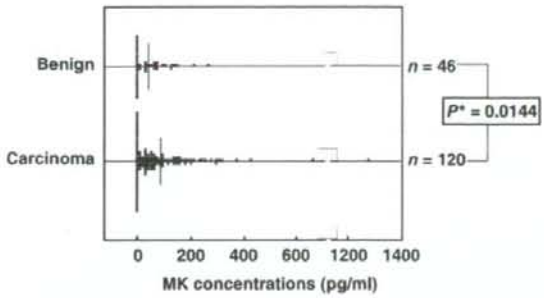

Fig. 3. Serum midkine (MK) protein concentrations from patients with benign gynecologic tumors or endometrial carcinoma. MK protein levels were measured by ELISA. The serum concentration for the carcinoma patients was significantly higher than that for the benign patients ($P = 0.014$, Mann–Whitney test).

esophageal squamous cell carcinoma, serum MK is a good marker of lymph node metastasis that correlates with serum levels of VEGF-C. Lymph node metastasis is a critical prognostic factor in endometrial carcinoma, and myometrial invasion and histological grade are correlated strongly with lymph node metastasis.[35,36] Thus, preoperative serum MK levels might prove to be useful for selecting high risk patients or predicting prognosis.

In conclusion, MK immunoreactivity in endometrial carcinoma is significantly higher than in normal endometrium. Additionally, preoperative serum MK levels are significantly correlated with prognosis and the presence of lymph node metastasis. Further, larger, prospective studies with longer follow-up periods are needed to fully understand the role of MK in endometrial carcinoma carcinogenesis.

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Expression of melatonin receptor (MT1) and interaction between melatonin and estrogen in endometrial cancer cell line

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Abstract

Aim: To determine the receptor subtypes of melatonin in estrogen receptor-positive endometrial cancer cell line, Ishikawa, and the influence of melatonin on chemosensitivity.

Methods: To confirm the subtype of melatonin on Ishikawa cells, cells were treated with melatonin alone and with antagonists against melatonin receptor luzindole and 4-phenyl-2-propionamidotetralin (4-P-PDOT). Expression of MT1/MT2 mRNA was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR). Immunocytochemistry of MT1/MT2 was also performed. The effect of melatonin against expression of MT1, MT2, and ERα-receptors mRNA was compared with RT-PCR. To determine whether melatonin enhances the effect of anticancer agents, chemosensitivity test was performed with or without melatonin.

Results: Our study revealed that Ishikawa cells express MT1 by both RT-PCR and immunocytochemistry. In contrast, expression of MT2 mRNA was not found. Furthermore, ERα mRNA expression was attenuated at melatonin level of 1×10^{-9} M. Chemosensitivity test revealed that melatonin enhanced anti-tumor effects of paclitaxel among anticancer drugs tested.

Conclusion: Based on the above results, MT1 receptor, but not MT2, is expressed in Ishikawa cells. It was also revealed that the cytostatic effect of melatonin is partly an action mediated by MT1 receptor, and attenuation of ERα expression was predicted as the mechanism of action. Clinical application of melatonin to biochemotherapy might be also expected.

Key words: endometrial cancer, estrogen, melatonin, MT1.

Introduction

Melatonin is a hormone synthesized from tryptophan in the pineal gland and has a variety of actions, such as circadian rhythm regulation, seasonal reproduction, immune mechanism integration and inhibition of tumorigenesis.[1] While the anticancer action of melatonin has been reported in a variety of solid cancers, there have been numerous reports on breast cancer, particularly on signaling mechanisms in the anticancer action of melatonin[2] and interactions between melatonin and estrogen in estrogen receptor-positive breast cancer.[3,4] Melatonin has been thought to control various second messengers in signaling mechanisms through receptors; however, although there are reports on the involvement of intracellular Ca^{2+} in mechanisms not mediated by a receptor, there is no well-established theory.[5]

Endometrial cancer, which has recently been on the increase, is classified into type 1, which occurs in younger people aged 40 years or younger; and type 2, which occurs in the elderly. Type 1 is induced by a

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persistent estrogen dominant endocrine environment due to ovulation disorder caused by hyperprolactinemia and obesity, etc.[6] Hormone therapy has been performed infrequently and its efficacy has been reported, but its side-effects, including thrombosis, and higher relapse rate have been a problem.[7] In contrast, there is no efficacy in postoperative adjuvant therapy,[8] and the response rate currently remains 25% for advanced and recurrent cancers.[9] Recently, type 1 endometrial cancer has been on the increase, and a novel therapeutic procedure for endometrial cancer is expected. Our previous reports demonstrated that melatonin has cytostatic effects on estrogen receptor-positive Ishikawa endometrial cancer cells and its mechanism of action is mediated by receptors on the cell membrane.[10,11] In the present study, analyses of melatonin receptors related to growth inhibition in Ishikawa cells and interactions of estrogen and estrogen receptors with melatonin were investigated.

Methods

Cell line and culture

Estrogen receptor-positive Ishikawa human endometrial cancer cultured cells[12] were obtained from Professor Nozawa (Keio University). Ishikawa strains were grown as a monolayer on a 90-mm diameter dish at 37°C in an atmosphere of 5% CO_2 using 10% inactivated fetal bovine serum (FBS, Hyclone, Utah, Canada) and phenol red-free RPMI1640 (Sigma-Aldrich, Steinheim, Germany) containing 1% penicillin G/streptomycin/amphotericin B-containing antibiotic (GIBCO, NY, USA). Following confluent state, 1 mL of 0.25% trypsin-EDTA (GIBCO, Grand Island, NY, USA) was added to each dish and left at 37°C for 10 min, after which the cells were separated and collected. Thereafter, the cells were suspended in culture media and centrifuged for 5 min at 190 *g* at 4°C. Cell aggregation was suspended to become uniform after precipitation, and the number of cells was measured by hemocytometer and coulter counter, and then adjusted so that the cell count was 1×10^5/dish in a 60-mm diameter dish. 10% charcoal-treated FBS (100 mL FBS, which is supplemented with FBS containing 25 mg dextran [Sigma Chemical, St Louis, MO, USA] and 250 mg charcoal [Sigma], was centrifuged at 190 *g* for 5 min after shaken at 55°C for 45 min in a thermostatic bath, then the supernatant was collected and after repeating these procedures, it was filtered through 0.45 and 0.22 μm filters) and used for serum in culture media. These were cultured at 37°C in an atmosphere of 5% CO_2 and a variety of drugs were administered after confirmation of cell adhesion (approximately 6 h later), then used for each experiment following 96 h of culture.

Influence of luzindole and 4-phenyl-2-propionamidotetralin (4-P-PDOT) on the cytostatic effect of melatonin in Ishikawa cells

Following confirmation of cell adhesion, the cells were divided into two groups: one supplemented only with 1×10^{-9} M melatonin (WAKO, Osaka, Japan) (M group); and one supplemented with melatonin and 5×10^{-6} M luziondole (MT1/MT2 antagonist [Sigma-Aldrich, Steinheim, Germany]) or 2×10^{-6} M 4P-PDOT (MT2 selective antagonist, TOCRIS, Missouri, USA) (M+LUZ group and M+4P group, respectively); and a group supplemented with 0.1 mL of 0.005% ethanol (control group). Following 96 h of culture at 37°C, cells were collected with 0.25% trypsin after washing three times with phosphate buffered saline (PBS), after which, the viable cell count was measured. Viability of cells was determined by dye exclusion test with trypan blue (GIBCO-BRL, Grand Island, USA).

