimulators and inhibitors in human thyroid tumors and correlation with clinical pathological features. Am J Pathol 1999;155:1967–76.
[44] Liotta LA. Tumor invasion and metastasis: role of the extracellular matrix. Cancer Res 1986;46:1–7.
[45] Yoshiji H, Harris SR, Thorgeirsson UP. Vascular endothelial growth factor is essential for initial but not continued in vivo growth of human breast carcinoma cells. Cancer Res 1997;57:3924–8.

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Carboplatin hypersensitivity induced by low-dose paclitaxel/carboplatin in multiple platinum-treated patients with recurrent ovarian cancer

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Abstract. Watanabe Y, Nakai H, Ueda H, Nozaki K, Hoshiai H. Carboplatin hypersensitivity induced by low-dose paclitaxel/carboplatin in multiple platinum-treated patients with recurrent ovarian cancer. *Int J Gynecol Cancer* 2005;15:224–227.

We report five cases of carboplatin (CBDCA) hypersensitivity after weekly low-dose paclitaxel (60 mg/m^2)/CBDCA (area under the concentration curve = 2) therapy in patients with recurrent ovarian cancer receiving multiple platinum-based chemotherapy. Paclitaxel and CBDCA therapy was indicated as second-line chemotherapy in one patient and as third line in four patients with recurrent disease. The range of previously administered total CBDCA was 2582–9589 mg, and the CBDCA area under the concentration curve of the first treatment exhibited appropriate intensity (mean, 1.92 ± 0.10; range, 1.76–2.10) in all patients. However, one patient exhibited severe hypersensitivity reactions including cardiac arrest and apnea, and another four patients developed eruptions, hypotension, and tachycardia soon after administration of CBDCA. Our report suggested that CBDCA hypersensitivity was correlated with the total dose of previously administered platinum agents and that CBDCA should be excluded in patients who have received multiple platinum-based chemotherapy, even in platinum-sensitive cases, because CBDCA hypersensitivity can occur even with low-dose CBDCA administration.

KEYWORDS: CBDCA, hypersensitivity, recurrent ovarian cancer, weekly low-dose chemotherapy.

Recent large phase-III comparative clinical trials have indicated that combination chemotherapy with carboplatin (CBDCA) and paclitaxel (TC therapy) is an effective chemoregimen for patients with advanced epithelial ovarian cancer[1]. Moreover, it has also been reported that TC therapy is effective even as a second-line therapy in recurrent epithelial ovarian cancer[2,3]. Generally, patients with advanced ovarian cancer have received multiple courses of platinum agent as first-, second-, and occasionally third-line platinum-based chemotherapy, and it is well known that the main adverse effects of platinum agents are bone marrow suppression, gastrointestinal toxicity, and neurotoxicity. Although they are rare events, previous reports[4–6] have described hypersensitivity reactions to platinum

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agents as an issue during multiple platinum-based chemotherapy regimens. They reported that the frequency of hypersensitivity reaction was 15% in patients receiving CBDCA for treatment every 3 weeks with the area under the concentration curve (AUC) = 5 or almost 300 mg/m^2 of CBDCA. Since 2000, we have indicated weekly paclitaxel and CBDCA therapy (WTC) with 3 weeks of administration followed by a 1-week break, for patients with recurrent epithelial ovarian cancer, as second-line chemotherapy. However, if patients requested WTC even as third-line chemotherapy, we administered this treatment under the condition that the platinum-free interval was over 6 months, to develop a therapeutic level of chemotherapy while preventing neurotoxicity under fully informed consent. We report the clinical features of those CBDCA hypersensitivity cases receiving weekly low-dose CBDCA-based chemotherapy.

Materials and methods

To begin WTC, a 60-min intravenous administration of 20 mg dexamethazone, 3 mg 5-HT$_3$ receptor antagonist, 50 mg ranitidine hydrochloride, and 50 mg of oral diphenhydramine was given before administration of paclitaxel and CBDCA. The treatment dose of paclitaxel and CBDCA was divided into three of the standard TC (paclitaxel at 180 mg/m^2, CBDCA AUC = 6): paclitaxel at 60 mg/m^2 (equal to the per-week paclitaxel dose intensity in the GOG 111[7] study), CBDCA AUC = 2 (Calvert's formula). After premedication, CBDCA was administered over 60 min followed by 60 min of paclitaxel treatment. All hypersensitivity cases had previously received platinum-based chemotherapy every 3 weeks for a total of six courses of 175 mg/m^2 paclitaxel and CBDCA AUC = 5, 500 mg/m^2 of cyclophosphamide and 70 mg/m^2 of cisplatin, or 500 mg/m^2 of cyclophosphamide and CBDCA AUC = 5 therapy. Four patients were receiving the present WTC as third-line chemotherapy, and one 72-year-old patient received this regimen as second-line chemotherapy for recurrent disease. Previously, three patients had received 18 courses of the same WTC schedule; the range of the previously administered total dose of CBDCA before hypersensitivity occurred was 2582–9589 mg, and all cases had platinum-free intervals longer than 6 months before WTC. Pretreatment performance status according to the criteria of the Eastern Cooperative Oncology Group and hematologic and nonhematologic status according to the criteria of the National Cancer Institute Common Toxicity were all within grade 1. The mean CBDCA AUC calculated by nine-point blood samples in the first opening treatment course was entirely within the approximate value (1.92 ± 0.01; range, 1.76–2.10).

