domain, which influences the phosphatase activity (Hinoda et al. 1998; Maehama and Dixon 2000; Parsons 1998; Tamura et al. 1999). PTEN has an antagonistic effect on intracellular signaling pathways induced by integrin or growth factors, and inhibits cell proliferation and finally induces apoptosis. One of the inhibitory mechanisms is that PTEN dephosphorylates focal adhesion kinase (FAK), which plays a major role in a transcription-regulatory signaling system. FAK is activated by integrin and growth factors, and induces focal adhesion, cytoskeletal formation, and cellular spreading, invasion and migration (Mochizuki 1999; Tamura et al. 1998a, 1998b, 1999). Another mechanism is that PTEN suppresses the signaling pathway that goes through protein kinase B (Akt/PKB) by dephosphorylating phosphatidylinositol 3,4,5-trisphosphate (PIP3). It thereby leads to apoptosis and inhibits cell proliferation (Gu et al. 1998; Tamura et al. 1999). PTEN also suppresses the activity of mitogen-activated protein kinase (MAPK) by dephosphorylating Src homologous and collagen (Shc) as an adaptor protein. Furthermore, PTEN also inactivates the stimulatory effect on cell growth induced by estrogen, and it has been suggested that this effect of PTEN is abolished by mutations of the PTEN gene(Mutter et al. 2000c).

In this study, we examined PTEN expression immunohistochemically in endometrioid adenocarcinoma of the uterine corpus as well as normal endometrium and endometrial hyperplasia, and examined the correlation of PTEN expression with the expression of cell cycle regulators, and with clinicopathological parameters, estrogen, and progesterone receptor levels, and p53 gene mutation.

Materials and methods

Tissue samples

Tissue samples of 19 normal endometria (eight cases of the proliferative phase and 11 of the secretory phase), 20 endometrial hyperplasias [nine cases of simple hyperplasia (SH), four of complex hyperplasia (CH) and seven of complex atypical hyperplasia (CAH)] and 117 endometrioid adenocarcinomas, including 67 well-differentiated (G1), 24 moderately differentiated (G2), and 26 poorly differentiated (G3) adenocarcinomas, were surgically obtained with informed consent at Kitasato University Hospital between 1983 and 2000. No patients received any therapy before surgery.

Immunohistochemistry

Immunohisitochemical staining for PTEN protein was performed with the labeled streptavidin-biotin (LSAB) method (LSAB-kit, DAKO, Kyoto, Japan) on formalin-fixed and paraffin-embedded tissue samples. Tissue samples were sectioned at 3-μm thickness and deparaffinized in xylene. Endogenous peroxidase activity was inhibited with 3% hydrogen peroxide for 15 min. Antigen retrieval was performed by autoclaving at 121 °C for 15 min in 0.01 mol/l citrate buffer (pH6.0). After the sections were incubated with 10% normal swine serum for 10 min, they were incubated with mouse monoclonal anti-PTEN antibody (clone 28H6, 1:400, Novocastra, Newcastle, UK) overnight at 4 °C. The sections were washed in 0.01 mol/l phosphate-buffered saline (PBS) and incubated with biotinylated anti-mouse goat immunoglobulin for 10 min, and then with horseradish peroxidase-labeled streptavidin for 10 min. The peroxidase reaction was developed in 0.02% 3,3'-diaminobenzidine tetrahydrochloride solution containing 0.003% hydrogen peroxide. The nuclei were lightly counterstained with Mayer's hematoxylin.

PTEN expression was compared with the expression of Ki-67, cdk2, cyclin A, cyclin D1, cyclin E, p27, and p53, which were also examined immunohistochemically. The staining methods were described elsewhere (Fujisawa et al. 2001; Kato et al. 2003; Kyushima et al. 2002; Watanabe et al. 2002). In brief, the antibodies used were those for Ki-67 (rabbit polyclonal, 1:50, Dako, Kyoto, Japan), cdk2 (rabbit polyclonal, 1:2000, Santacruz, Calif., USA), cyclin A (clone 6E6, 1:100, Novocastra), cyclin D1 (clone DCS-6, 1:80, Oncogene, Mass., USA), cyclin E (clone 13A3, 1:40, Novocastra), p27 (clone 1B4, 1:200, Novocastra) and p53 (clone DO-7, 1:80, Novocastra).

Evaluation of immunohistochemical staining

The level of PTEN protein was expressed as the PTEN staining score, which was calculated using both the labeling index (LI) and staining intensity. LI was defined as the percentage of cells positive for PTEN among approximately 1,200 cells in three randomly selected high-power fields. LIs were classified into four groups: group 1 (0% ≤ LI < 25%), group 2 (25% ≤ LI < 50%), group 3 (50% ≤ LI < 75%) and group 4 (75% ≤ LI ≤ 100%), and these groups were given scores of 1, 2, 3, and 4 points (LI score), respectively.

The staining intensity of the nuclei of tumor cells, which was compared with that of adjacent stromal cells taken as a control with intensity of +, was also classified into four groups with intensity judged to be −, ±, +, or + +, and these groups were scored as 1, 2, 3, and 4 points (staining intensity score), respectively.

The product of LI score times staining intensity score was used to evaluate PTEN expression as the PTEN staining score, which ranged from 1 to 16 points. The expression levels of cell cycle regulators were evaluated by calculating LI by the same method as described above (Kato et al. 2003; Kyushima et al. 2002; Watanabe et al. 2002).

p53 and PTEN gene mutation analysis

Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis was performed to analyze mutations of the p53 and PTEN genes. In brief, DNA of endometrial cancer tissues was extracted by a phenol chloroform method (Uchida et al. 1993). The oligonucleotide primer pairs located in exons 5 to 8 of the p53 gene and the PCR conditions also conformed to the methods of Uchida et al. The primer sets used for the p53 gene were as follows: Exon5(sense,antisense): 5'-TGTTCACTTGTGCCCT GACT-3', 5'-CAGCCCTGTCGTCTCTCCAG-3'; Exon6:5'-TGT TTGCCCAGGGTCCCCAG-3', 5'-GGAGGGCCACTGACAAC CA-3'; Exon7:5'-CTTACCACAGGTCTCCCCAA-3', 5'-AGGGG TCAGCGGCAAGCAGA-3'; Exon8:5'-TTGGGAGTAGATGG AGCCT-3', 5'-AGTGTTAGACTGGTAAACTTT-3'.

The oligonucleotide primer pairs located in exons 1 to 9 of the PTEN gene and the PCR conditions conformed to those used in the method of Steck et al. (Steck et al. 1997). The primer sets used for the PTEN gene were as follows: Exon1 (sense,antisense): 5'-CAGCCGTTCGGAGGATTA-3',5'-ATATGACCTAGCAAC CTGACCA-3'; Exon2:5'-TGACCACCTTTTATTACTCC-3', 5'-TACGGTAAGCCAAAAAATGA-3'; Exon3:5'-ATATTCTC TGAAAAGCTCTGG-3', 5'-TTAATCGGTTTAGGAATACAA-3'; Exon4:5'-TTCAGGCAATGTTTGTTA-3', 5'-CTTTATGCAATA CTTTTTCCTA-3'; Exon5:5'-AGTTTGTATGCAACATTTCTAA-3', 5'-TTCCAGCTTTACAGTGAATTG-3'; Exon6:5'-ATATGTTCT TAAATGGCTACG-3', 5'-AGCAACTATCTTTAAAACCTGT-3'; Exon7:5'-ACAGAATCCATATTTCGTGTA-3', 5'-TAATGTCT

CACCAATGCCA-3'; Exon8:5'-TGCAAAATGTTTAACATAG GTGA-3', 5'-GTAAGTACTAGATATTCCTTGTC-3'; Exon9:5'-AAGATGAGTCATATTTGTGGGT-3', 5'-GACACAATGTCC TATTCCAT-3'.

The 5'-end of each primer was labeled with [γ-^{32}P]ATP. SSCP was performed according to the method of Orita et al. (Orita et al. 1989). In brief, electrophoresis was performed at 40 W for 3 h on a 5% polyacrylamide gel. The gel was dried at 80 °C for 45 min and exposed to Kodak XAR film at room temperature for 15 min to 24 h with an intensifying screen. DNA extracted from lymphocytes of a normal woman whose menstrual cycle was regular was used as a normal control. Aberrant bands or mobility shift indicated gene mutations. p53 and PTEN gene analysis was performed randomly in 56 cases in the present series.