Immunocytochemistry of melatonin receptor

Cultured cells on the 35-mm diameter dish were washed three times with PBS and fixed with 4% paraformaldehyde (PFA) for 30 min. Thereafter, these cells were washed three times (5 min each time) with PBS after PFA was removed, then blocked with PBS containing 3% bovine serum albumin for 1 h. Anti-MT1 antibody and anti-MT2 antibody (both of which were anti-rabbit polyclonal antibody; MBL, Japan) were used as primary antibodies to adjust each concentration to 0.4 μg/mL for anti-MT1 and 1 μg/mL for anti-MT2, respectively. Primary antibody was allowed to react at room temperature for 12 h, then washed three times (5 min each) with PBS after the antibody solution was removed. Thereafter, 2.5 μg/mL of AP-labeled anti-rabbit IgG antibody (Invitrogen, OR, USA) was used as secondary antibody and allowed to react at room temperature for 2 h. Nucleus was stained with ProLong Gold antifade reagent with DAPI (Invitrogen) and examined by fluorescence microscopy.

Detection of melatonin receptor mRNA by polymerase chain reaction (RT-PCR)

Ishikawa cells were adjusted to 1×10^5/dish on a 60-mm diameter dish. Melatonin and 17β-estradiol

were added after cell adhesion. The final concentrations of melatonin were changed to 0, 1×10^{-12}, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, and 1×10^{-6} M; whereas the final concentration of 17β-estradiol was set to 1×10^{-10} M. Cells were cultured at 37°C in an atmosphere of 5% CO_2 for 96 h, then total-RNA was extracted according to the acid guanidinium-phenol-chloroform (AGPC) method. DNase was used to remove genome DNA following quantitative determination with absorptiometer (SmartSpec 3000, BIO-RAD, Hercules, CA, USA). The RNA content of each sample was set to 500 μg. T3000 thermocycler (Biometra, Goettingen, Germany) and TaKaRa RNA PCR kit version 3.0 (TaKaRa, Siga, Japan) was used for RT-PCR following the protocol of the manufacturer. Expression of MT1, MT2, and ERα was examined at each melatonin level. PCR was performed using a set of primers for MT1 [5′ CCT GGT CAT CCT GTC GGT GTA TC 3′ (forward primer) and 5′ TGC TGC TGT ACA GTT TGT CGT ACT T 3′ (reverse primer)] and for MT2 [5′ CCA GCG CTG TCC GCG GT 3′ (forward primer) and 5′ CCA GAT GAG GCA GAT GTG CAG A 3′ (reverse primer)] and for ERα [5′GCA CCC TGA AGT CTC TGG AA 3′ (forward primer) and 5′ TGG CTA AAG TGG TGC ATG AT 3′ (reverse primer)] (OPERON Biotechnologies, Tokyo, Japan). PCR conditions were as follows: 30 cycles of 94°C for 30 s; 60°C for 30 s; and 72°C for 30 s. For MT1 and MT2, a second PCR was performed to detect band using one-tenth of the first PCR products under the same conditions. PCR products were electrophoresed on a 1.8% agarose gel containing ethidium bromide in the TAE buffer (Mupid 21; CosmoBio, Tokyo, Japan) for 25 min and the bands were detected with a UV transilluminator and photographed with a digital camera.

Influence of melatonin addition in anticancer drug susceptibility test

Experiments were performed using 24-well plates (International Frontier Technology Laboratory, Tokyo, Japan) containing TGP (themoreversible gelation polymer) in accordance with the protocol of the Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan). The details of experimental procedures have been reported[13,14] and are briefly discussed below. 300 μL of culture media was injected into each well and then the wells were allowed to stand at 37°C in an atmosphere of 5% CO_2 for 12 h. After the plates were fully cooled to 4°C, cell suspension was injected so that the number of cells were 5×10^4/well on ice, followed by 1 h of culture at 37°C in an atmosphere of 5% CO_2. 450 μL (440 μL for melatonin additive group) of culture media was added after gelatinization of medium. After 24 h of culture under the same conditions, 50 μL of each anticancer drug was added, followed by 96 h of culture under same conditions. Thereafter, 50 μg of WST-8 (2-[2-methoxyl-4-nitrophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetra-zorium, monosodium salt) and 50 μL of 0.1% sodium succinate (WAKO, Tokyo, Japan) were added, then allowed to develop at 37°C in an atmosphere of 5% CO_2 for 24 h. The optical density of each plate was measured using a microplate reader (Labsystems Multiscan MS; Dainippon Pharmaceutical, Osaka, Japan) at 450 nm after each plate was cooled to 4°C for 1 hour. IC_{50} (50% inhibitory concentration) value for each drug and peak plasma concentration (PPC) of each anticancer drug was compared, and susceptibility was defined as positive when IC_{50} was lower than PPC. The following five anticancer drugs were used in the experiment: cisplatin (CDDP [Pfizer, Tokyo, Japan]), mitomysin C (MMC) (Kyowa Hakko Kogyo, Tokyo, Japan), 5-fluorouracil (5-FU [Kyowa Hakko Kogyo, Tokyo, Japan]), paclitaxel (PTX [Bristol-Myers K.K., Tokyo, Japan]) and docetaxel (DOC [Sanofi Aventis, Tokyo, Japan]). PPC of each anticancer drug was determined with 4.98 μg/mL for CDDP, 3 μg/mL for MMC, 120 μg/mL for 5-FU, 20 μg/mL for PTX, and 2.27 μg/mL for DOC. Mean IC_{50} values of the control group and melatonin additive group were compared in the experiment, and melatonin was added at the time of administration of anticancer drug and final concentration was adjusted to 1×10^{-9} M.

Statistical analysis

Analyses were conducted three times each for all experimental systems. All results were analyzed using Statcel2 (OMS Publishing, Tokyo, Japan) Stat View version 5.0 and were expressed as mean ± SE. Mann–Whitney *U*-test was used for the analysis of cell proliferation and anticancer drug susceptibility test, and statistical significance was set at $P < 0.05$.

Results

The influence of luzindole and 4P-PDOT addition on cytostatic effect of melatonin in Ishikawa cells is shown in Figure 1. The viable cell count was significantly decreased in the M group compared to the control group; however, the antiproliferative effect of melatonin disappeared even after the addition of luzindole. In contrast, the cytostatic effect of melatonin did not

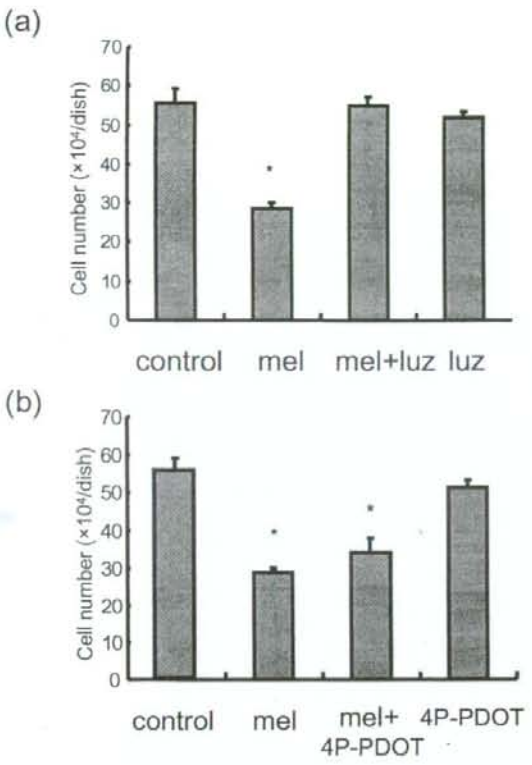

Figure 1 To monitor receptor antagonist induced cell proliferation, Ishikawa cells were incubated with 1×10^{-9} M melatonin (mel) and luzindole (luz), an MT1/MT2 antagonist (A), or 4-phenyl-2-propionamidotetraline: 4-P-PDOT(4P), an MT2 selective antagonist (B), for 96 h. Data are shown as mean ± SE. $^{*}P < 0.05$ compared with control.

disappear due to the addition of 4P-PDOT. Thus, the cytostatic effect of melatonin is mediated by MT1 receptors, suggesting no involvement of MT2.