Results

A total of 23 patients were treated with WTC (14 patients as second-line and nine as third-line chemotherapy). The effects of WTC for patients with recurrent ovarian cancer were 64.3% in second-line (complete response: 5, partial response: 3) and 33.3% in third-line (PR: 3) treatments, while five cases (four patients in third-line and one patient in second-line chemotherapy) developed CBDCA hypersensitivity among those receiving WTC (Table 1). All the hypersensitivity reactions occurred immediately after CBDCA administration but not during paclitaxel administration. Four patients recovered from symptoms within 10 min after stopping CBDCA administration, but one patient required respiratory assistance with intubation in an intensive care unit due to sudden apnea and cardiac arrest after fainting. Except in one patient with a severe hypersensitivity reaction, symptoms were hypotension (mean drop in blood pressure, 29 + 3 mm Hg in systolic, 14 ± 8 mm Hg in diastolic), dyspnea (mean SaO_2, 67.3 ± 3.7 cm H_2O), nausea, and eruption. Except for the one severe hypersensitivity patient, the other four patients were treated with a 2-week break after the hypersensitivity reaction with an initial 60 mg/m^2 of single paclitaxel therapy after giving the informed consent. These four patients have been able to continue treatment with more than six courses weekly of 80 mg/m^2 of single paclitaxel therapy without further hypersensitivity reactions, and two patients (one second line and one third line) achieved PR from the weekly single paclitaxel treatment. The frequency of CBDCA hypersensitivity by weekly low-dose therapy has been estimated as 44.4% (4/9) in third-line chemotherapy and 7.1% in second-line (1/14) chemotherapy.

Discussion

Primary ovarian epithelial cancer is known as a chemosensitive gynecological malignancy, and recent regimens have achieved an almost 75% overall response rate and a 40% complete response rate[1]. Therefore, patients with ovarian cancer occasionally receive multiple platinum-based chemotherapy, especially patients with a platinum-free interval of longer than 6 months. However, after multiple platinum-based chemotherapy, hypersensitivity reaction to CBDCA is a noteworthy adverse effect. Although the details of the cause remain unknown, recent studies have reported

Table 1. Characteristics of CBDCA hypersensitivity cases

Case	Age	Symptoms	Number of chemotherapy (prior regimens)	PFI	Total CBDCA (Total CDDP)	AUC of CBDCA
1	58	Eruption Hypotension Tachycardia Dyspnea	Third line (CP, TC-3)	7 months	6991 mg (1300 mg)	2.10
2	53	Eruption Hypotension Tachycardia	Third line (CC, WTC)	8 months	3780 mg	1.98
3	62	Eruption Hypotension Tachycardia	Third line (CP, WTC)	10 months	5344 mg (1036 mg)	1.92
4	72	Eruption Hypotension Tachycardia Dyspnea	Second line (TC-3)	9 months	2582 mg	1.76
5	62	Cardiac arrest Hypotension Apnea	Third line (TC-3, WTC)	15 months	9589 mg	1.81

WTC, weekly taxol (60 mg/m^2) + carboplatin (AUC = 2); CP, cyclophosphamide (500 mg/m^2) + cisplatin (70 mg/m^2); CC, cyclophosphamide (500 mg/m^2) + carboplatin (AUC = 5); TC-3, every 3 weeks taxol (175 mg/m^2) + carboplatin (AUC = 5). PFI, platinum-free interval; CBDCA, carboplatin; CDDP, cisplatin; Total CBDCA, prior total carboplatin administration before hypersensitivity; Total CDDP, prior total cisplatin administration before hypersensitivity; AUC, area under the concentration curve.

interesting facts regarding CBDCA hypersensitivity. Polyzos *et al.*[8] reported that CBDCA hypersensitivity was found in 16% and indicated that 62.5% of these cases showed mild reactions. They also reported that there was no hypersensitivity reaction in a group receiving intraperitoneal CBDCA treatment, and in 33.3% of these patients, treatment could be replaced by cisplatin. Furthermore, Robinson *et al.*[9] reported the utility of desensitization for CBDCA hypersensitivity by low-dose exposure to CBDCA. However, Markman *et al.*[4] expressed some doubts about desensitization and re-administration of platinum agents based on clinical experiences. In our cases, the mean practical weekly administered CBDCA during the course that caused a hypersensitivity reaction was 195.6 ± 26.3 mg (range, 160–260 mg), and all cases exhibited hypersensitivity reactions within 1 min after CBDCA administration (roughly estimated mean administered CBDCA dose until hypersensitivity reaction occurred was 2.17 ± 0.29 mg). Moreover, although CBDCA AUC was examined when initiating the course of WTC, the administered CBDCA AUC was confirmed as the approximate dose. Therefore, it was thought that the utility of CBDCA desensitization could not be expected because the present cases exhibited CBDCA hypersensitivity after administration of less than 2 mg of CBDCA. The incidence of CBDCA hypersensitivity after weekly low-dose CBDCA therapy remains unknown because all previous reports regarding CBDCA hypersensitivity involved a CBDCA treatment showing AUC 5–6. Yu *et al.*[10] reported that the risk of hypersensitivity in children with brain tumors treated weekly with 175 mg/m^2 of CBDCA would increase because CBDCA hypersensitivity was observed in 11.1% receiving weekly treatment and in 0.7% on the monthly schedule. However, in the present series of adult patients with recurrent ovarian cancer, hypersensitivity reaction occurred in only one elderly patient receiving second-line chemotherapy, and the frequency of hypersensitivity was increased in third-line chemotherapy. These results suggest that the risk of CBDCA hypersensitivity is not correlated to a definite amount or administration schedule but to the prior accumulated dose of the CBDCA. Moreover, it was also suggested that CBDCA hypersensitivity could be considered as platinum toxicosis because all the patients showed hypersensitivity reaction after administration of premedication, including dexamethazone for preventing allergic reactions. Although the efficacy of third-line chemotherapy for patients with recurrent ovarian cancer has not been established, the present report suggests that WTC is a safe treatment regimen as second-line chemotherapy for such patients after considering the risk of CBDCA hypersensitivity. Moreover, it remains unknown whether the pretreatment dose of CDDP affects CBDCA hypersensitivity and how much CBDCA will influence the hypersensitivity reaction because five out of nine (55.6%) patients who received third-line WTC treatment did not sustain CBDCA hypersensitivity even

though they received full courses of WTC. However, consideration should be given to the fact that among the present CBDCA hypersensitivity cases, four patients (80.0%) were receiving WTC as third-line chemotherapy and three (60.0%) had previously received over 5000 mg of CBDCA. Therefore, intensive observation is necessary during CBDCA-based chemotherapy in such patients. Furthermore, exclusion of CBDCA might be recommended in patients who have already received multiple platinum-based chemotherapies, for treatment safety, even if the patients are identified as platinum sensitive.