ER and PR expression analysis

Estrogen receptor (ER) and Progesterone receptor (PR) expression was analyzed with a radioreceptor assay or enzyme immunoassay at Kitasato Biochemical Laboratory (Sagamihara, Kanagawa, Japan). Expression of 5.0 fmol/mg cytosol protein was the cut-off value.

Comparison with clinicopathological parameters

Clinicopathological parameters of the patients were obtained from the tumor registry of the Department of Gynecology, Kitasato University Hospital, and compared with PTEN expression.

Statistical analysis

Statistical analysis of the correlation between the PTEN staining score and the LI of each cell cycle regulator was conducted with Spearman's rank correlation test. The Mann Whitney U-test was used to examine the correlation of the PTEN staining score with clinicopathological parameters, p53 mutation, and ER and PR levels. The correlation between PTEN gene mutation and grade was analyzed with Fisher's exact test. P-values less than 0.05 were considered statistically significant.

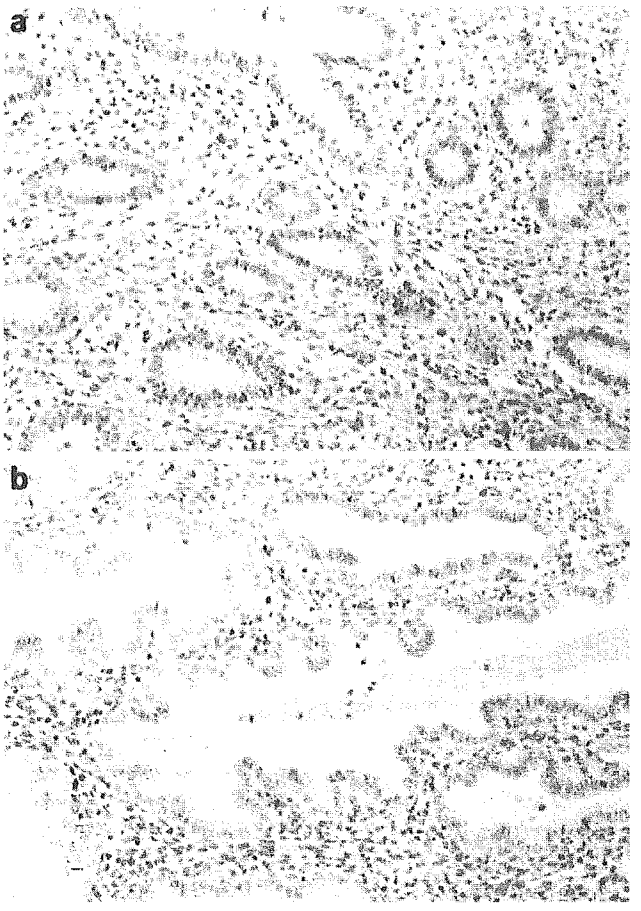

Fig. 1a,b **a** PTEN protein expression in the proliferative phase of normal endometrium. Almost all nuclei of glandular cells show the immunoreaction (PTEN staining score 16, ×200); **b** In the secretory phase, the glandular cells are slightly positive for PTEN in the nuclei (PTEN staining score 4, ×200)

Results

PTEN protein in the proliferative and secretory phase endometria was detected in the nuclei of endometrial columnar cells and adjacent stromal cells (Fig. 1a,b). The PTEN staining scores of columnar cells in the proliferative and secretory phases were 13.3 ± 3.5 and 9.0 ± 3.1, respectively (Table 1). The former was significantly higher than the latter.

In endometrial hyperplasias, PTEN protein expression showed the same pattern as in normal endometria (Fig. 2a–c). The PTEN staining scores of SH, CH and CAH were 10.1 ± 4.4, 12.3 ± 2.9, and 11.6 ± 1.1, respectively (Table 1), and were not significantly different from each other. The PTEN staining scores were not significantly different between normal endometria and endometrial hyperplasias.

The PTEN staining in a case of G1 adenocarcinoma was entirely negative, (Fig. 3a). In a case of G3 adenocarcinoma, almost all nuclei of the cancer cells appeared positive for PTEN (Fig. 3b). The PTEN staining scores of G1, G2, and G3 endometrioid adenocarcinomas were 7.6 ± 5.2, 9.6 ± 5.2, and 11.9 ± 3.7, respectively. The score of G1 adenocarcinomas was significantly lower than that of G3 adenocarcinomas (Table 1), and was also significantly lower than those of endometrial hyperplasia and the proliferative phase endometrium (Table 1).

PTEN staining score was positively correlated with the LIs of cell cycle regulators such as Ki-67, cdk2, cyclin A, cyclin D1, cyclin E, p27, and p53 (Table 2).

PTEN staining score was not significantly associated with clinicopathological parameters such as FIGO stage, myometrial invasion, lymph-vascular space invasion (LVSI), lymph node metastasis or group (group 1, cancer with coexisting endometrial hyperplasia; group 2, cancer with coexisting normal endometrium; group 3, only cancer; Ohkawara et al. 2000) (Table 3).

The PTEN staining scores in cases with wild-type and mutant p53 genes were 7.4 ± 5.3 and 11.9 ± 4.6, respectively, and the former was significantly lower than the latter (Table 4). In contrast, the PTEN staining scores in cases with wild-type and mutant PTEN genes were 8.8 ± 5.3 and 7.7 ± 6.0, respectively, showing no

Table 1 The correlation between PTEN staining score and normal endometrium, endometrial hyperplasia, and endometrioid adenocarcinoma of the uterine corpus

	No.of cases	PTEN staining score Mean	±	SD	P-value			
Proliferative phase	8	13.3	±	3.5	0.0208*			
Secretory phase	11	9.0	±	3.1		N.S.		
Endometrial hyperplasia, simple(SH)	9	10.1	±	4.4	N.S.			
Endometrial hyperplasia, complex(CH)	4	12.3	±	2.9	N.S.	N.S.		0.0046*
Atypical endometrial hyperplasia, complex(CAH)	7	11.6	±	1.1	N.S.		0.0101*	
G1	67	7.6	±	5.2				
G2	24	9.6	±	5.2	N.S.	0.0004*		
G3	26	11.9	±	3.7	N.S.			

*p < 0.05 ; significant, N.S. ; not significant, Mann-Whitney U test

significant difference between them. When analyzed in relation to pathological grade, PTEN staining scores with or without p53 and PTEN gene mutation were not significantly different except these between G1 vs G2 with p53 mutation ($P=0.04$). PTEN expression was high in G2 and G3 with PTEN gene mutation, although statistical analysis could not be conducted because of the limited number of cases. PTEN expression with or without PTEN gene mutation was not significantly correlated in each grade examined by Fisher's exact test (Table 4).

The PTEN staining scores were 6.5 ± 5.3 in the cases with ER $\geq$ 50 f mol/mg protein and 9.5 ± 4.9 in cases with ER $<$ 50 f mol/mg protein, and were 6.6 ± 5.5 in cases with PR $\geq$ 100 f mol/mg protein and 9.7 ± 4.8 in cases with PR $<$ 100 f mol/mg protein. PTEN expression was significantly lower in cases with either a high level of ER or PR than in their counterparts with low receptor levels. PTEN staining scores of each grade were not significantly correlated each other in either high or low ER and PR groups. G1 with high ER ($P=0.079$) and PR ($P=0.026$) groups showed lower PTEN expression than those with low their groups (Table 5).

Discussion

The PTEN staining score was significantly higher in the proliferative endometrium than in the secretory endometrium in this study. Mutter et al. reported that all endometrial columnar and stromal cells in the proliferative phase were positive for PTEN, and that PTEN expression was decreased or absent in the secretory phase (Mutter 2000a; Mutter et al. 2000c). That result is similar to ours in this study. This indicates that PTEN protein may be induced in the proliferative phase as a negative feedback response to the stimulatory effect of estrogen on proliferation, and may be decreased in the secretory phase due to antagonism of estrogen's action by progesterone (Mutter 2000a; Mutter et al. 2000b, 2000c).

PTEN gene mutation in endometrial hyperplasia with or without atypia has been detected in 19–55% (Ellenson 2000; Maxwell et al. 1998; Mutter et al. 2000b). In contrast, in this study, the level of PTEN expression in endometrial hyperplasia as examined immunohistochemically was not different from that in proliferative phase endometrium and also showed no significant correlation with the subtype of hyperplasia. It is suggested that PTEN staining using the present antibody might not be associated with PTEN gene mutation in endometrial hyperplasia, although we have not examined the mutation.