The immunocytochemical staining tests of MT1 and MT2 yield positive result for MT1 and negative result for MT2 (Fig. 2).

Analyses of MT1- and MT2-receptor mRNA by RT-PCR demonstrated that expression of MT1 receptor mRNA was seen, but expression of MT2 was not seen, regardless of addition of melatonin (Fig. 3a). In addition, studies looking for the effect on expression of MT1, MT2, and ERα when cultured Ishikawa cells in the presence of various concentrations of melatonin revealed that expression of MT1 receptor mRNA was not affected by melatonin level; however, expression of ERα receptor mRNA was attenuated when melatonin level was 1×10^{-9} M (Fig. 3b).

Experimental results that observed the effect of melatonin addition in the cytotoxicity reaction of a variety of anticancer drugs for Ishikawa cells are shown in Figure 4. It was determined that Ishikawa cells are susceptible to anticancer drugs tested other than DOC. In addition, the observation of influence of melatonin addition showed that a significant decrease in IC_{50} value is found in PTX, indicating enhancement of PTX action. However, enhancement of cytotoxicity reaction due to addition of melatonin was not seen in the other three anticancer drugs (CDDP, MMC and 5-FU).

Discussion

Melatonin receptor mainly exists in two subtypes: MT1 (Mel 1a); and MT2 (Mel 1b), in mammals.[15] Both are membrane-surface receptors, and *in vivo*, their expression in the suprachiasmatic nucleus, retina and cerebellum, as well as in brown fat cell, prostate, lymphocyte, small intestine and colon has been reported. In tumor cells, expression of melatonin receptors has been reported in breast cancer, ovarian cancer,[16] choriocarcinoma,[17] prostate cancer,[18] colon cancer[19] and pancreatic cancer,[20] and in particular, there have been many reports of expression in hormone-dependent tumor. However, analyses in endometrial cancer cells have been little studied. Our previous studies have revealed that melatonin has an anti-proliferative effect on ER-positive endometrial cancer cells. Its effect is inhibited by estrogen, and its mechanism of action is mediated by receptors on the cell membrane.[10,11]

Intrinsic and common actions are present for each of the actions via MT1 and MT2, and its anti-tumor effect is generally considered to be an action via MT1.[21] In Ishikawa endometrial cancer cells, although the results from analysis of receptor mRNA in the present study confirmed MT1 expression in protein and at gene level, MT2 expression was not found. We previously reported that MT2 expression may be associated with growth inhibition in Ishikawa cells,[11] and such discussion resulted from the report demonstrating that luzindole is an MT2-selective antagonist at that time of publication.[22] However, 4P-PDOT,[23] a selective antagonist of MT2, was also used to investigate cytostatic effect in this study because thereafter it was reported that luzindole is an antagonist for both MT1 and MT2, but not an MT2-selective one.[24] As a consequence, expression of MT1 rather than MT2 was seen in

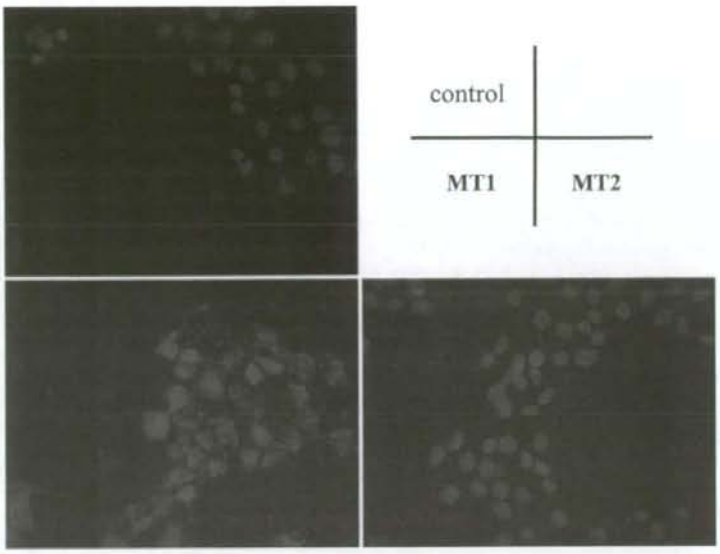

Figure 2 Immunocytochemistry of MT1 and MT2. Ishikawa cells were stained with MT1 or MT2 antibodies or phosphate buffered saline (control), followed by a second antibody labeled by AP (alkaline phosphatase). Nuclei were stained with DAPI.

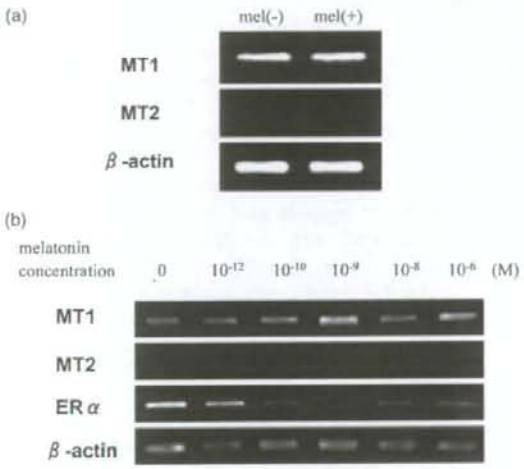

Figure 3 (A) Expression pattern of the melatonin receptor subtype mRNA on Ishikawa cells. Ishikawa cells were incubated with or without 1×10^{-9} M melatonin for 96 h. Each group has an equal amount of total RNA. (B) Expressions of MT1, MT2 and ERα mRNA on Ishikawa cells. Ishikawa cells were incubated with various concentrations of melatonin and 1×10^{-10} M 17β-estradiol for 96 h. Each group has an equal amount of total RNA.

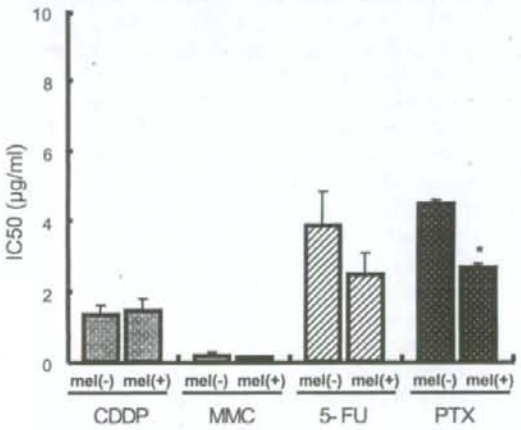

Figure 4 Effects of melatonin for anticancer drugs (cisplatin, CDDP; mitomysinC, MMC; 5-fluorouracil, 5-FU; paclitaxel, PTX; docetaxel, DOC). Ishikawa cells were incubated with or without 1×10^{-9} M melatonin for 96 h. We compared the average IC_{50} with peak plasma concentration (PPC) to determine whether or not each drug was effective on Ishikawa cells. All of the drugs except for docetaxel (DOC) were sensitive to Ishikawa cells. Data are shown as mean ± SE. $P < 0.05$ compared without melatonin (DOC are not shown).