References

1 du Bois A, Luck HJ, Meier W *et al.* Carboplatin/paclitaxel versus cisplatin/paclitaxel as first-line chemotherapy in advanced ovarian cancer: an interim analysis of a randomized phase III trial of the Arbeitsgemeinschaft Gynecologishe Onkologie Ovarian Cancer Group. *Semin Oncol* 1997;**24**:44–52.

2 Dizon DS, Hensley ML, Poynor EA *et al.* Retrospective analysis of carboplatin and paclitaxel as initial second-line therapy for recurrent epithelial ovarian carcinoma: application toward a dynamic disease state model of ovarian cancer. *J Clin Oncol* 2002;**20**:1238–47.

3 The ICON and AGO Collaborators. Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the ICON4/AGO-OVAR-2.2 trial. *Lancet* 2003; **361**:2099–106.

4 Sood AK, Gelder MS, Huang SW, Morgan LS. Anaphylaxisis to carboplatin following multiple previous uncomplicated courses. *Gynecol Oncol* 1995;**57**:131–2.

5 Markman M, Kennedy A, Webster K *et al.* Clinical features of hypersensitivity reactions to carboplatin. *J Clin Oncol* 1999;**17**:1141–5.

6 Dison DS, Sabbatini PJ, Aghajanian C, Hensley ML, Spriggs DR. Analysis of patients with epithelial ovarian cancer or fallopian tube carcinoma treated with cisplatin after the development of a carboplatin allergy. *Gynecol Oncol* 2002;**84**:378–82.

7 McGuire WP, Hoskins WJ, Brady MF *et al.* Cyclophosphamide and cisplatin versus paclitaxel and cisplatin: a phase III randomized trial in patients with suboptimal stage III/IV ovarian cancer (from the Gynecologic Oncology Group). *Semin Oncol* 1996;**23**:40–7.

8 Polyzos A, Tsavaris N, Kostama C *et al.* Hypersensitivity reactions to carboplatin administration are common but not always severe: a 10-year experience. *Oncology* 2001;**61**:129–33.

9 Robinson JB, Singh D, Bodurka-Bevers DC *et al.* Hypersensitivity reactions and the utility of oral and intravenous desensitization in patients with gynecologic malignancies. *Gynecol Oncol* 2001;**82**:550–8.

10 Yu DY, Dahl GV, Shames RS, Fisher PG. Weekly dosing of carboplatin increases risk of allergy in children. *J Pediatr Hematol Oncol* 2001;**23**:349–52.

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BRIEF REPORT

The effect of granisetron on *in vitro* metabolism of doxorubicin, irinotecan and etoposide

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Key words: CYP3A – Doxorubicin – Etoposide – Granisetron – Irinotecan

ABSTRACT

Objective: Doxorubicin, irinotecan and etoposide are all associated with the debilitating side-effects of nausea and vomiting, thereby necessitating concomitant antiemetic therapy. However, this may increase the potential for drug–drug interactions by inhibition or induction of the cytochrome P450 enzymes. A study was undertaken to investigate the effects of the 5-HT_3-receptor antagonist granisetron on the metabolism of doxorubicin, irinotecan and etoposide *in vitro* in human liver microsomal preparations.

Research design and methods: Doxorubicin, 20 μM, irinotecan, 10 μM, and etoposide, 50 μM, were incubated in the presence of granisetron, 0 nM, 20 nM, 200 nM and 2000 nM, in human liver microsomal preparations (250 μg). The levels of unchanged doxorubicin, irinotecan and etoposide in the incubation mixture were determined by high-performance liquid chromatography. Positive controls were ketoconazole, 20 μM, a potent inhibitor of CYP3A metabolism, for irinotecan and etoposide and quercitrin, 2 mM, a potent inhibitor of aldo-keto reductase, for doxorubicin.

Results: In the absence of granisetron, unchanged doxorubicin, irinotecan and etoposide levels decreased by 34.2 ± 5.5%, 21.3 ± 2.9% and 13.4 ± 1.6% of control, respectively. Ketoconazole prevented the breakdown of both irinotecan and etoposide, while quercitrin inhibited the breakdown of doxorubicin. Granisetron had no effect on the rate of reduction of doxorubicin, irinotecan or etoposide.

Conclusions: The results from this study suggest that granisetron neither inhibits nor induces the enzymes involved in the metabolism of doxorubicin, irinotecan or etoposide. Thus, granisetron can be used safely to treat nausea and vomiting induced by these agents with minimal risk of drug–drug interactions.

Introduction

The anthracycline doxorubicin has been used for many years in combination with other cytotoxic agents for the treatment of a wide range of solid tumours and haematologic malignancies. More recently, liposomal doxorubicin has appeared as a new cytotoxic agent for the treatment of patients with advanced[1] or relapsed[2] ovarian cancer. Moreover, etoposide has been recognized as an effective agent for the treatment of lymphoma[3], metastatic testicular cancer[4], small cell lung cancer[5] and relapsed ovarian cancer[6], while irinotecan hydrate is widely used with 5-fluorouracil for the treatment of advanced colorectal cancer[7] and advanced ovarian cancer[8]. Furthermore, all these antineoplastic agents are recommended as salvage chemotherapy for relapsed epithelial ovarian cancer by the National Comprehensive Cancer Network Treatment Guidelines. However, all these agents may induce the debilitating symptoms of nausea and vomiting that are commonly associated with antineoplastic chemotherapy. Fortunately, the development of the 5-HT_3-receptor antagonists for the

prevention and treatment of chemotherapy-induced emesis has enabled many patients to achieve good control of this side-effect[3].

Granisetron is a potent and selective antagonist at the 5-HT_3 receptor[10]. Clinical trials have shown that it controls nausea and vomiting in patients undergoing moderately or severely emetogenic chemotherapy[9,11], while granisetron given with a corticosteroid enhances antiemetic efficacy further[9]. However, concomitant administration of supportive care agents with antineoplastic drugs increases the risk of drug–drug interactions via the cytochrome P450 (CYP) system[12]. Furthermore, the majority (60%) of cancer patients are aged 65 years and over[13] and approximately 80% of them have comorbid conditions for which they are taking prescription medications [4,15]. A recent study in patients with advanced cancer indicated that the potential for drug–drug interactions increases with age, number of concomitant medications and length of hospital stay[16]. Although all the patients in the study were suffering from advanced cancer, and may have been experiencing specific problems related to the stage of their disease, the study indicates that there is considerable potential for drug–drug interactions in elderly patients with cancer. This may compromise treatment efficacy or increase drug-related toxicity.