It has been suggested that there may be two different sequences of the development of endometrioid adenocarcinoma; one develops through endometrial hyperplasias and mainly consists of well-differentiated cancer and coexists with endometrial hyperplasia (Ohtani et al. 1999; Fujimoto et al. 1998). The other is an estrogen-unrelated type that originates de novo from atrophic endometrium and develops into poorly differentiated cancer without endometrial hyperplasia, and is associated with gene mutation of p53 and c-erbB2/neu amplification (Sherman 2000; Ohtani et al. 1999; Bussaglia et al. 2000). The latter type of carcinoma occurs not infrequently in post-menopausal women and shows aggressive behavior. The former is known to be promoted by an unopposed estrogen environment (Fujimoto et al. 1998; Sherman 2000). ER is first phosphorylated after being combined with estrogen and is

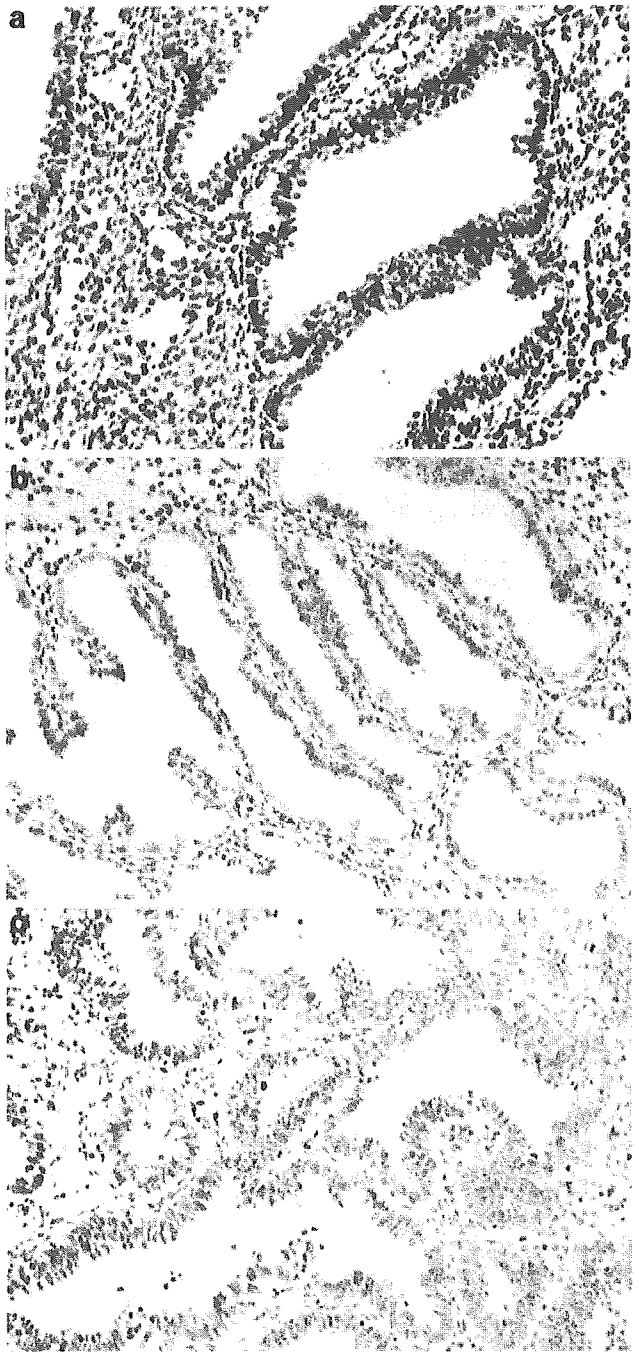

Fig. 2a–c **a** PTEN protein expression in endometrial hyperplasia, simple type. Almost all nuclei of glandular cells show the immunoreaction (PTEN staining score 16, ×200); **b** PTEN protein expression in endometrial hyperplasia, complex type. Almost all nuclei of glandular cells show the immunoreaction (PTEN staining score 16, ×200); **c** PTEN protein expression in endometrial atypical hyperplasia, complex type. Almost all nuclei of glandular cells show the immunoreaction (PTEN staining score 12, ×200)

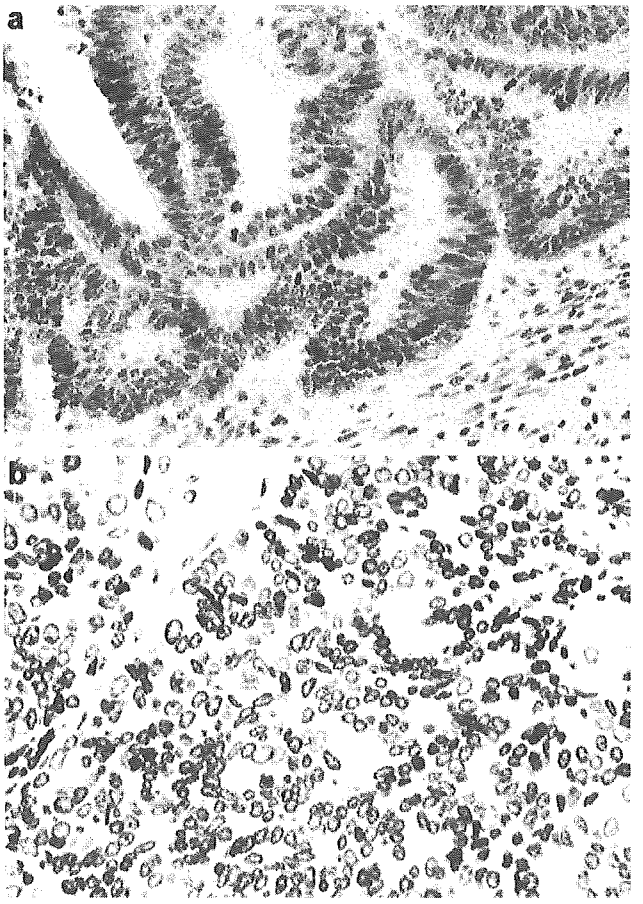

Fig. 3a,b a Negative PTEN protein expression in endometrioid adenocarcinoma (G1) (PTEN staining score 1, ×200); b PTEN protein expression in endometrioid adenocarcinoma (G3) (PTEN staining score 12, ×200)

Table 2 The correlation between PTEN staining score and LIs of cell cycle regulators in endometrioid adenocarcinopma of the uternine corpus (*LI* labeling index)

Cell cycle regulator	*r*	P-value
Ki-67	0.32	0.0006*
cdk2	0.21	0.0289*
Cyclin A	0.34	0.0005*
Cyclin D1	0.19	0.0428*
Cyclin E	0.24	0.0090*
p27	0.22	0.0208*
p53	0.44	0.0014*

*$P < 0.05$ significant; Spearman's rank correlation test

then activated by changing its conformation. Activated ER combines with the estrogen response element in the nucleus and induces the expression of transforming growth factor-1 (TGF-1), epithelial growth factor (EGF) receptor and cyclin D1 (Hata et al. 1998; Kato et al. 1998; Weng et al. 2001). Subsequently, it activates a PIP3-Akt pathway that causes cell growth and inhibits apoptosis. Then, by activation of the estrogen receptor through GRB2-Sos-Ras, resulting in activation of the Shc-MAPK or Raf-MAPKK-MAPK pathway, cell growth is further promoted. In normal endometrial cells, PTEN suppresses the estrogen-stimulated cell proliferation by dephosphorylating Shc, FAK, and PIP3 (Gu et al. 1998; Mochizuki 1999; Tamura et al. 1998a, 1998b,

Table 3 The correlation between PTEN staining score and clinicopathological parameters in endometrioid adenocarcinoma of the uterine corpus

Clinicopathological parameter		No.of cases	PTEN staining score Mean	±	SD		P-value
Stage	FIGO I	76	8.9	±	4.9	I vs II	N.S.
	FIGO II	12	9.7	±	6.3	I vs III	
	FIGO III	26	8.9	±	5.6	I vs IV	
	FIGO IV	3	9.3	±	4.6	I vs II, III, IV	
Myometrial invasion	< 1/3	53	9.7	±	4.8		N.S.
	1/3≦	56	8.2	±	5.5		
LVSI	−	80	8.5	±	5.3		N.S.
	+	28	9.9	±	4.6		
Lymph node metastasis	−	92	8.9	±	5.2		N.S.
	+	13	10.5	±	4.7		
Group	1	49	7.9	±	5.4	1 vs 2	N.S.
	2	50	9.2	±	4.9	1 vs 3	
	3	15	10.9	±	4.6	2 vs 3	

LVSI ; Lymph-vascular space invasion, N.S. ; not significant, Mann-Whitney U test

Table 4 The correlation between PTEN staining score, and p53 and PTEN mutation in endometrioid adenocarcinoma of the uterine corpus