Ishikawa cells similar to other solid cancers, such as breast cancer, and inhibition of cell proliferation via MT1 has been confirmed. MT1 expression has also been confirmed by immunocytochemistry.

In the experiment that analyzed the influence of melatonin addition on mRNA expression in MT1, MT2 and ERα, attenuation of mRNA expression in ERα was found in the group supplemented with 1×10^{-9} M melatonin. These observations suggested that ERα expression in Ishikawa cells is inhibited in the presence of melatonin concentration at the physiological level. Molis *et al.* demonstrated that cell proliferation is inhibited optimally by melatonin addition with concentration at the physiological level in breast cancer cell line, MCF-7, and that its mechanism is an attenuation of mRNA expression level in ERα.[25] Furthermore, it was suggested that the steady state of ER mRNA level is mainly determined by transcriptional regulation of genes, and only melatonin at physiological concentration provides its control, which is not provided by other concentrations.[25] Kiefer *et al.* reported that melatonin at physiological concentration attenuates the ER-dependent transcriptional activity of ER genes in MCF-7.[26] These results suggest that melatonin may indirectly inhibit the proliferative action of E2 on Ishikawa cells by attenuation of ERα expression in Ishikawa endometrial cancer cells. However, our previous studies confirmed that melatonin also inhibits proliferation of Ishikawa cells in a culture condition in the absence of E2;[10] therefore, all mechanisms of action of growth inhibitory effect on endometrial cancer cell could not be explained. Mechanisms of action, including the effect on other growth factors other than E2 and cell cycle, is also suggested.[27] Further study is warranted to analyze these mechanisms.

Recently, the usefulness of concomitant use of melatonin and chemotherapy[28] (i.e. biochemotherapy) has been reported. Although postoperative chemotherapy for endometrial cancer and chemotherapy for the advanced/recurrent cases are still less than well-established, potential candidates include taxan-based antineoplastic drugs.[29] Although the mechanism is unclear, our *in vitro* study demonstrated that melatonin could enhance the cytocidal effect of paclitaxel, suggesting a potential use of melatonin for biochemotherapy of endometrial cancer. The antitumor effect of melatonin is based on mechanisms such as (i) direct induction of apoptosis in tumor cells and inhibition of apoptosis in normal cells; (ii) activation of antitumor immune system; and (iii) regulation of oncogene.[30] Lissoni has reported that cases with complete response plus partial response were significantly increased in a group receiving chemotherapy plus oral melatonin compared with chemotherapy alone for patients with a variety of solid cancer, suggesting the results were due to oncostatic, immunomodulating, and antioxidant properties of melatonin.[28] Based on our results, it is necessary to prove the role of melatonin for biochemotherapy and biochronotherapy through further *in vitro* studies.

Inhibition of E2-dependent tumor cell proliferation by melatonin includes the pathway that influences tumor cells directly via receptors and the pathway that inhibits E2 secretion from local ovaries.[3] The former is thought to involve the mechanism that inhibits the action of aromatase, which generates E2 from androgen secreted by the adrenal gland (SEEM: selective estrogen enzyme modulator) and the mechanism that inhibits the action and expression of ER in the nucleus (SERM: selective estrogen receptor modulator). *In vivo* experiments in an animal model and *in vitro* experiments in a breast cancer cell line supported the hypothesis that the action of melatonin is mainly due to the former.[3] Because ovarian estrogen secretion diminishes after menopause, the hypothesis supports the premise that the action of melatonin can be expected even after menopause. Melatonin tends to decrease after peaking in puberty;[31] thus, sufficient secretion of melatonin may not be expected at the perimenopausal age.

Our study revealed that melatonin inhibits the expression of ERα in Ishikawa endometrial cancer cultured cells and that melatonin may enhance the antitumor effect by chemotherapy, especially in combination with paclitaxel. Alonso *et al.* demonstrated that direct exposure to E2 reduces melatonin synthesis and release in female rat pinealocytes.[32] Moreover, a recent study showed that women who work rotating night shifts for a long duration have a significant increased risk of endometrial cancer,[33] which suggests that insufficient secretion of melatonin as a result of night work might be a risk in endometrial cancer. Thus, administration of melatonin may prevent the onset of endometrial cancer in early postmenopausal women or women working at night who have decreasing melatonin. In the future, melatonin should be investigated as a possible biochemotherapeutic modulator in endometrial cancer.

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Heparanase expression in endometrial cancer: Analysis of immunohistochemistry

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Summary

The human enzyme heparanase has been shown to function in tumour progression, metastatic spread and tumour angiogenesis. The aim of the present study was to assess heparanase expression assessed by immunohistochemical staining (IHC) in endometrial cancer in correlation with clinicopathological factors. A total of 52 endometrial cancers were obtained from previously untreated patients (median age, 56 years, range, 35–80 years). The expression of heparanase was evaluated by using IHC with anti-heparanase polyclonal antibody. This antibody was raised by immunising a rabbit with a peptide containing the amino acid residues from 238 to 250 of the heparanase. The IHC data were used to determine the relationship between heparanase expression, and clinicopathological parameters. IHC showed that the heparanase was expressed in 23 of 52 (44.2%) endometrial cancers. Heparanase was abundantly and equally expressed in both the cytoplasm and the cell membrane of the cells in endometrial cancer. Strong heparanase-positive staining was also seen at the invasive front of the tumour into myometrium. The expression was significantly related to lymph-vascular space involvement ($p = 0.0028$), depth of myometrial invasion ($p = 0.0026$), and histological tumour grade ($p = 0.0135$). In six tumours with positive lymph nodes, the heparanase expression was observed as being higher compared with tumours with negative lymph nodes, which was not significant ($p = 0.2349$). In terms of peritoneal cytology, ovarian metastasis, and cervical invasion, we observed no significant difference in the heparanase expression assessed by IHC. These results suggest that the expression of heparanase may promote tumour invasion into myometrium and lymph vascular space in endometrial cancer.

Keywords

Endometrial cancer, heparanase, immunohistochemistry, polyclonal anti-heparanase antibody, tumour invasion

Introduction

The human enzyme heparanase, an endo-beta-glucuronidase that cleaves heparan sulfate (HS) at specific intrachain sites, has been shown to function in tumour progression and metastatic spread. (Vlodavsky et al. 1999; Hulett et al. 1999) Degradation of heparan sulfate proteoglycans by heparanase appears to play an important role in the invasiveness of tumour cells through the basement membrane and into the extracellular matrix. Tumour spread involves degradation of various components of the extracellular matrix and blood vessel wall. Among these is heparan sulfate proteoglycan, which plays a key role in the self-assembly, insolubility and barrier properties of basement membranes and extracellular matrices (Marchetti et al. 2000; Ilan et al. 2006). Expression of heparanase, which degrades heparan sulfate correlates with the metastatic potential of tumour cells, and treatment with heparanase inhibitors markedly reduces the incidence of metastasis in experimental animals (Vlodavsky et al. 1999; Nakajima et al. 1984; Toyoshima and Nakajima 1999). Heparanase may thus facilitate both tumour cell invasion and neovascularisation, two critical steps in tumour progression.