Doxorubicin is metabolized to doxorubicinol by cytoplasmic aldo-keto reductase, and both doxorubicin and doxorubicinol are further metabolized by CYP enzymes[17]. The major metabolic pathway of etoposide is 3′-demethylation by CYP3A4[18], while oxidation of the piperidinylpiperidine side chain of irinotecan by CYP3A4 produces the relatively inactive oxidative metabolites 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin and 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin[19]. The metabolism of granisetron is also mediated via the CYP3A subfamily to produce the major metabolite 7-hydroxygranisetron and also a smaller amount of 9′-desmethylgranisetron[20]. The role of the CYP3A isoenzymes in the metabolism of granisetron, therefore, raises the potential for drug–drug interactions between granisetron and any other agent metabolized through the same enzyme pathway.

Granisetron does not appear to inhibit the activities of CYP1A2, CYP2A6, CYP2B6, CYP2C9/8, CYP2C19, CYP2D6, CYP2E1 or CYP3A at concentrations up to 250 μM[20]. Furthermore, experience from our laboratory suggests that granisetron has no effect on the *in vitro* metabolism in human liver microsomes of either paclitaxel or docetaxel, both of which are also metabolized by CYP3A4[21]. To further evaluate the potential for granisetron interactions with chemotherapeutic agents, the present study investigated the effects of granisetron on the *in vitro* metabolism of doxorubicin, irinotecan and etoposide in human liver microsomes.

Methods

Chemicals

Granisetron was obtained from Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). Irinotecan and SN-38, a potent inhibitor of topoisomerase I, were obtained from Yakult Honsha Co., Ltd. (Tokyo, Japan). Doxorubicin, etoposide, (*S*)-(+)-camptothecin, ethyletoposide, daunorubicin and quercitrin were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and ketoconazole was from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA, USA). All other reagents were purchased from commercial sources and were of the highest grade.

Human liver microsomes

Four batches of pooled human liver microsomes (International Institute for the Advancement of Medicine: IIAM, Scranton, PA, USA), collected from 15 donors, were used. They were preserved at –80°C until the time of use.

Analytical procedures

Human liver microsomes, 250 μg, were incubated at 37°C in the presence of 0.1 M potassium phosphate buffer (pH 7.4) and 0.1 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH) in a final volume of 0.5 mL. Preliminary experiments determined the incubation time and the concentration of doxorubicin, irinotecan and etoposide. Based on the findings of these studies, granisetron (final concentration 20 nM, 200 nM and 2000 nM) was incubated with the human liver microsome preparation in combination with either doxorubicin, 20 μM, for 4 h, irinotecan, 10 μM, for 60 min or etoposide, 50 μM, for 2 h. The levels of unchanged doxorubicin, irinotecan and etoposide in the incubation mixtures were determined by high-performance liquid chromatography (HPLC). Ketoconazole, 20 μM, a potent inhibitor of CYP3A4, served as a positive control for irinotecan and etoposide, while quercitrin, 2 mM, a potent inhibitor of aldo-keto reductase, served as a positive control for doxorubicin.

HPLC conditions

The LC-10A System (Shimadzu Co., Tokyo, Japan) was used as the determination device with an Inertsil separation column (150 × 4.6 mm internal diameter, 5 μm, GL Sciences Inc., Tokyo, Japan) with a guard column (Guard Pal Inserts Nova Pak C18, Waters Co., Milford, CT, USA).

HPLC for determination of doxorubicin was performed according to the method of de Bruijn *et al.*[22]. The flow

rate of the mobile phase was set at 1.25 mL/min, column temperature was 50°C and the wavelength for UV detection was 480 nm. Retention times for unchanged doxorubicin and daunorubicin in the human liver microsome preparations were 8.5 min and 22 min, respectively, and the concentrations of doxorubicin and daunorubicin were calculated using the area under the peak. The limit of detection for doxorubicin was 0.3 μM.

HPLC for determination of irinotecan was performed according to the method of Haaz *et al.*[23]. The flow rate of the mobile phase was set at 1.0 mL/min, column temperature was room temperature and the wavelength for UV detection was 355 nm. Retention times for unchanged irinotecan, its active metabolite SN-38 and camptothecin in the human liver microsome preparations were 17 min, 29 min and 28 min, respectively, and the concentrations of irinotecan, SN-38 and camptothecin were calculated using the area under the peak. The limit of detection for irinotecan was 0.3 μM.

HPLC for determination of etoposide was performed according to the method of Kawashiro *et al.*[18]. The flow rate of the mobile phase was set at 1.0 mL/min, column temperature was room temperature and the wavelength for UV detection was 288 nm. Retention times for unchanged etoposide and ethyletoposide in the human liver microsome preparations were 8.5 min and 16 min, respectively, and the concentrations of etoposide and ethyletoposide were calculated using the area under the peak. The limit of detection for etoposide was 1.5 μM.

Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA), followed by Bonferroni's multiple *t*-test. A value of $p < 0.05$ was classed as significant.

Results

All data are the mean ± SE of four determinations.

Doxorubicin

Preliminary experiments established optimum conditions of 4-h incubation time with doxorubicin concentrations of 10 μM (Figure 1A). In the presence of NADPH, quercitrin, 2 mM, inhibited aldo-keto reductase metabolism and prevented the breakdown of doxorubicin in the human microsomal preparations (Figure 2A). In the absence of granisetron, the amount of unchanged doxorubicin (10 μM) in the incubation mixture decreased by 34.2 ± 5.5% (Figure 2A). Granisetron, 20 nM, 200 nM and 2000 nM, had no effect on the rate of doxorubicin reduction ($p > 0.05$; Figure 2A).