Mutation		No.of cases	PTEN staining score Mean	±	SD	P-value	Grade	No.of cases	PTEN staining score Mean	±	SD	P-value
p53	−	44	7.4	±	5.3	0.0094*	G1	34	6.6	±	5.5	N.S.
							G2	6	9.2	±	4.3	
							G3	4	12.0	±	0.0	
	+	11	11.9	±	4.6		G1	4	9.8	±	4.5	0.04*
							G2	4	15.0	±	2.0	
							G3	3	10.7	±	6.1	
PTEN	−	37	8.8	±	5.3	N.S.	G1	22	7.3	±	5.5	N.S.
							G2	9	11.4	±	4.8	
							G3	6	10.7	±	3.3	
	+	19	7.7	±	6.0		G1	17	6.9	±	5.9	
							G2	1	12.0			
							G3	1	16.0			

*p < 0.05 ; significant, N.S. ; not significant, Mann-Whitney U test

1999; Weng et al. 2001). It is thought that Shc, FAK, and PIP3 cannot be dephosphorylated when the PTEN gene is mutated and cell growth cannot be inhibited. (Gu et al. 1998; Mochizuki 1999; Tamura et al. 1998a, 1998b, 1999). Mutation of PTEN has been analyzed in various advanced cancers (Steck 1997), and detected in 34–83% of endometrial adenocarcinomas (Bussaglia et al. 2000; Ellenson 2000; Kurose et al. 1998; Levine et al. 1998; Maxwell et al. 1998; Mutter 2000a). In the present study, PTEN gene mutation was seen in 19 of 56 cases (34%). Our data showed that PTEN expression was decreased in G1 more than in G3, endometrial hyperplasia and proliferative phase endometrium. There are reports that PTEN gene mutation was detected in well-differentiated carcinomas, including brain tumors (Sano et al. 1999; Steck et al. 1997), and carcinomas of the prostate (Girl and Ittamann 1999), breast (Perren et al. 1999), and thyroid (Gimm et al. 2000).

No correlation between PTEN gene mutation and PTEN protein expression was observed in our study and there was also no difference when examined depending on each histological grade. The reason for this may be that the PTEN gene is frequently mutated as a frame shift in the phosphatase domain (Hinoda et al. 1998;

Table 5 The correlation between PTEN staining score, and estrogen and progesterone receptor expression in endometrioid adenocarcinoma of the uterine corpus

	f mol/mg protein	No.of cases	PTEN staining score Mean ± SD	P-value	Grade	No.of cases	PTEN staining score Mean ± SD	P-value	
ER	High(≧50)	18	6.5 ± 5.3	0.0241*	G1	17	6.2 ± 5.2	0.07	N.S.
					G2	0	-		
					G3	1	12.0		
	Low(<50)	77	9.5 ± 4.9		G1	39	8.6 ± 5.0		
					G2	19	9.7 ± 5.4		
					G3	19	11.2 ± 3.8		
PR	High(≧100)	22	6.6 ± 5.5	0.0256*	G1	18	5.6 ± 4.8	0.026*	N.S.
					G2	2	4.0		
					G3	2	14.0		
	Low(<100)	72	9.7 ± 4.8		G1	37	9.0 ± 5.1		
					G2	17	9.8 ± 5.2		
					G3	18	10.9 ± 3.7		

*$p < 0.05$; significant, N.S.; not significant, Mann-Whitney U test

Maehama and Dixon 2000; Parsons 1998; Tamura et al. 1999), whereas the epitope recognized by the antibody that was used in this study was located around 200 amino acids from the C-terminus. Therefore, cases of cancers with PTEN gene mutation might not have been detected by immunohistochemical staining. At least some PTEN gene mutations are not expected to be detected by this antibody.

In our study, high expression of PTEN protein was observed in G3 endometrial carcinomas, and was significantly correlated with the LIs of cell cycle regulators such as Ki-67, cdk2, cyclin A, cyclin D1, and cyclin E. We have demonstrated that these cell cycle regulators were positively correlated with histological grade of endometrial adenocarcinoma (Watanabe et al. 2003). It has also been reported that the high expression of cell cycle regulators occurred in poorly differentiated cancers (Sherr 1996; Weng et al. 2001). Therefore, it has been suggested that PTEN protein is expressed as a negative feedback response to control cellular overgrowth (Campbell et al. 2001; Kato et al. 1998).

PTEN expression was not significantly associated with clinicopathological parameters that we examined. However, as it was correlated with cell cycle regulators indicating higher proliferative activity, it will be necessary to follow these patients for a longer period to evaluate PTEN expression as a prognostic factor.

In the present study, PTEN expression was decreased in well-differentiated adenocarcinoma and wild type p53, high ER, and PR groups. It is known that p53 mutation is a late event in endometrial carcinogenesis (Kohler et al. 1992) and expression of both ER and PR is decreased or abolished in poorly differentiated endometrial cancer (Ohtani et al. 1999). This may indicate that decreased PTEN expression is involved in the early stage of carcinogenesis of the endometrium. PTEN expression was high in poorly differentiated cancers. This suggests that PTEN protein may have been induced to inhibit the aggressive growth of the poorly differentiated carcinomas, whereas in well-differentiated cancers PTEN may have been expressed at a low level. It is likely that in poorly differentiated cancers, the mutation of more critical genes than the PTEN gene such as the p53 gene are involved in the acquisition of more aggressive malignancy.

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特　集　婦人科癌化学療法　新しい展開

子宮頸癌に対する手術前化学療法(NAC)は予後改善に有効か？

Dose neoadjuvant chemotherapy followed by surgery give the impact on survival of advanced cervical cancer patients?

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Friedlanderら[1]により子宮頸癌の局所進行例に対して主治療たる手術や放射線治療に先行した形で行う化学療法 neoadjuvant chemotherapy (NAC) が導入されて20年が経とうとしている．現在までに，放射線治療に先立って行われるNACに治療的意義が乏しいことは，randomized studyを含めた多くの報告のほぼ一致した見解となっている．一方，手術に先立って行われる「術前NAC」は原発病巣に対して70%をこえる高い奏効率を示し，手術適応例を増加させることができるだけでなく，リンパ節転移などの微小転移巣に対してもある程度の効果が期待できる．しかし，これがはたして患者の長期予後を向上させているのかについての明確な答えは得られてはいない．その原因の一つとして，NACがsurgical stagingの前に行われるために，「どのような病期の，どのような病態をNACで治療しているのか？」という常に投げかけられる疑問がその評価を複雑にしているためと思われる．本稿では手術を前提として行われる術前NACをめぐる最近の動向とその予後向上への意義についてレビューする．

Key Words　子宮頸癌，手術前化学療法（NAC），化学療法の奏効率，予後

NACに用いられるレジメンと奏効率

シスプラチンをkey drugとして，ほかのいくつかの薬剤と組み合わせた併用療法が多く用いられている[2)12)]（表1）[13)]．ほとんどのレジメンにより70%以上の高い一次奏効率が得られており，子宮頸癌が化学療法に感受性の高い固形癌であることをあらためて認識させられる．このような高い一次効果に永続性はないとしても，再発例に対する化学療法の奏効率がたかだか30%に過ぎないことを考えると，初回治療として有効性の高い化学療法を用い，手術へと導入する治療過程は集学的治療の観点からも魅力的である．代表的なNACレジメンであるBOMP療法[14)]のプロトコールを表2に示した．最近では，後述するようにpaclitaxel，irinotecan，gemcitabineなども導入されつつあり，やはり高い奏効率が示されている．

NACの投与法と期間

NACの薬剤投与ルートとして，静脈内投与（静注）と動脈内投与（動注）とが行われている．動注は薬剤の腫瘍内濃度を上げて，しかも副作用を軽減できるとされるが[15)]，手技が煩雑である．欧米では静注が主流であり，動注を主流としてきた日本においても最近は静注が用いられるようになってきた．

NACの投与方法はweeklyから21日周期までさまざまで，その期間も1ヵ月間の短期から3ヵ月

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表1 進行子宮頸癌に対するシスプラチンをkey drugとした術前NACの有効性（Gadducci A. et al. 2001[13]より改変）

著者	文献	患者数	Chemotherapy regimen	奏効率	CR率
Dottino	2	28	CDDP + VCR + MIT + BLM	100%	35%
Leone	3	56	CDDP + IFO	54%	7%
Benedetti-Panici	4	75	CDDP + BLM + MTX	83%	15%
Benedetti-Panici	5	26	CDDP + BLM	88%	19%
Bolis	6	79	CDDP + IFO	69.6%	5.1%
Marth	7	15	CDDP + 5 − FU	93%	27%
Sugiyama	8	23	CDDP + CTP −11	78%	13%
Lai	9	59	CDDP + VCR + BLM	81.4%	18.6%
Serur	10	20	CDDP + MTX + BLM or CDDP + VCR + BLM	90%	10%
Colombo	11	100	CDDP + VCR + BLM	96%	15%
Pignata	12	27	CDDP + VNL	81.5%	25.9%

CDDP, cisplatin ; VCR, vincristine ; MIT, mitomycin-C ; BLM, bleomycin ; IFO, ifosfamide ; MTX, methotrexate ; EPI, epirubicin ; CLB, chlorambucil ; 5-FU, 5-fluorouracil ; CTP-11, irinotecan ; VNL, vinorelbin.
Studies assessing neodjuvant chemotherapy before surgery.