A majority of patients with endometrial cancer have a 5-year survival rate of approximately 90%. However, 15–25% of patients with a tumour extending outside the uterus but limited to the true pelvis (FIGO stage III), have an estimated 5-year survival of 40–70% (Wolfson et al. 1992; Greven et al. 1993). Also, in patients with stage III disease, lymph vascular space involvement, deep myometrial invasion and lymph node metastasis are reported to be independent prognostic factors (Aoki et al. 2001, 2004). It is quite interesting to investigate the relationship between heparanase expression and tumour aggressiveness in association with the prognostic variables in endometrial cancer.

Materials and methods

Tissue samples

A total of 52 endometrial cancers were obtained from previously untreated patients (median age, 56 years, range, 35–80). Tissues obtained at laparotomy were formalin-fixed and paraffin-embedded for standard H&E staining and immunohistochemical study. Informed consent for using tumour tissues obtained for scientific research in the future was obtained from the patients. All cases underwent

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curative operations for endometrial cancer and were surgically categorised into different stages according to International Federation of Gynecology and Obstetrics (FIGO) staging.

Anti-heparanase antibody generation

The anti-heparanase polyclonal antibody was raised by immunising a rabbit with a peptide containing the amino acid residues from 238 to 250 the 50-kDa heparanase subunit and is able to detect both the 50-kDa and 65-kDa forms of expressed heparanase (Maxhimer et al. 2005). Specificity of this antibody was well characterised by Western blot (data not shown).

Immunohistochemistry

Tissue sections were dewaxed with xylene and rehydrated in alcohol. Slides were then heat inactivated in 10 mmol/l sodium citrate (pH 6.0) in a microwave for 3 min. Cooled slides were rinsed with PBS and then incubated with 1% H_2O_2 in methanol for 30 min at room temperature. Sections were then blocked with 5% normal goat serum in PBS for 30 min at room temperature followed by a 1-h incubation with an anti-heparanase rabbit serum (1:500 dilution) in PBS. Slides were washed and then incubated with goat antirabbit IgG-biotin conjugate (PharMingen) diluted at 1:300 in 5% human serum in PBS. Streptavidin-heparanase conjugate (Zymed Laboratories) diluted at 1:200 in PBS with 5% normal human serum was added and incubated for 45 min at room temperature. Colour was developed by DAB substrate (Sigma) followed by 3,3 = diaminobenzidine enhancer (Vector Laboratories). Slides were counterstained with Mayer's haematoxylin for 2 min, dehydrated, and mounted. Heparanase expression was determined by a board-certified pathologist and corroborated independently by three investigators in this study, all blinded to other clinicopathologic information. Heparanase expression was judged as positive by the presence of brown staining, specifically within 20% of the tumour areas. In the majority of specimens, heparanase staining was present in all tumour cells. A graded scoring system was not used because of the variation in intensity of heparanase signal between the experiments conducted at different times.

Results

IHC analysis was performed to analyse heparanase expression

As shown in Figures 1–3, heparanase was abundantly and equally expressed in both the cytoplasm and the cell membrane of the cells in endometrial cancer (Figure 1), and no heparanase expression was detected (Figure 2). Strong heparanase-positive staining was also seen at the apex of cancer invasion into myometrium (Figure 3). Of specimens staining positively for heparanase, the majority expressed heparanase uniformly throughout the tumour.

Correlation between heparanase expression and clinicopathological factors

The IHC positive for heparanase in each clinicopathological factors are presented in Table I. Tumours with deep myometrial invasion (outer half) expressed significantly higher heparanase positive rate compared with those in tumours without or with inner half myometrial invasion ($p = 0.0026$). Grade 2 and 3 tumours showed a higher heparanase expression than those of grade 1 tumours, significantly ($p = 0.0135$). In respect to lymph vascular space involvement (LVSI), tumours with positive LVSI had

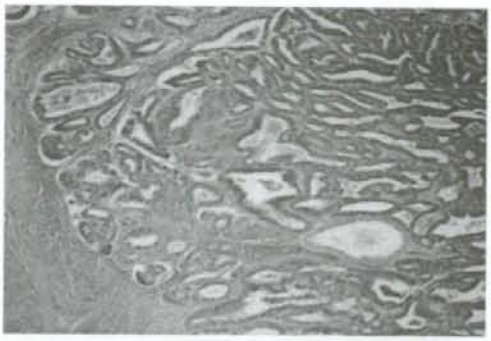

Figure 1. Heparanase abundantly and equally expressed in both the cytoplasm and the cell membrane of the cells in endometrial cancer (× 40). Of specimens staining positive for heparanase, the majority expressed heparanase uniformly throughout the tumour.

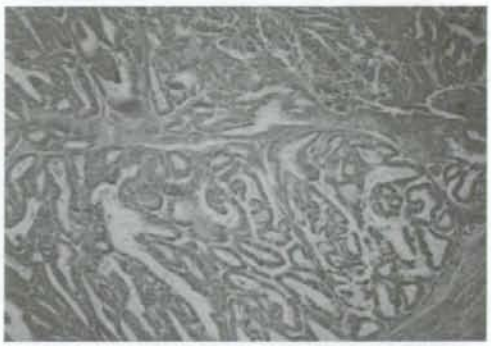

Figure 2. No heparanase expression detected (× 40).

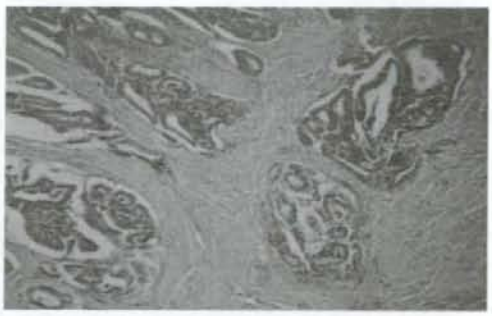

Figure 3. Strong heparanase-positive staining seen at the apex of cancer invasion into myometrium (× 40).

Table I. The correlation between heparanase expression by IHC and clinicopathological factors.

Variables	No. of patients	Hep positive	*p*-value[c]
Median age: 56 years (range, 35–80 years)			
FIGO clinical stage			
IA	6	1	
IB	27	9	
IC	7	5	
II	4	2	
III	7	5	
IV	1	1	
Tumour grade			0.0135
1	40	14	
2 + 3	12	9	
Myometrial invasion			0.0026
a + b	36	11	
c	16	12	
LVS involvement[a]			0.0028
No	34	10	
Yes	18	13	
Lymph node metastasis			0.2394
No	46	19	
Yes	6	4	
Peritoneal cytology			0.0666
Yes	2	2	
No	50	21	
Ovarian metastasis			0.1933
Yes	4	3	
No	48	20	
Cervical invasion			0.0849
Yes	5	4	
No	47	19	

a significantly higher expression rate of heparanase than those with negative LVSI ($p = 0.0028$). In six tumours with positive lymph nodes, the heparanase expression was observed as being higher compared to tumours with negative lymph nodes, which was not significant ($p = 0.2349$). In terms of peritoneal cytology, ovarian metastasis, and cervical invasion, we observed no significant difference in the heparanase expression assessed by IHC.