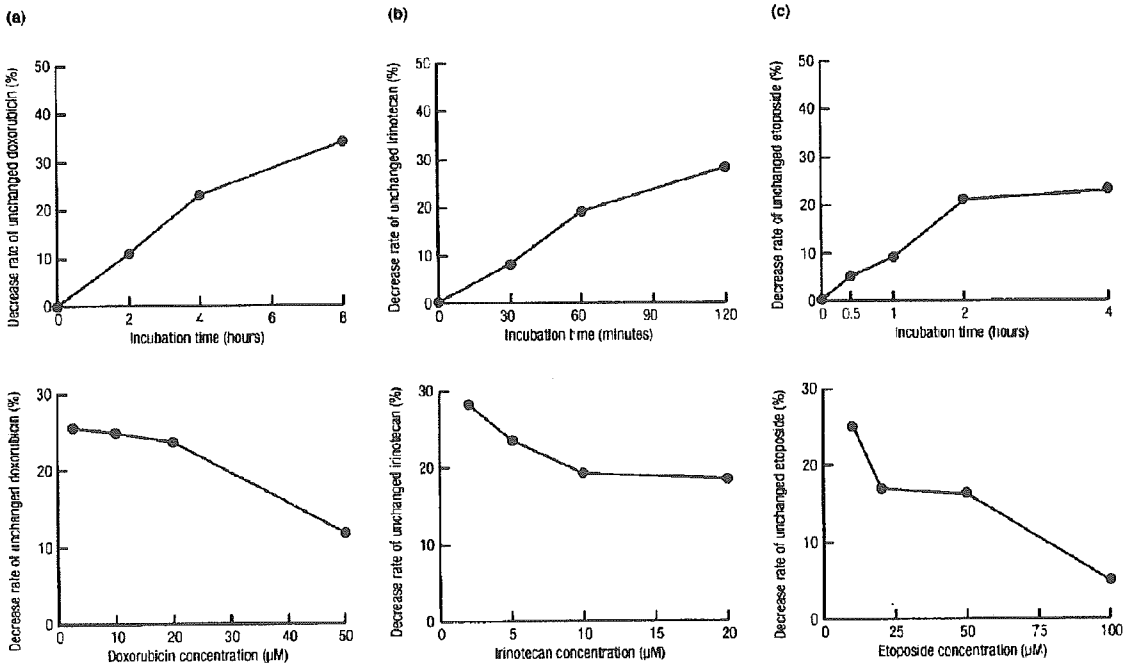

Figure 1. Effect of incubation time on metabolism (upper panel) and concentration in human liver microsomes (lower panel) of (a) doxorubicin, (b) irinotecan and (c) etoposide

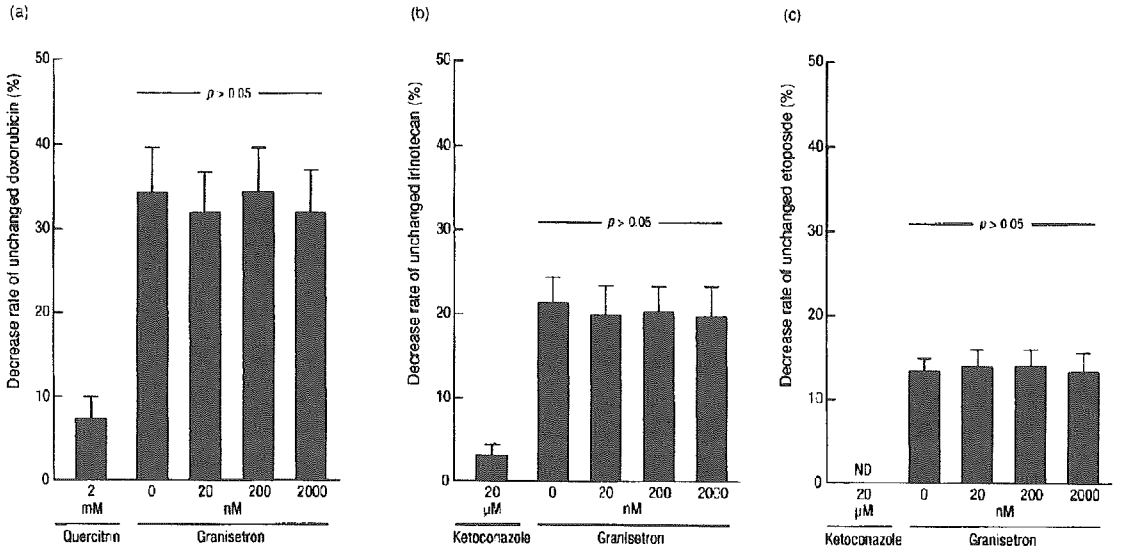

Figure 2. Effect of granisetron on the metabolism of (a) doxorubicin, (b) irinotecan and (c) etoposide in human liver microsomes. Data are the mean ± SE of 4 determinations (ND, not detected; $p > 0.05$ *vs no granisetron)*

Irinotecan and etoposide

Optimum conditions established by preliminary experiments were 60-min incubation time with concentrations of irinotecan, 10μM (Figure 1B), and 2-h incubation time with concentrations of etoposide, 50μM (Figure 1C). In the presence of NADPH, ketoconazole, 20μM, inhibited CYP3A4 metabolism and prevented the breakdown of irinotecan (Figure 2B) and etoposide (Figure 2C) in the human microsomal preparations. In the absence of granisetron, the amount of unchanged irinotecan (10μM) in the incubation mixture decreased by 21.3 ± 2.9% (Figure 2B), while that of unchanged etoposide (50μM) decreased by 13.4 ± 1.6% (Figure 2C). Granisetron, 20nM, 200nM and 2000nM, had no effect on the rates of reduction of irinotecan ($p > 0.05$; Figure 2B) or etoposide ($p > 0.05$; Figure 2C).