表2 BOMP療法

	day 1	2	3	4	5
BLM (7mg/m²)	↓	↓	↓	↓	↓
VCR (0.7mg/m²)					↓
MMC (7mg/m²)					↓
CDDP (50mg/m²)					↓

BLM：ブレオマイシン　VCR：ビンクリスチン
MMC：マイトマイシンC　CDDP：シスプラチン
上記療法を3～4週ごとに施行

間投与まで行われ，標準的プロトコールはない．しかし，NACの施行期間はその意義を何に求めるかに関わる重要な問題である．NACには大きく二つの臨床的意義が期待されている．一つは，手術適応を目指した局所的な原発病巣の縮小効果であり，今一つはリンパ節転移などの微小転移巣への全身的効果である．局所的効果を第一義的に考えるなら，手術可能な腫瘍縮小効果が得られ次第に化学療法を打ち切るべきであるが，あわせて全身的効果をも期待するのなら，CRを目指して長期に行われるべきであろう．リンパ節転移などの微小病巣への効果は化学療法のサイクル数との相関が推定されているからである[16]．

しかし，NACが主治療たる手術への導入療法である以上，これが有効でない場合には手術療法への早急な切り替えが必要であるし，放射線療法への移行も早い方がよい．すなわち，NACレジメンとしては原発局所に対する奏効率が高く，しかも効果の発現が迅速であることが望まれる．NACに関する先駆的報告を行っているSardiら[17]が“quick VBP”と名づけた短期NACはその代表的レジメンといえる．そのプロトコールはCDDP + vincristine (VCR) + bleomycin (BLM) を10日間隔で3コース施行するものである（表3）．ほかの短期NACとしては，CDDP (50mg/m², day1) + VCR (1mg/m², day1) + BLM (25mg/m², day1) をweeklyで3コース施行する方法[18]などが報告されている．いずれの場合にも，NAC期間（約30～40日間）の終了後2～3週間以内に手術療法が施行されている．

われわれもirinotecan (CPT-11) + mitomycin C (MMC) による短期NAC[19]（表4）を試みている．

表3 Quick VBP 療法

		day	1	2	3	
VCR	(1 mg/m²)		↓			15分
BLM	(25mg/m²)		↓	↓	↓	6時間
CDDP	(50mg/m²)		↓			15分

VCR：ビンクリスチン　BLM：ブレオマイシン
CDDP：シスプラチン
上記療法を10日間隔で3コース施行

表4 CPT-11+MMC 療法

			1コース				2コース		
		day	1	8	15		29	36	43
CPT-11	(100mg/m²)		↓	↓	↓	休薬	↓	↓	↓
MMC	(10mg/m²)								↓

CPT-11：イリノテカン　MMC：マイトマイシンC
上記療法を4週ごとに施行

このレジメンを短期術前NACに導入した理由は，

①効果発現が迅速で，とくにNACではわずか1コース/1ヵ月で65%の高い奏効率が得られる，

②腎不全をきたした進行子宮頸癌患者にも適応できる，

③手術を待つ患者の精神的QOLのため，

などである．しかし，子宮頸癌に対するkey drugであるCDDPを含まないことから初回治療としてのNACレジメンとしては問題が残り，今後の検討が待たれる．

術前NACが長期予後に与える影響を検討したnon-randomized study

Serurら[20]によるコホート研究では，頸部扁平上皮癌stage Ⅰb2を対象に，NAC＋根治術群（20人）と根治術単独群（32人）が比較された．NACはCDDP＋BLM＋MTXあるいはVBP療法を用いた．NACの奏効率は90%で，腫瘍径の大きい症例がNAC群に多く含まれていたにもかかわらず，5年生存率はNAC群80%，根治術群69%で有意差があったと報告している．Benedetti-Paniciら[21]は128人の局所進行した頸部扁平上皮癌に対してNAC＋根治術を行った結果，10年生存率はstage Ⅰb2～Ⅱa bulky：91%，Ⅱb：80%，Ⅲ：34.5%であり，標準的治療法を行った群よりも予後良好であった．Hwangら[22]は80人の腫瘍径4 cm以上の頸癌stage Ⅰb～Ⅱbに対してNAC（BVP療法）＋根治術＋RTを施行した結果，5年，10年無病生存率はそれぞれ82%，79.4%と良好な予後であったと報告している．

以上の論文をはじめ多くのnon-randomized studyがいずれも術前NACが予後向上をもたらす可能性を示唆してはいるが，どの論文でも最後の文章はいつも「この結果は大規模randomized studyにより裏づけられる必要がある」と結ばれている．

術前NACに関するrandomized study

NACの有効性を評価したrandomized studyはきわめて少ない．アルゼンチンのSardiら[23]は，309人の頸部扁平上皮癌stage Ⅱbを次の4群に分けてrandomized studyを行った．①放射線治療（RT）群（体外50Gy＋腔内照射），②根治術＋RT群，③NAC（quick VBPx3コース）＋RT群，④NAC＋根治術群．その結果，84ヵ月の平均観察期間後の生存率は，①群48%，②群41%，③群：54%，④群65%であった．NACを含んだ③④群と他群との間に有意差はなかったが，④群と②群の間と④群と①群の間には有意差があった．手術完遂率は④群80%，②群56%であった．結論として，NACにより予後は向上し，手術時のリスク因子である傍結合織浸潤，脈管侵襲，リンパ節転移などを減少させることができるとした．

Changら[24]も頸部扁平上皮癌stage Ⅰb2，Ⅱaを対象に，前述のSardiら[17]とまったく同様のNAC（quick VBP）を3コースの後に根治術を行ったNAC群68例とRT単独群52例との間でrandomized studyを行った．NAC後の手術でリンパ節転移などのリスク因子が確認された症例（28%）のみが補助放射線療法を受けた．その結果，中央値39ヵ月の観察期間で，2年生存率はNAC群81%とRT群84%，5年生存率はNAC群70%とRT群61%で，ともに有意差を認めなかった．

Benedetti-Panici ら[25]によるrandomized studyは頸部扁平上皮癌stage Ⅰb2～Ⅲに対してCDDPをベースとしたNACの後に根治術を行ったNAC群（160名）と，体外照射と腔内照射を行ったRT群（143名）を比較した第3相試験である．NACのレジメンは一定したものではなく，総投与量が240mg/m² 以上のCDDPを含んだ多剤併用療法であることを必要条件とした．術後のリスク因子に対する補助療法の（化学療法，RT，無治療など）の選択は主治医のポリシーに委ねられた．その結果，全体の5年生存率はNAC群56.5%とRT群44.4%で有意差があった．また，臨床期別の5年生存率で見ると，stage Ⅰb2～ⅡaではNAC群68.9%とRT群50.7%で有意差があったが，stage ⅡbではNAC群58.6%とRT群56.5%で有意差なし，stage Ⅲでもそれぞれ NAC群41.6%とRT群36.7%で有意差なしであった．

Napolitano ら[26]は頸部扁平上皮癌stage Ⅰb～Ⅲbに対して，NAC（VBP×3コース）＋根治術群102人と根治術単独群（C群）64人の間でのrandomized studyを行った．術後の病理学的リスク因子があった場合には放射線治療が追加されている．その結果，5年生存率は，stage Ⅰb～ⅡaではNAC群78.6%とC群：73.2%で有意差なし，stage ⅡbでもNAC群68.7%とC群64.3%で有意差なしであったが，5年無病生存率で見ると，stage Ⅰb～ⅡaがNAC群77.1%とC群64.3%で有意差あったが，stage ⅡbではNAC群56.2%とC群57.1%で有意差はなかった．結論はNACにより多くの患者が手術可能となりその予後を向上させたとした．