Discussion

In the present study, we tested whether there was a direct correlation between heparanase expression assessed by IHC and tumour invasion or spread in endometrial cancer. Heparanase was abundantly and equally expressed in both the cytoplasm and the cell membrane of the cells in endometrial cancer. Strong heparanase-positive staining was also seen at the invasive front of the tumour into the myometrium, supporting a role for heparanase in cell invasion. IHC showed that the heparanase was expressed in 23 of 52 (44.2%) endometrial cancers, and that its expression was closely associated with depth of myometrial invasion, LVSI, and tumour grade. Tumours with deep myometrial invasion (outer half) expressed significantly higher heparanase positive rate, and tumours with positive LVSI had a significantly higher expression rate of heparanase. The enzyme may play an important role in tumour invasion, metastatic spread of the cancerous cells, and neovascularisation as previously reported in several types of cancer, such as melanoma (Marchetti et al. 2000), gastric cancer (Endo et al. 2001), bladder cancer (Gohji et al. 2001), pancreas cancer (Rohloff et al. 2002; Koliopanos et al. 2001), leukaemias (Bitan et al. 2002), oral carcinoma (Ikuta et al. 2001), hepatocellular carcinoma (El-Assal et al. 2001), and colon cancer (Friedmann et al. 2000). Heparin-binding angiogenic proteins are stored as a complex with heparan sulfate in the microenvironment of tumours. These proteins are released and can induce new capillary growth when heparan sulfate is degraded by heparanase. Heparanase may influence the bioavailability of different growth factors including FGFs, VEGF, HGF, and PDGF, which are stored in heparan sulfate and possess heparan sulfate-binding sequences (Folkman et al. 1988; Raines and Ross 1992; Vlodavsky et al. 1990; Mizuno et al. 1994). Altogether, heparanase not only enhances cell dissemination, but also promotes the establishment of a vascular network that accelerates primary tumour growth and invasion, and provides a gateway for invading metastatic cells into lymph-vascular space.

Grade 2 and 3 tumours showed a significantly higher heparanase expression. The degree of histological differentiation of endometrial cancer has long been accepted as one of the most sensitive indicators of prognosis. Also, the grade of tumour correlates with other prognostic factors. As the tumour loses its differentiation, the chances of survival decrease (Creasman et al. 1987). Interestingly in our study, as the tumour becomes less differentiated, both the heparanase expression increase. This may be one of the reasons that the histological tumour grade in endometrial cancer is a strong prognostic variable.

In conclusion, our study demonstrated the biological importance of heparanase expression in endometrial cancer. The expression of heparanase was found to influence different malignant behaviour in endometrial cancer, including lymph-vascular space involvement, myometrial invasion, and tumour grade. These results suggest that the expression of heparanase may promote tumour invasion into myometrium and lymph vascular space in endometrial cancer.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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感染症

平川 誠* 長井 裕* 久高 亘* 稲嶺盛彦* 青木陽一*

術後感染症は手術療法に関連する最も一般的な合併症であり，入院日数の延長や薬剤の追加投与による医療費の増大，また社会復帰の遅れによる社会コストの増大をもたらすため，手術後感染防止は重要である。ガイドラインにより術後感染予防の標準化がなされ，加えて滅菌・消毒法の発達や抗菌薬の新たな開発と改良，そしてディスポーザブル製品の開発利用などによってさらに改善がなされてきている。現在の術後感染症予防管理について検証する。

はじめに

術後感染症は手術療法に関連する最も一般的な合併症であり，婦人科手術での入院の約8～10%に認められる[1]。術後感染症は入院日数の延長や薬剤の追加投与による医療費の増大，また社会復帰の遅れによる社会コストの増大をもたらすため，手術後感染防止は重要である。術後感染症は手術操作を直接加えた部位に起こる手術部位感染（surgical site infection；SSI）と呼吸器感染や尿路感染など手術操作とは離れた部位の感染とに大別される。術中・術後を通じてSSIの予防に十分注意を払う必要があり，1999年にアメリカ疾病対策センター（CDC）からの手術部位感染予防に関する勧告（guideline for prevention of surgical site infection；SSI）がなされており[2]，アメリカ産科婦人科学会（American College of Obstetricians and Gynecologists；ACOG）からは2006年に婦人科手術における予防的抗菌薬投与に関するPractice Bulletinが発表され[3]わが国でも1997年に「術後感染症防止抗菌薬の臨床評価に関するガイドライン」が日本化学療法学会より発表されている[4]。それにより，術後感染予防の標準化がなされ，その後の滅菌・消毒法の発達や抗菌薬の新たな開発と改良，そしてディスポーザブル製品の開発利用などによってさらに改善がなされてきている。医療の進歩はとどまることがないが，現在のSSI予防管理についてevidenceを検証する。

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Ⅰ. SSIの定義

CDCのガイドラインではSSIを定義しており，切開部の創感染（wound infection）に加えて手術時に触れた臓器や腔の感染も含めてSSIと表現されている。切開部の感染は皮膚と皮下組織に限局するもの（切開部表層創感染）と，深部の軟部組織に波及するもの（切開部深層創感染）がある[2]（表1）。

Ⅱ. SSIのリスクファクター

種々の報告では創感染の危険性を増大させるおそれのある因子として，遠隔部位の感染または菌の定着，糖尿病，喫煙，全身的なステロイド投与，理想体重より20%以上の肥満，高齢，低栄養状態，特定の血液製剤の使用および長時間手術などが挙げられている。健常者の清潔創

表 1　手術部位感染（SSI）の定義（CDC1999）

1）切開部表層創感染（superficial incisional SSI）
術後 30 日以内に発症し，切開部の皮膚または皮下組織に限定しており，下記のうち少なくとも 1 項目に該当するもの
① 切開部表層から排膿がある
② 創浸出液から微生物が分離される
③ 発赤，腫脹，発熱のうち，少なくとも一つの感染徴候を認め，切開排膿の必要性があり，培養によって菌が検出される
④ 医師が表層創感染であると診断した場合
なお，縫合糸膿瘍や感染した熱傷，会陰切開創，新生児の環状切開術創および切開部深層感染は除外される
2）切開部深層創感染（deep incisional SSI）
術後 30 日以内の感染であり，異物（インプラント）がある場合には術後 1 年以内に発生する感染をいう。感染は筋膜，筋層などに達し，下記のうち少なくとも 1 項目に該当するもの
① 切開部の筋膜や筋などの深層からの排膿
② 創部の自然離開または発熱や圧痛を認め外科医が開放創としたもので，培養陽性のもの
③ 組織学的もしくは放射線診断で膿瘍や感染が明らかなもの
④ 医師が切開部深層創感染であると診断したもの
注）切開部の表層と深層の双方に及ぶ感染は切開部深層創感染とする
3）臓器/体腔創感染（organ/space SSI）
埋入物（インプラント）のない場合は手術 30 日以内に，埋入物を置いた場合には 1 年以内に発生した感染で，その感染が手術手技に関連していると考えられ，さらに，下記のうち少なくとも 1 項目に該当するもの
① 臓器/体腔に創部以外から挿入したドレーンからの排膿がある
② 臓器/体腔から無菌的に採取した体液や組織から微生物が検出される
③ 臓器/体腔の感染の証拠が検査や手術および放射線検査で証明される
④ 医師が臓器/体腔創の感染であると診断した場合
臓器/体腔の感染が切開創を通して排膿された場合は，一般に再手術を必要としない切開創の合併症と考えられるため，切開部深層創感染に分類する

の感染頻度が 1.8％であるのに対し，低栄養状態者では 16.6％，肥満者では 13.5％も増加するという報告もある[5]。糖尿病は確定的な感染のリスクとはいえないが，糖尿病患者の SSI も 10.7％と報告されており，その原因は糖尿病による長期的代謝障害と微小循環障害や周術期の高血糖と考えられている[6]。術後の血糖値が 200 mg/dl 以上であると，SSI の危険性が増大するとの報告もある[7][8]。よって周術期の徹底した血糖コントロールは非常に重要である。喫煙に関してはニコチンを摂取していると創の一次治癒が遅れて，SSI の危険性が増大するとの報告や[9]，術前 4 週間の禁煙で SSI が有意に減少するとの報告がある[10]。しかし，喫煙量や喫煙歴の定義が曖昧であり，さらなる検討が必要である。手術前にステロイド投与その他の免疫抑制剤を投与された患者は，SSI のリスクがあるとされているが，術前のステロイド減量が SSI の予防になるという証拠はない。手術時間は，清潔創の SSI では時間ごとに倍増し，2 時間以上の手術では有意に増加するといわれている[2]。これらのリスクファクターを減らすことで SSI の予防がさらに可能となる。