Discussion

The 5-HT_3-receptor antagonist, granisetron, is metabolized primarily via the CYP3A isoenzymes. Bloomer *et al.* have previously demonstrated that granisetron at concentrations between 0.5μM and 250μM did not inhibit the activity of a number of CYP isoenzymes[20], and the present study has confirmed that it neither inhibits nor induces the enzymes involved in the metabolism of doxorubicin, irinotecan and etoposide in an *in vitro* human liver microsomal preparation. These results further extend the knowledge from a previous study, which found that granisetron did not alter the *in vitro* metabolism of docetaxel or paclitaxel in human liver microsomes[21]. In the present study, granisetron did not interact with the breakdown of doxorubicin, irinotecan or etoposide, even when present at concentrations up to 60-fold higher than the maximum plasma concentrations that have been reported *in vivo*[24].

Ketoconazole is known to be a potent, selective inhibitor of the CYP3A isoenzymes, with a K_i of less than 1μM[25]. Consistent with this, the present study found that ketoconazole, 20μM, was a potent inhibitor of the metabolism of irinotecan and etoposide, both of which are metabolized by CYP3A4. Additionally, quercitrin, which is an inhibitor of aldo-keto reductase, completely inhibited the reduction of doxorubicin. In contrast, granisetron had no effect on the metabolism of the three study agents.

Sixty per cent of cancer patients are classed as elderly (over 65 years of age) and, as such, have an increased risk of suffering from declining organ function and a number of comorbid conditions[13,14]. As a result, they will often consume multiple medications[5,6]: indeed, 78% of patients aged over 65 years are estimated to be taking prescription medications, while 39% consume five or more drugs[15]. The risk of drug–drug interactions has been shown to increase with age and concomitant medications[16], and is estimated to be over 50% when a patient is receiving five medications, rising to a 100% probability with seven or more medications[26]. It is, therefore, essential that the potential for drug–drug interactions is minimized through identification of offending agents or the use of agents with no known drug–drug interactions.

Current antiemetic guidelines recommend the prophylactic administration of a 5-HT_3-receptor antagonist

in combination with dexamethasone and aprepitant for highly emetogenic chemotherapy, and a 5-HT_3-receptor antagonist in combination with dexamethasone for moderately emetogenic chemotherapy[27]. Among the currently available 5-HT_3 antagonists, granisetron is metabolized exclusively by the CYP3A isoenzymes. In contrast, ondansetron is metabolized by CYP2D6, CYP1A2 and to a small extent CYP1A1, in addition to the CYP3A isoenzyme[12]. Consequently, there is a potential for multiple drug–drug interactions between ondansetron and other agents that are also metabolized by any of these isoenzymes, and there have been a number of documented drug–drug interactions with this agent[28-31]. Furthermore, dolasetron and tropisetron are also both metabolized in part by members of the CYP3A group, in addition to CYP2D6. This is of particular concern given that CYP2D6 is subject to genetic polymorphism[32,33].

As doxorubicin, irinotecan and etoposide are all associated with the debilitating side-effects of nausea and vomiting, an antiemetic agent is indicated for patients undergoing treatment with any of these drugs. However, consideration must be given to the choice of antiemetic agent and its potential for drug–drug interactions. The present study suggests that granisetron can be used to treat nausea and vomiting induced by antineoplastic regimens that include doxorubicin, irinotecan or etoposide, with minimal risk of drug–drug interactions.

Conclusions

This study indicates that the 5-HT_3-receptor antagonist granisetron neither inhibits nor induces enzymes involved in the metabolism of doxorubicin, irinotecan and etoposide. The use of granisetron for the treatment of nausea and vomiting should, therefore, limit the potential for drug–drug interactions.

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References

1. Copeland LJ, Booxman M, Trimble E. Clinical trials of newer regimens for treating ovarian cancer: the rationale for Gynecologic Oncology Group Protocol GOG 182-ICON5. Gynecol Oncol 2003;90:S1-7
2. Gordon AN, Fleagle JT, Guthrie D, Parkin DE, Gore ME, Lacave AJ. Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. J Clin Oncol 2001;19:3312-22
3. Greco FA. Oral etoposide in lymphoma. Drugs 1999;58 (Suppl 3):35-41
4. Shelley MD, Burgon K, Mason MD. Treatment of testicular germ-cell cancer: a Cochrane evidence-based systematic review. Cancer Treat Rev 2002;28:237-53
5. Sandler AB. Chemotherapy for small cell lung cancer. Semin Oncol 2003;30:9-25
6. Alici S, Saip P, Eralp Y, Aydiner A, Topuz E. Oral etoposide (VP16) in platinum-resistant epithelial ovarian cancer (EOC). Am J Clin Oncol 2003;26:358-62
7. Benson 3rd AB, Goldberg RM. Optimal use of the combination of irinotecan and 5-fluorouracil. Semin Oncol 2003;30 (Suppl 6):68-77
8. Bodurka DC, Levenback C, Wolf JK, et al. Phase II trial of irinotecan in patients with metastatic epithelial ovarian cancer or peritoneal cancer. J Clin Oncol 2003;21:291-7
9. Gralla RJ, Osoba D, Kris MG, et al. Recommendations for the use of antiemetics: evidence-based, clinical practice guidelines. An ASCO special article. J Clin Oncol 1999;17:2971-94
10. Van Wijngaarden I, Tulp MTM, Soudijn W. The concept of selectivity in 5-HT receptor research. Eur J Pharmacol 1990;188:301-12
11. del Giglio A, Soares HP, Caparroz C, Castro PC. Granisetron is equivalent to ondansetron for prophylaxis of chemotherapy-induced nausea and vomiting: results of a meta-analysis of randomized controlled trials. Cancer 2000;89:2301-8
12. Blower PR. 5-HT_3-receptor antagonists and the cytochrome P450 system: clinical implications. Cancer J 2002;8:405-14
13. Yancik R, Ries LAG. Aging and cancer in America. Hematol Oncol Clin North Am 2000;14:17-23
14. Yancik R, Ganz PA, Varricchio CG, Conley B. Perspectives in comorbidity and cancer in older patients: approaches to expanding the knowledge base. J Clin Oncol 2001;19:1147-54
15. Jorgensen T, Johansson S, Kennerfalk A, Wallander MA, Svardsudd K. Prescription drug use, diagnoses, and healthcare utilization among the elderly. Ann Pharmacother 2001;35:1004-9
16. Riechelmann RP, Smaletz O, Saad ED. Drug interactions in cancer patients. Proc Am Soc Clin Oncol 2004;23:761 [abstract 8146]
17. Lee HJ, Lee MG. Effects of dexamethasone on the pharmacokinetics of adriamycin after intravenous administration to rats. Res Commun Mol Pathol Pharmacol 1999;105:87-96
18. Kawashiro T, Yamashita K, Zhao XJ, et al. A study on the metabolism of etoposide and possible interactions with antitumor or supporting agents by human liver microsomes. J Pharmacol Exp Ther 1998;286:1294-300
19. Santos A, Zanetta S, Cresteil T, et al. Metabolism of irinotecan (CPT-11) by CYP3A4 and CYP3A5 in humans. Clin Cancer Res 2000;6:2012-20
20. Bloomer JC, Baldwin SJ, Smith GJ, Ayrton AD, Clarke SE, Chenery RJ. Characterisation of the cytochrome P450 enzymes involved in the in vitro metabolism of granisetron. Br J Clin Pharmac 1994;38:557-66
21. Watanabe Y, Nakajima H, Nozaki K, Hoshiai H, Noda K. The effect of granisetron on in vitro metabolism of paclitaxel and docetaxel. Cancer J 2003;9:67-70
22. de Bruijn P, Verweij J, Loos WJ, et al. Determination of doxorubicin and doxorubicinol in plasma of cancer patients by high-performance liquid chromatography. Anal Biochem 1999;266:216-21
23. Haaz MC, Rivory L, Riche C, Vernillet L, Robert J. Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. Cancer Res 1998;58:468-72
24. Kumakura H, Koyanagi J, Nisioka Y, et al. Phase I study of granisetron (second report) pharmacokinetics of granisetron following single and repeat intravenous drip infusion in Japanese healthy volunteers. J Clin Ther Med 1990;6:25-34
25. Maurice M, Pichard L, Daujat M, et al. Effects of imidazole derivatives on cytochromes P450 from human hepatocytes in primary culture. FASEB J 1992;6:752-8
26. Karas S. The potential for drug interactions. Ann Emerg Med 1981;10:627-30
27. MASCC. Antiemetic guidelines from the consensus conference on antiemetic therapy. Perugia International Cancer Conference VII, 29–31 March 2004, Perugia, Italy. Available at www.mascc.org [accessed January 2005]