腫瘍サイズはNACの効果に影響するか？

以上のrandomized studyでは，いずれも80%を超えるNACの高い一次奏効率が得られてはいる．しかし5年生存率では，NAC＋根治術群がRT単独群や根治術＋RT群に比べてやや優れている傾向にはあるものの，明らかな有意差が示されているわけではない．ここで興味深いことは，Sardi ら[23]が腫瘍サイズの大きい進行例ほどNAC＋根治術の有効性が高いとしているのに対して，Benedetti-Panici ら[25]とNapolitano ら[26]は腫瘍サイズの小さい早期例に対するほど有効性が高い，と相反する結果となっていることである．最近のHuang らの報告[27]でも5cm以上の腫瘍サイズは術前NAC療法のリスク因子であるとしている．Sardi ら[23]だけがNAC＋根治術群の全症例に対して補助放射線療法を行っている点がその原因となっているかは判然としない．はたしてNACがどのような臨床進行期や腫瘍サイズの頸癌に対してより有効であるのかは最も重要な今後の検討課題である．

NAC後の縮小手術の是非は？

NACにより著明な腫瘍縮小効果が得られた（down staging）場合には，手術は完遂度を増し，局所制御と根治性を高めることができることには十分なコンセンサスが得られている．すなわち，NACによりⅢb期がⅡb期とdown stageして広範子宮全摘術が可能となったり，Ⅱb期がⅠa～Ⅰb期となって準広範術式で切除可能となることが示されている[28]．しかし，現時点でのNACの主たる目的は，手術への適応例を増加させることや，手術の根治性を高めることにあり，縮小手術を可能とすることにはないと思われる．何故なら，NACのリスク因子への影響は広範子宮全摘術を行ってはじめて確認できるからである．とくにリンパ郭清術に関しては，NACがどの程度までリンパ節転移巣を消滅させるかが分からない以上，リンパ節郭清を省略できるというエビデンスは得られない．

NACはリンパ節転移を減少させるか？

最近の画像診断技術の進歩をもってしても，治療前にリンパ節転移の有無を正確に評価することは困難であり，生検を行わない限り微小転移巣の判定はできない．さらに，化学療法により消失したリンパ節転移巣を術後の病理所見で証明するこ

とも容易ではない．したがって，NACがリンパ節転移巣に与える影響についての客観的評価は難しいが，NACが骨盤内リンパ節転移の陽性率を減少させたとする多くの報告がある．それらをまとめると，NAC後の骨盤内リンパ節郭清により確認された転移陽性率は，Ⅰb2～Ⅱb期10～25%，Ⅲb期30～50%で，NAC前のそれぞれの臨床期から推定される陽性率よりも低いと報告されている[29)～32)]．

腫瘍のリンパ節への転移には，臨床期，原発巣の腫瘍サイズ，腫瘍の分化度などの関与が指摘されているが，NAC後においてもリンパ節転移の陽性率は治療前の腫瘍サイズと相関することが報告されている．Giaroliら[33)]は，頸部扁平上皮癌（stage Ⅰb bulky～Ⅲ）に対して，NAC（modified VBP）を行った後の骨盤内リンパ節転移の陽性率を調べた．その結果，リンパ節転移陽性率は腫瘍径が3cmを下回る症例で9%，3～4cmで10%，4～5cmでは25%であった．一方，5cmを上回る症例では60%の高い陽性率であったが，NACにより3cmとなった場合には14%に低下した．また，NACによりCRとなった56例中ではリンパ節転移陽性はわずか1例のみであったのに対して，stable diseaseであった36人中24人（66.7%）が陽性であったことから，NAC後の手術におけるリンパ節転移の陽性率や個数は，NAC前の腫瘍サイズに比べ化学療法に対する感受性に依存する可能性がより高いことを示唆した．また，予後的にもリンパ節転移が陰性であった場合の2年無病生存率は89.2%であったのに対して，1～2個では約70%，3個以上ではわずか25%と大きな有意差があった．以上の結果から，NAC後に残存腫瘍径が2cm以下になり，リンパ節転移陰性かつ傍結合織陰性のものは手術により最良の予後が得られるが，残存腫瘍径2cm以上でリンパ節転移が陽性であれば傍結合織浸潤はどうあれ，きわめて予後不良であると結論づけた．

以上のことから，NACがどの程度のリンパ節転移を消滅させているか，またそのことが長期予後の改善に寄与しているかについては具体的に示されてはいないものの，大きなリスク因子であるリンパ節転移の陽性率をNACが減少させていることが事実なら，十分に意義深いことと思われる．しかし，このことはNACがより完全なリンパ節郭清を可能にするということであり，これを省略できることを意味せず，予後の推定や向上のために必要な手術操作であることに変わりはない．

NACを先行させた手術後の補助療法は必要か？

手術後の病理検索により，傍結合織浸潤，高度の間質浸潤や脈管侵襲，切除断端陽性，リンパ節転移などのリスク因子が認められれば，NACの有無によらず補助療法としての放射線療法あるいは化学療法の追加が通常行われている．しかし，問題はNAC前には存在したと推定されるリスク因子が手術後には確認されなかった場合である．つまり，術前NACによりリスク因子が消失したと考えられる場合の補助療法をどうするかは難しい判断である．Sardiら[17)]のプロトコールでは，術後の病理所見がどうあろうと全例に対して放射線療法が追加されている．一方，Napolitanoら[26)]のプロトコールでは，リスク因子の認められた症例に対してのみ放射線療法が施行されている．予後的には，前者の方が良好な生存率を報告しているが，過剰治療の可能性も危惧される．また，NAC奏効例に対しては，術後に同じ化学療法を追加する試み[34)]も報告されている．

現時点では，NAC前の進行期に対応して術後の補助療法が行われることが標準的であり，ほとんどの場合で放射線治療が選択されている．また，これに化学療法を併用するか否かは今後の検討課題である．

頸部腺癌に対する術前NAC

前述してきたNACの成績のほとんどが頸部扁平上皮癌に対するものであり，頸部腺癌に対する術前NACに関する報告は少ない．頸部腺癌の予後は不良であることから，化学療法が期待されて

いるが，NACとしての奏効率は扁平上皮癌に比べて同等か低いことが報告されている．Paniciら[35]は42例の頸部腺癌stage Ⅰb2～Ⅲに対してNACを施行し，79%（33例）の奏効率（CR7%）を得た．33例中29例で根治的手術が可能となり，術後の病理所見ではCR7%，PR57%で，骨盤内リンパ転移率は15%であった．その結果，NAC奏効例の5年生存率は84%と扁平上皮癌の場合と同程度に良好であった．Zanettaら[36]は21人の進行頸部腺癌に対して，CDDP（50mg/m², weekly）＋epirubicin（70mg/m², every 3 weeks）を施行し，奏効率67%（CR19%）であった．82%が手術を受けたが，病理学的CRはなかった．Iwasakaら[37]は16例の頸部腺癌stage IB-IVに対して，CDDP＋MMC＋etoposideによるNACをおこなったが，50%の奏効率（CR19%）であったと報告している．

ほかの報告[38]を含め，頸部腺癌に対する現行のNACには扁平上皮癌に対するほどの有効性はないというのが現時点での一般的見解であると思われる．予後も不良であることから化学療法に過大な期待をかけずできるだけ迅速な手術が望ましいと考えられる．

日本におけるNACの多施設共同研究

本邦でも，婦人科がん化学療法共同研究会（JGOG）において1991年から1997年まで頸部扁平上皮癌stage Ⅱに対して，術前NAC群（34例）と手術単独群（22例）の間で封筒法によるpilot studyが行われた．NAC群ではBOMP療法2コースの後，広範子宮全摘術が施行された．また，両群ともに病理学的リスク因子陽性の症例に対しては放射線治療が追加されている．その結果，NACの奏効率は61%（CR9%）であり，間質浸潤と傍結合織浸潤はNAC群で有意に低率であったが，リンパ節転移率には有意差は認められなかった．ところが5年生存率を見ると，手術群の90%に対してNAC群は67%と有意に低率となった．このように期待を裏切る結果となった理由として，症例割りつけを封筒法としたために，より重篤な症例が主治医により恣意的にNAC群に割りつけられた可能性が高い．とはいえ，この術前NAC療法に大きな予後改善は望めぬと判断され，臨床試験は中止された．

現在，日本臨床腫瘍研究グループ（JCOG）により，頸部扁平上皮癌stage Ⅰ（bulky）/stage Ⅱに対して，術前NAC（BOMP）群と根治術群の間でrandomized studyが進行中である．両群ともに術後補助療法として放射線療法が追加されるプロトコールである．世界でも数少ないNACのrandomized studyであり，多くの症例登録が期待される．