III. 術前処置

手術前のシャワー浴または入浴は，皮膚についた微生物のコロニー数を減少させることから CDC のガイドラインでは推奨されているが，明らかに SSI の率を低下させるかどうかは明確になっていない[11]~[17]。

手術前夜に行う手術部位の剃毛は，脱毛剤を

表 2　手術前の手洗い方法について（CDC2002）

The Surgical site infection guideline was published by the Centers for Disease Control and Prevention (CDC) in 1999. Three years later, in 2002, the CDC published an update of its guideline on hand hygiene. The following recommendations on surgical hand antisepsis update the recommendations in the SSI guideline：

- Surgical hand antisepsis using either an antimicrobial soap or an alcohol-based hand rub with persistent activity is recommended before donning sterile gloves when performing surgical procedures.
- When performing surgical hand antisepsis using antimicrobial soap, scrub hands and forearms the length of time recommended by the manufacturer, usually 2 to 6 minutes. Long scrub times (eg, 10 minutes) are not necessary.
- When using an alcohol-based surgical hand-scrub product with persistent activity, follow the manufacture's instructions. Before applying the alcohol solution, prewash hands and forearms completely. After application of the alcohol-based product as recommended, allow hands and forearms to dry thoroughly before donning sterile gloves.

使用した場合やもしくは除毛しなかった場合よりもSSIの危険性は大きい。従って，手術前の除毛はいかなる方法でもSSIの増加に結びつくため，除毛しないように提案されている[18]。カミソリによる剃毛でのSSIの危険性の増大は，皮膚に生じた微細な切創が，細菌の増殖と密接に関連していることにより説明されている。SSIの発生率はカミソリで剃毛した場合は5.6%であるのに対し，剃毛しなかった，あるいは脱毛剤を使用した場合が0.6%と報告されている[18]。もし，体毛が手術の支障となる場合は手術直前に電気バリカンを使用して皮膚損傷を起こさないように慎重に行うことが推奨されている。手術直前の剃毛は手術前24時間以内や，24時間以上前と比べるとSSIの発生率はそれぞれ3.1%，7.1%，20%以上とされ[18]，また手術直前のバリカンでの除毛は手術前夜の剃毛や除毛よりもSSIの危険性が低いと報告されている[18]。

IV. 手術者，手術野の消毒

無菌の手術野や滅菌された器械，材料に直接接触する術者は手と前腕をよく洗浄消毒しなければならない。手指の一過性菌のみならず常在菌をも可及的に少なくする必要があり，使用する最適の消毒薬は理想的に抗菌スペクトルが広くて，速効性と持続性を持ち合わせているものがよいと思われる。消毒薬としてアルコール類，クロルヘキシジン，ヨード/ヨードホール製剤などがある。2002年にCDCは手術前の手洗いに関するガイドラインを発表している（表2）。手洗いの時間は最低2分間でも，手の細菌コロニー数減少は10分間法と同程度に有効であるといわれている。ブラッシングと手揉み洗いに関してはブラッシングが有意に手の細菌コロニーを減らすことはなく，ブラッシングによる手の微細な切創を作るデメリットも指摘されており，現在は手揉み洗いが推奨されている。以上より手洗い方法は「グルコン酸クロルヘキシジンによる手揉み洗いを2〜6分行う」ことであり，3時間以上の長時間の手術の場合は手術途中での再度の手洗いをする必要がある。手術野の皮膚消毒に関しては，クロルヘキシジンかまたはポビドンヨードにて手術野全体をよく清拭する。通常消毒薬を手術野の加刀部位周囲から塗布し，次第に周辺に広げていくように塗布する。膣洗浄はポビドンヨード20倍希釈液や，塩化ベンザルコニウム0.02〜0.05%水溶液，塩化ベンゼトニウム0.025%水溶液，クレゾール石鹸液0.1%水溶液，滅菌生理食塩水などを用いる[18]。

術中の腹腔内洗浄や閉腹時の創部の滅菌生理食塩水などでの洗浄が術後のSSIの予防になるというevidenceは認められないが，手術部位の異物・壊死組織の残存を減らすという観点

表 3　手術創の清潔度分類（NNIS1999）

NNIS system	手術例	ターゲットとなる細菌
clean operation (class Ⅰ)	開腹による卵巣嚢腫核出術，卵管留水腫，乳腺の手術など，手術野の消毒を含め手術中にも無菌的な操作が可能な手術	黄色ブドウ球菌などのグラム陽性菌
clean contaminated operation (class Ⅱ)	子宮全摘出術など手術前は無菌手術と同じ処置が可能であるが，常在菌の存在する臓器にメスを加える手術のうち，術中の汚染が少ないもの	大腸菌，肺炎桿菌，エンテロバクター属，プロテウス属などのグラム陰性菌，バクテロイデスフラジリスグループなどの嫌気性菌
contaminated operation (class Ⅲ)	同上であるが，術中に大きな汚染が生じる手術。多くの場合，拭除，洗浄などの対策がすぐに立てられる	
dirty-infeted operation (class Ⅳ)	骨盤内腫瘍，卵巣腫瘍，ダグラス窩膿瘍の手術などすでに感染が成立しているか，汚染が手術前にすでに起こっているもの	さらに腸球菌，緑膿菌などに拡大

から有効である可能性がある[19]。ただし，腹腔内洗浄に関しては大量洗浄による体温低下や末梢循環不全により麻酔覚醒後の振戦などの副作用を起こさないように洗浄液を体温程度に温めるなどの注意が必要である。

V. 予防的抗菌薬投与について

外科手術の周術期予防的抗菌薬投与は，適切に行えばSSIの予防方法として大変有効な手段である。予防的抗菌薬はもちろん原則的に無菌手術ではあえて投与する必要はない。女性生殖器は気道，消化管などと同様に常在細菌を有する。これらの細菌がSSIの原因となりうるために，national nosocomial infection surveillance system（NNIS）の手術創の清潔度を示す創分類ではclean-contaminated woundと分類されている[20]（表3）。感染は術中に曝露する汚染菌の量と生体防御能に関係するため，無菌操作にて菌量を少なく維持し，その上で起炎菌に抗菌活性を有する薬剤を適切なタイミングで投与し，的確な投与間隔で維持することが重要である。婦人科手術においても予防的抗菌薬投与がSSIを有意に低下させると報告されている[21][22]。

1. 抗菌薬投与のタイミング

周術期予防投与に用いられる抗菌薬は，執刀開始前60分以内に投与を開始し，執刀時に投与を完了しておくべきである[3]。この論理的根拠は，SSIを起こす要因で最も重要なのが皮膚切開時の菌の創部への混入とその後の感染の成立である。菌の定着後3時間以内に感染が成立するので，まず麻酔導入直前または直後（手術開始前のおよそ30分前）に投与を開始し，執刀時に血中の抗菌薬の濃度を最高にしておくことにより，軟部組織への移行が速やかであるCefazolinなどは組織濃度も最高に保たれるため，その結果抗菌活性が最大限に発揮される。そして抗菌薬の血中濃度を手術中と切開部位の閉創後数時間維持すればよいことになる[6]。投与方法は経静脈的経路で点滴静注もしくはワンショット静注で行う[2]。