28. Blum RA, Majumdar A, McCrea J, et al. Effects of aprepitant on the pharmacokinetics of ondansetron and granisetron in healthy subjects. Clin Ther 2003;25:1407-19
29. Stanford BJ, Stanford SC. Postoperative delirium indicating an adverse drug interaction involving the selective serotonin reuptake inhibitor, paroxetine. J Psychopharmacol 1999;13:313-7
30. Cagnoni PJ, Matthes S, Day TC, Bearman SI, Shpall EJ, Jones RB. Modification of the pharmacokinetics of high-dose cyclophosphamide and cisplatin by antiemetics. Bone Marrow Transplant 1999;24:1-4
31. DeWitte JL, Schoenmackers B, Sessler DI, Deloof T. The analgesic efficacy of tramadol is impaired by concurrent administration of ondansetron. Anesth Analg 2001;92:1319-21
32. Kaiser R, Sezer O, Papies A, et al. Patient-tailored antiemetic treatment with 5-hydroxytryptamine type 3 receptor antagonists according to cytochrome P-450 2D6 genotypes. J Clin Oncol 2002;20:2805-11
33. Kim M-K, Cho J-Y, Lim H-S, et al. Effect of the CYP2D6 genotype on the pharmacokinetics of tropisetron in healthy Korean subjects. Eur J Clin Pharmacol 2003;59:111-16

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Evaluation of weekly low-dose paclitaxel and carboplatin treatment for patients with platinum-sensitive relapsed ovarian cancer

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Abstract

Objective. Although paclitaxel and carboplatin therapy (TC) is an established effective standard regimen for patients with ovarian cancer, both treatment delay for hematologic toxicity and discontinuation of treatment due to neurotoxicity have occasionally been reported. To achieve therapeutic density, we evaluated the usefulness of weekly low-dose TC therapy (WTC) in patients with platinum-sensitive (median PFI was 11.4 ± 2.7 months) recurrent epithelial ovarian cancer.

Methods. A total of 25 patients were treated with paclitaxel at 60 mg/m^2 and carboplatin at AUC = 2 using 3 weekly courses with a 1-week break schedule. Eighteen patients had assessable tumors for response, and the other seven patients were evaluated by CA-125-based response. All of the patients were assessable for toxicity.

Results. The overall response rate (OR) based on WHO criteria was 84.2% (95% CI; 0.65–0.98), including nine complete responses (CR); OR based on CA-125 was 85.7% (95% CI; 0.42–0.99), including 3 CR. The total response rate was 88.0% (95% C.I.; 0.68–0.97). The median progression-free survival of the patients was 13.5 months during the mean follow-up period of 21.9 ± 9.2 months. No patients had grade 1 or higher thrombocytopenia, and although 44% of the patients developed neurotoxicity, all cases remained grade 1. Treatment delay of over 7 days due to toxicity was observed in only two patients (16.0%) and in six cycles (1.3%) in a total of 451 cycles.

Conclusion. WTC combination, as used in this study, produced a high response rate with acceptable toxicity, and the optimal combination in a weekly regimen remains to be determined.

Keywords: Weekly administration; Paclitaxel; CBDCA; Recurrent ovarian cancer

Introduction

Recently, the ICON 4 trial [1] demonstrated that paclitaxel/platinum therapy was significantly superior in achieving progression-free survival (PFS) and overall survival compared to those achieved by conventional nonpaclitaxel/platinum chemotherapy in patients with platinum-sensitive recurrence. Therefore, paclitaxel and carboplatin (CBDCA) therapy (TC) is being developed as an effective regimen not only for patients with primary epithelial ovarian cancer but also for platinum-sensitive relapsed epithelial ovarian cancer. However, with treatment by TC therapy, thrombocytopenia, alopecia, and neurotoxicity are frequently experienced as uncontrolled adverse effects. In our department, several patients rejected continuation of TC therapy due to neurotoxicity, and several patients rejected even starting TC therapy due to their anxiety about neurotoxicity. These trends indicate that thrombocytopenia, alopecia, and neurotoxicity, especially sensory neurotoxicity, should be resolved while maintaining therapeutic effects, dose intensity, and providing an incentive for treatment and maintaining the patient's quality of life. Recently, several studies reported the efficacy of weekly 1-h paclitaxel infusion therapy for advanced or recurrent cancer.