NACに関する米国GOGトライアル

米国GOGではNACに関するrandomized studyは行われていないが，1995年にpilot studyとして，頸部扁平上皮癌stage Ib bulkyに対して術前NAC（CDDP＋VCR）3コースを行って82%の高い奏効率を発表した[39]．ところが，米国では局所進行頸癌に対しては放射線療法が主たる治療法となっているうえに，おりしも公表されたconcurrent chemoradiationの良好な成績から，これがNCIアナウンスメントにより推奨されるに及んで，術前NACに関する臨床試験は中断されたままとなっている．

新しいNACレジメン

最近，タキサン類を頸癌に対する術前NACに導入し，高い奏効率が示されている．Zanettaら[40]らは，CDDP（50mg/m², day1）＋ifosfamide（5 g/m², day1）＋paclitaxel（175mg/m², day 1）からなるNAC（every 21days, 3cycle）を38人の頸部扁平上皮癌stage Ⅰb2～Ⅳaに施行し，奏効率84%（CR29%）が得られている．さらに根治術を施行した結果，16%が病理学的CR，18%に微小浸潤癌の残存と，高い病理学的効果が確認された．

Duenas-Gonzalez ら[30] は43人の頸部扁平上皮癌（腺癌を含む）stage Ⅰb2～Ⅲbに対して CBDCA (AUC6) ＋paclitaxel（175mg/m²）によるNACを3コースの後，根治術を行い，術後補助療法として6週ごとのCDDP 40mg/m²による chemoradiation を施行した．NACの奏効率は95%（CR 9%）と高く，手術による病理所見では，CR17%，nearly CR20%であった．切除端陽性は12%，骨盤リンパ節陽性は20%であり，26人が術後放射線治療を受けた．

以上のように，paclitaxel を含んだNACレジメンは高い一次効果を示してはいるが，それが長期予後の改善につながるかは今後の検討を待たねばならない．

NACは長期予後を改善させているか？

NACの最終ゴールが予後の向上にあるのは勿論である．NACに感受性があり，根治術が可能であった頸癌患者の予後がもっとも良好であることは多くの報告の示すところである．しかし，化学療法に感受性の乏しい症例や過去の症例との比較で予後を評価することは誤った結論を導く可能性がある．なぜなら，化学療法に対して不応性の頸癌患者は手術や放射線療法によってもまた本質的に難治性であることは十分にあり得るからである．したがって，NACの正しい評価には大規模な第3相 randomized study が必須であるが，これがほとんど行われてこなかったのは述べてきた通りである．最近，英国で行われたNACに関するメタアナリシス[41] によると，NACがstage Ⅰb/Ⅱa bulky，Ⅱbに対して高い臨床的，病理学的な奏効率を示し，手術適応例を増やすことが明らかとなったものの，残念ながら2～3年の生存率でみた場合にはNAC群と標準的治療群の間に有意差を見出してはいない．対象論文数があまりに少ないとしながらも，現時点で予後向上の面においてNACが標準的治療に優るエビデンスはないとしている．

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Phase I study of daily cisplatin and concurrent radiotherapy in patients with cervical carcinoma

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Abstract

Objective. Chemoradiation based on cisplatin is the standard treatment for locally advanced cervical carcinoma; however, the optimal scheduling and dosing have still not been established. This study was conducted to determine the maximum-tolerated dose (MTD) of cisplatin for daily administration during pelvic radiotherapy (RT).

Methods. Fourteen patients with locally advanced cervical carcinoma and 13 who required postoperative RT were registered. A low dose of cisplatin was given daily concurrently with RT. Cisplatin dosing was started at 6.0 mg/m^2/day, which was incremented by 0.5 mg/m^2/day. RT was delivered at 2 Gy/day to a total dose of 50 Gy. The MTD was defined as the dose level immediately below that causing dose-limiting toxicity (DLT) in over one-third of treated patients.

Results. Twenty-five patients were treated with a maximum of six escalating dose levels. In 22/25 patients (88%), cisplatin was administered continuously as planned without interruption. The MTD was determined to be 8 mg/m^2 and the DLT was indicated by the onset of neutropenia.

Conclusion. Daily cisplatin, at 8 mg/m^2/day, is a well-tolerated radiosensitizer in cervical carcinoma patients.

Keywords: Cervical carcinoma; Phase I; Cisplatin; Chemoradiation

Introduction

Cervical carcinoma is the most frequent cause of death by cancer in women worldwide [1]. Radiation therapy is considered to be the gold standard of treatment for stage IIB–IVA patients. Recently, several phase III studies showed that concurrent chemoradiation could improve outcomes more than radiotherapy alone [2–6]. Cisplatin and cisplatin plus 5-fluorouracil have been the two most common radiosensitizer regimens used in cervical cancer. However, the Gynecologic Oncology Group 120 study showed that 40 mg/m^2 of cisplatin weekly for 6 weeks was as effective as, yet less toxic than, a combination of cisplatin plus 5-fluorouracil. Thus, weekly 40 mg/m^2 cisplatin with concurrent radiotherapy seems to have the better therapeutic ratio [5]. Although the new paradigm of cisplatin-based concurrent chemoradiotherapy is a step forward, questions remain regarding optimal scheduling, dosing, and systemic agents.

In non-small-cell lung cancer, phase III studies demonstrated that radiotherapy combined with daily administration of 6 mg/m^2 cisplatin offered improved local control and improved actuarial survival in comparison with the radiation alone group (significantly) and the weekly administration group (not significantly) in inoperable patients [7]. Several

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authors who combined high-dose radiotherapy with 6 mg/m^2 cisplatin daily did not observe either renal or severe hematological toxicity in head, neck, or non-small-cell lung cancers [8–11].

On these grounds, we thought that daily administration of cisplatin was as effective as weekly administration given concurrently with pelvic radiation in patients with cervical carcinoma. We also initiated a phase I study to evaluate the maximum tolerated dose of daily cisplatin given concurrently with pelvic radiation to patients with cervical carcinoma.

Methods

Patient selection

Fourteen patients with locally advanced cervical carcinoma and 13 who required postoperative RT were entered in this study. Eligibility criteria for postoperative radiation included the presence of at least one of the following: positive pelvic lymph node metastasis, a positive surgical margin, deep stromal invasion, and parametrium invasion. Patients with either disease outside the pelvis or para-aortic lymph node swelling were not eligible. The following were the other inclusion criteria: (1) aged ≤ 75 years; (2) ECOG performance status ≤ 2; (3) no previous chemotherapy or radiotherapy; (4) leukocytes ≥ 3000/mm^3; (5) neutrophils ≥ 2000/mm^3; (6) platelets ≥ 100.000/mm^3; (7) serum creatinine ≤ 1.5 mg/dl; (8) normal chest radiograph and electrocardiogram; and (9) informed consent.

This study was approved by the Institutional Review Board of Chiba University.

Radiotherapy

Patients were treated with 10 MV X-rays from a linear accelerator using four-field box technique, with the fields encompassing the whole pelvis extending from the lower margin of the obturator foramen to the upper margin of the fifth lumbar vertebra, and laterally to at least 1.5 cm outside of the true pelvis. Anterior and posterior borders of lateral fields were carefully determined based on the pretreatment diagnostic imaging such as CT and MRI, with an adequate coverage of the pelvic lymph node area and the primary tumor bed. Typically, the anterior margin was placed just anterior to the symphysis pubis and the posterior margin included the anterior aspect of the entire sacrum. A CT-simulator with three-dimensional treatment planning system was used for all patients.

No attempt was made to irradiate the para-aortic lymph node region. A total dose of 50 Gy was delivered in 25 daily fractions of 2.0 Gy, administered on 5 days a week (from Monday to Friday). All fields were treated each day. Low dose-rate brachytherapy was applied for curative cases 1–2 weeks after the end of external-beam radiotherapy. Brachytherapy was not performed in the adjuvant setting.

Chemotherapy

Each dose of cisplatin was administered i.v. over 30 min, and was completed 1 h before irradiation. The daily dose of cisplatin was reconstituted in 100 ml of normal saline. All patients received 5 mg of granisetron 1 h before cisplatin to prevent emesis. Post-cisplatin hydration was performed with 1 L of normal saline given over 2 h.