2. 抗菌薬の術中追加投与

手術時間が長くなり，皮膚切開から時間が経過した場合には組織中の抗菌薬濃度が低下し有効域を下回る。そのため薬剤の半減期を考慮

表 4 Antimicrobial Prophylactic Regimens by procedure (ACOG 2006)

Procedure	Antibiotic	Dose
Vaginal/abdominal hysterectomy	Cefazolin	1- or 2-g single dose IV
	Cefoxitin	2-g single dose IV
	Metronidazole	1-g single dose IV
	Tinidazole	2-g single oral dose (4〜12 hours before surgery)
Laparoscopy	None	
Laparotomy	None	
Hysteroscopy	None	
Hysterosalpingogram	Doxycycline	100 mg orally, twice daily for 5 days
IUD insertion	None	
Endometrial biopsy	None	
Induced abortion/D & C	Doxycycline	100 mg orally 1 hour before procedure and 200 mg orally after procedure
	Metronidazole	500 mg orally twice daily for 5 days
Urodynamics	None	

し，手術時間が3時間以上経過した場合，または半減期の2倍以上経過した場合は抗菌薬の追加投与が推奨されている[23]。具体的にはCefazolinであれば手術開始後3時間で追加投与を行うべきであるとされている。

3．抗菌薬の術後投与期間

従来抗菌薬の投与期間はまちまちであり伝統的に3〜5日間の長期間投与が行われてきた。これに関しては再検討が行われ，抗菌薬の予防投与に関しては24時間以内に投与を終了してもそれ以上継続した場合と比べて効果に差がないことが証明されている[24][25]。また，長期間の投与は抗菌薬耐性菌の検出およびそれらの耐性菌による術後感染のリスクが上がることが報告されている[26][27]。むろん，医療経済的にも投与期間が短いほうがよいことは自明である。こうした背景から，諸外国では投与期間は24時間以内であり，ほとんどが皮膚切開時と術中の追加投与のみの投与が行われている。

ただし，もともと手術部位に感染がある場合の手術（腹腔内膿瘍，子宮内感染症）や汚染手術（腸管穿孔など）が認められる場合は感染に対する治療が必要であり，その治療に必要な期間の投与を必要とする。

4．抗菌薬の選択

Cefazolinは半減期が2.46時間と長く，SSIの起炎菌として頻度の高いブドウ球菌（*Staphylococcus aureus*, *Staphylococcus hemolyticus*など）に強い抗菌活性があることと，静注後の臓器への移行が速やかで，高い血中濃度を比較的長時間維持する特徴を持っているため好んで用いられている[28]。一方，婦人科手術の特徴としては悪性腫瘍の場合や腟内細菌叢による汚染の可能性がある場合は嫌気性菌（特に*Bacteroides fragilis*）が起炎菌となるSSIが多い。また腹式子宮全摘術におけるSSIの75%が子宮頸部細菌叢からの感染であるとの報告もある[3]ことからCefazolinの抗菌スペクトラムに加えて嫌気性菌に強い抗菌活性のある第二世代セフェム系のセファマイシン系のCefmetazoleやオキサセフェム系のFlomoxefを選択する場合もある。この両者は腹腔内浸出液への移行も優れていることが特徴である[29]。ただし，血中半減期が両者とも短いため，追加投与には十分注意して行う必要がある[29]。2006年のACOG Practice Bulletinにおける婦人科手術の感染予防として推奨される抗菌薬を表4に示した[3]。加えて，周術期予防投与に用いるべきでない抗

菌薬は第三世代セフェム系抗菌薬や第四世代セフェム系抗菌薬などである。理由としては，①これらの抗菌薬はCefazolinと比較して*Staphylococcus aureus*に対する抗菌力が明らかに落ちることと，②抗菌スペクトラムが広すぎるためSSIの原因となりえない菌までカバーすることで菌交代現象を起こし，難治性感染症を引き起こす可能性があるからである。

VI. 術後処置

1. 創部の被覆

術後は手術創を一時閉鎖した場合は滅菌した被覆材（ドレッシング）にて覆っておき，24～48時間はそのままにしておく。それ以降は被覆材をはがし，シャワーや入浴を行った後，浸出液が出てくる間は被覆材にて覆って観察していく。創傷治癒には創面から出てくる浸出液が重要である。この浸出液には創傷治癒を促進させる細胞成長因子（サイトカインの一種）が豊富に含まれているため，創面が浸出液で覆われているような環境，つまり湿潤状態を作り出すことにより早期に治癒が得られることになる。しかし，創を閉鎖することにより感染の危険性を指摘する声もあるが，実際には感染は細菌の存在のみでは起こらず，創内に血腫や縫合糸などの異物や壊死組織が混在して初めて起こるものであり，術後の創閉鎖の際に創部の血液や壊死組織などを十分取り除けば，その後には細菌は存在しても（colonizaiton）感染（infection）が成立する可能性は低いと考えられる。加えてガーゼで創面を被覆することは創面から浸出液を奪い取り，乾燥状態を作ることにより細菌の増殖を低下させるが創傷治癒も遅延してしまうため，結果的に感染の機会を増やしてしまうことになる[30]。

2. 創部の消毒

創部縫合前後の消毒液による消毒は現在ではすべきではないと考えられている。イソジンヨード液を例にとると，血液や浸出液などの有機物の存在下では殺菌力のもとである遊離ヨードは急速に減少し失活することが知られている。また殺菌効果は1%に希釈されるとほとんど期待できなくなる。しかし，創傷治癒に重要な線維芽細胞や上皮細胞，好中球などは0.1%に希釈されたイソジンでも全滅されるなど，消毒液は人体細胞にとって極めて強力な障害性を持っているのである[30]。

VII. 創部離開と創感染

現在まで創部離開と創感染が同じ意味で使われてきた感があるが実際は異なる。創部離開の原因は大きく分けて三つある。

(1) 感染が原因の創部離開
(2) 創縁の過緊張や肥満による血流不全が原因の創部離開
(3) 創部縫合直前後の消毒による創部離開

実際に多いのは2番目の血流不全によるものである。そのような場合には創縁の壊死組織を除去し被覆材による湿潤療法を行えばよいと考える。もし感染が原因の創部離開が疑われた場合は創傷治癒よりも感染を抑えることが先決であり，感染を抑えるために創部離開した直後はガーゼドレーンやデブリードマンなどで排膿を図りつつ，抗生剤の投与を行う。

それ以降は創部を生理食塩水で洗浄しつつ被覆材にて湿潤療法を行っていく。再縫合は逆に感染を増悪させてしまうことが多く，症例を慎重に選択する必要がある。近年，持続陰圧吸引療法が行われるようになり，閉鎖された湿潤環境のなかで創傷治癒が促進される方法である[31]。

創部離開を認めない創部深部創感染の場合も基本的には同じで，切開排膿後デブリードマンを行いつつ抗生剤を使用していく。

おわりに

近年の滅菌・消毒法の発達や抗菌薬の新たな開発と改良，そしてディスポーザブル製品の開発は目覚ましいものがあるが，依然SSIを完全

にコントロールできたとはいい難い状況である。特に悪性疾患においてはSSIが術後の追加治療の開始を遅らせることにより予後にまで影響する可能性がある。上記を踏まえた上で今後も十分な観察と予防を行うことが大切である。

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