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Weekly administration regimens afford theoretical advantages based on cell cytokinetic effects of drug-resistant clones by frequent exposure of cancer cells to anti-neoplatic agents [2], while the weekly schedule was also expected to offer less toxicity than the standard schedule. Seidman et al. [3] treated 30 patients with metastatic breast cancer with weekly paclitaxel infusion at an initial dose of 100 mg/m^2 until disease progression, and reported that the OR was 53%, the complete response rate was 10%, 13.3% of grade 3/4 neutropenia, and 9.5% grade 3 neuropathy. They concluded that weekly paclitaxel infusion therapy will be an attractive treatment alternative for patients with metastatic breast cancer due to its high therapeutic index, manageable toxicity, and convenient administration schedule. As a second-line chemotherapy for relapsed ovarian cancer, Abu-Rustam et al. [4] studied the effects of weekly paclitaxel (60–100 mg/m^2) as salvage therapy for relapsed ovarian cancer, and reported an OR of 28.9%. Moreover, Markman et al. [5] evaluated a phase II study of weekly single-agent at 80 mg/m^2 of paclitaxel, and reported that the OR was 25%. Recently, Havrilesky et al. [6] performed a phase II study of WTC (T: 80 mg/m^2, C: AUC = 2) in recurrent ovarian and peritoneal cancers, and reported an 82.8% OR and a 13.7-month median interval until progression in platinum-sensitive patients and an 11.5-month overall median interval until progression in platinum-resistant patients. Moreover, Wu et al. [7] also reported the results of WTC (T: 60 mg/m^2, C: AUC = 2) for Chinese patients with advanced ovarian cancer in comparison with those on the monthly regimen. They reported that WTC achieved 71.4% OR, and although there were no significant differences in nonhematological toxicities between WTC and the monthly regimen, delayed treatment, unanticipated hospitalization, and G-CSF support were much less frequent with WTC. Weekly fractionated platinum for recurrent ovarian cancer has also been studied by several groups [8–10]. Colombo et al. [9] and Cocconi et al. [10] conducted randomized trial, compared weekly cisplatin to every-3-week dosing, and reported the potential role for weekly platinum therapy for recurrent ovarian cancer. Recently, Belani et al. [11] reported that weekly paclitaxel with nonfractionated CBDCA administration achieved the superior effects than weekly paclitaxel with fractionated CBDCA in patients with stage IIIB or IV non-small-cell lung cancer. According to those reports, although the addition of CBDCA will be needed to achieve a better response rate, in the chemotherapy for advanced non-small-cell lung cancer, it has been thought that nonfractionated CBDCA administration was more favorable in weekly TC regimen. However, in recurrent epithelial ovarian cancer, phase II study of WTC with nonfractionated CBDCA was just now underway, and only overall response was reported [Rose et al., Proc Am Soc Clin Oncol 2003, Abstr. 1932]. Moreover, prior studies of WTC with fractionated CBDCA [6,7] [Dunton CJ et al., Proc Am Soc Clin Oncol 2003, Abstr. 1876] have achieved satisfactory effects both in the OR and the PFS. According to the results of those trials, the optimal dose and schedule for WTC therapy for recurrent ovarian cancer remain unknown. Furthermore, there is some possibility that the clinical role of fractionated CBDCA administration would be different in chemosensitive tumor, such as epithelial ovarian cancer. Thus, we have conducted phase II study and pharmacokinetic study of WTC using fractionated CBDCA subjected the same as in the recent reports [6,7] from 2000 in Japanese patients with platinum-sensitive recurrent ovarian cancer for further comparative study to evaluate the clinical role of WTC.

Patients and methods

Treatment regimen

From October 2000 to August 2003, 25 patients with relapsed epithelial ovarian cancer who had a greater than 6-month platinum-free interval were treated using the WTC regimen at the Department of Obstetrics and Gynecology at Kinki University School of Medicine. In this study, we only utilized patients who were first-line failures and platinum-sensitive (platinum-free interval > 6 months) with a minimal survival of 6 months. Fig. 1 shows the patient characteristics of recurrent ovarian cancer. The treatment dose and schedule of WTC consisted of paclitaxel (Taxol, Bristol Meiers) at 60 mg/m^2 for 60 min, and CBDCA (Paraplatin, Bristol Meiers) at AUC = 2 (Carvert's formula) for 60 min, at a weekly administration, for 3 weeks with a 1-week break. The treatment dose was calculated by dividing the

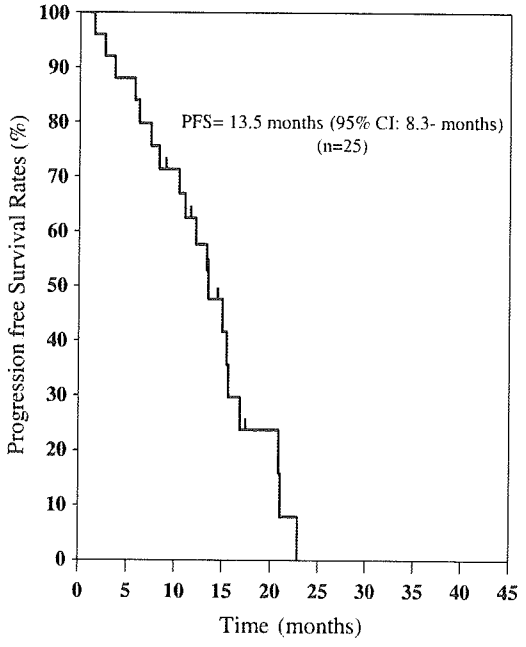

Fig. 1. Progression-free survival of subjected patients. PFS: progression-free survival. Mean follow-up period of patients was 21.9 ± 9.1 months (range: 6–37 months).