Study design

A phase I study was designed to define the MTD of daily cisplatin and pelvic radiotherapy. The starting dose of cisplatin was 6 mg/m^2/day and increments of 0.5 mg/m^2/day were planned at each level until DLT occurred. The MTD was defined as the highest safely tolerated dose with toxicity levels that did not exceed the DLT. DLT was defined as grade 3 or 4 neutropenia or thrombocytopenia and grade 3 or 4 nonhematologic toxicity except for alopecia, nausea, and vomiting. Toxicity was evaluated according to National Cancer Institute common toxicity criteria and the Radiation Therapy Oncology Group toxicity criteria. Cisplatin was suspended if grade ≥3 toxicity appeared, and was resumed once the counts rose above grade 3 levels at the dose level below that which produced DLT. Radiotherapy was suspended if grade 4 hematological toxicity appeared or in the event of grade 4 radiation-related gastrointestinal or genitourinary toxicity, and treatment was resumed once the counts rose above those levels.

The dose was escalated to the next level if none of the patients experienced DLT. If the incidence of DLT was >33% (seen in 2 or 3) at a given dose level, then dose escalation was stopped. If one of three patients at any level developed treatment-related DLT, three additional patients were then treated at the same dose level. The MTD was defined as the dose level below that which produced DLT in more than one-third of the treated patients. If DLT appeared in only one or two of the six patients, the dose was escalated to the next level.

Laboratory studies, including chemistry panels and a complete blood cell count, were obtained twice weekly, or more frequently if clinically indicated.

Chemoradiation with weekly cisplatin

From December 1999 to March 2002, 10 patients with cervical carcinoma, stages IIB–IIIB, were treated with five weekly courses of cisplatin 40 mg/m^2 during standard pelvic radiation. Radiation was administered according to the same schedule as daily cisplatin. Cisplatin was withheld in any case of grade 3 toxicity (except nausea/vomiting) until the toxicity regressed to

less than grade 3. If grade 3 neutropenia appeared, G-CSF was administered.

Results

Between April 2002 and December 2003, a total of 27 patients were enrolled in the study. (Table 1). The mean age was 51.0 (range 29–71) years. The mean BMI was 24.1 (range 16.8–30.6). Two were not eligible because of non-dose-related toxicity (grade 2 nausea/vomiting) and were refused chemotherapy (2× and 15× cisplatin). Twenty-five patients were evaluable for toxicity analysis. Six dose levels were studied (Table 2). DLT was observed in six patients: in two patients at level 3, in one at level 5 and in three at level 6 (Table 3). Thus, the MTD of daily cisplatin was defined 8 mg/m^2/day.

In 22/25 patients (88%), daily cisplatin could be administered continuously as planned with no interruption. Cisplatin administration had to be interrupted in only two patients and terminated in only one.

Hematological toxicity was mild overall. As shown in Table 3, grade 3 or 4 leukopenia or neutropenia was recorded in nine cases (including five after treatment). Only one patient was treated with G-CSF because of grade 4 leukopenia (level 6); in no case was febrile neutropenia recorded. Grade 3 thrombocytopenia was observed in one patient after treatment. No grade 3 nonhematological toxicity was seen. Four patients observed grade 2 nausea and vomiting. Grade 1 and 2 diarrhea was frequent, being recorded in almost all patients. But no grade 3 diarrhea was not found. No late toxic event was observed during follow-up of patients. There was no correlation between BMI and side effects.

Fourteen patients were receiving primary treatment and were evaluated for response. Thirteen patients achieved responses: 11 (78.6%) complete responses and 2 (14.3%) partial. At the median follow-up period of 14.2 months (range 7–26), one patient with progression had died of the disease, and two patients suffered relapses at sites outside radiation field.

Table 1
Patient characteristics

Number of patients	27
Age(years)	
Mean	51.0
Range	29–71
BMI	
Mean	24.1
Range	16.8–30.6
Histology	
Squamous	19 (8)[a]
Adeno	6 (4)[a]
Small cell	1 (1)[a]
Carcinosarcoma	1 (0)[a]
Stage	
IB	6 (6)[a]
IIB	9 (7)[a]
IIIB	11 (0)[a]
IVA	1 (0)[a]
Radiation	
Adjuvant	13
Primary therapy	14

[a] Parentheses indicate members of the adjuvant group.

Table 2
Toxicity and dose levels

Toxicity	Dose levels of cisplatin (mg/m^2/day)					
	1 (n = 5)[a]	2 (n= 3)	3 (n = 7)[a]	4 (n = 3)	5 (n = 6)	6 (n = 3)
Hematological						
Leukopenia						
1	1	1	3	1	1	0
2	2	1	0	1	3	0
3	0	0	4	0	2	2
4	0	0	0	0	0	1
Neutropenia						
1	1	1	3	1	2	0
2	2	0	2	1	3	1
3	0	0	2	0	1	1
4	0	0	0	0	0	1
Thrombocytopenia						
1	2	4	6	2	4	2
2	0	0	0	0	0	0
3	0	0	0	0	0	1
4	0	0	0	0	0	0
Nonhematological						
Nausea/vomiting						
1	3	2	3	2	4	1
2	1	0	1	0	0	2
3, 4	0	0	0	0	0	0
Diarrhea						
1	1	3	2	2	4	1
2	3	0	4	1	1	2
3, 4	0	0	0	0	0	0

[a] One patient from this group was not eligible for this study.

Table 3
Dose regimens administered, toxicity, and interruption of administration

Dose level	Dose of cisplatin (mg/m^2/day)	No. of patients with DLT[a]	DLT[a]	Interruption of cisplatin administration
1	6	0/4		
2	6.5	0/3		
3	7	2/6	grade 3 neutropenia	D-21, 23,24
			grade 3 neutropenia	D-22, 24
4	7.5	0/3		
5	8	1/6	grade 3 neutropenia	7 days after treatment
6	8.5	3/3	grade 4 neutropenia	D-24
			grade 3 neutropenia	4 days after treatment
			grade 3 thrombo-cytopenia	4 days after treatment

[a] DLT: dose-limiting toxicity.

Chemoradiation with weekly cisplatin; the mean course of cisplatin was 4.2 cycle (mean total dose 168 mg). The proportion of patients who received the total course of treatment was 30%. Grade 3 and 4 hematologic toxicity was recorded in six cases (60%): Five cases of grade 3 and one of grade 4 leukopenia/neutropenia and two cases of grade 4 thrombocytopenia. Grade 3 nonhematologic toxicity occurred in one patient.

Discussion

In the present study, we sought the MTD of daily 8 mg/m^2 administration of cisplatin given concurrently with pelvic radiotherapy in patients with cervical cancer. Neutropenia was the DLT at daily cisplatin dose level of 8.5 mg/m^2.

Cisplatin-based concurrent chemoradiation was regarded as standard treatment for locally advanced cervical carcinoma. Despite the increasing use of cisplatin to exploit its powerful radiosensitizing properties, its nephrotoxicity has been recognized as its main dose-limiting feature since its early clinical trials. Therefore, another agent, namely carboplatin, was tried as a radiosensitizer [12–14]. In this study, no patient recognized any alteration of renal function. Even if the patient has a urinary tract obstruction, as long as the serum creatinine level <1.5, daily administration of cisplatin is considered to be safe. Other authors, who combined radiotherapy with 6 mg/m^2 cisplatin daily in the treatment of lung carcinoma, observed neither renal nor severe hematological toxicity [8–11].

Daily cisplatin administration led to milder adverse side effects than weekly cisplatin. Weekly cisplatin 40 mg/m^2 was accompanied with grade 3 or 4 gastrointestinal and hematological side effects, in 14% and 28.3% of patients, respectively [4]. In our study, weekly cisplatin 40 mg/m^2 caused grade 3 or 4 gastrointestinal and hematological side effects, in 10% and 60% of patients, respectively. However, only 30% of patients received the entire course of weekly cisplatin 40 mg/m^2, and a complete course of daily cisplatin 8 mg/m^2 could be administered to Japanese women. There was no phase I study of weekly cisplatin concurrent with radiotherapy in Japanese cervical carcinoma patients. We suggest that 40 mg/m^2 of cisplatin weekly is not the optimal dose for Japanese women. With daily cisplatin (≤8 mg/m^2), grade 3 gastrointestinal side effects were uncommon and 6 of 22 patients had grade 3 or 4 hematological toxicity (27.3%). We regard the daily administration of cisplatin to be more tolerable than its weekly administration. Moreover, we considered that 8 mg/m^2 could be administered daily in an outpatient situation.

Although the evaluation of response was not the primary objective of this study, the overall response rate was higher than 90%, which suggests that this treatment is clinically relevant. However, the small sample size of this phase I study precludes any conclusions about the response. The results of the present study warrant further phase II study of cervical cancer using a daily administration of 8 mg/m^2 cisplatin concurrently with pelvic radiotherapy.

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