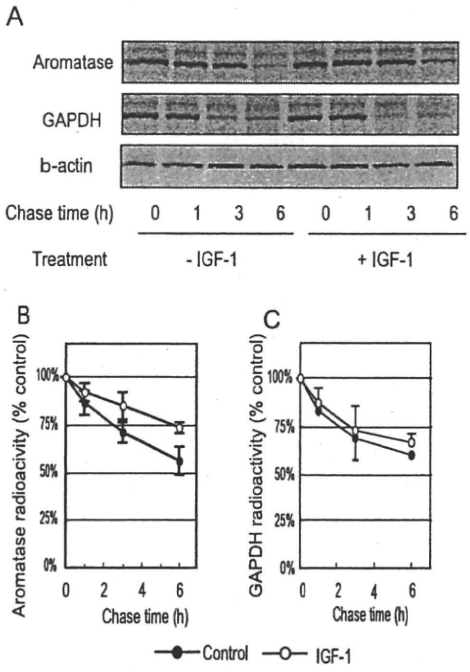


**FIG. 2.** Effect of IGF-I on the degradation rate of pulse-labeled aromatase protein in THP-1 cells. A, A representative autoradiogram of immunoprecipitated proteins; B and C, time course of radioactivity of aromatase and GAPDH obtained from four independent experiments.

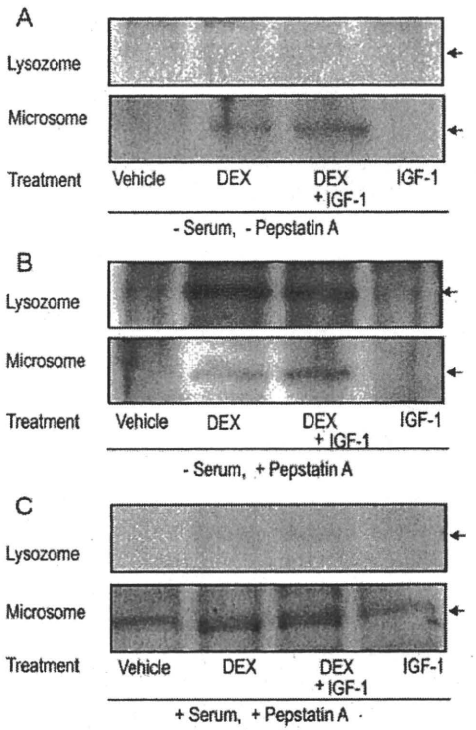


**FIG. 3.** The subcellular localization of aromatase in the presence of pepstatin A. Cellular fractions were prepared from THP-1 cells incubated with vehicle alone (A), pepstatin A (B), or pepstatin A plus 10% FBS (C). The *arrows* indicate the size of the placental microsome that run in the same gel.

lysosomal cathepsin families, such as cathepsin D (which is sensitive to pepstatin A), are primarily involved in the degradation of aromatase.

## IGF-I reduces DEX-dependent accumulation of aromatase in lysosomes

We thereafter determined the *in vivo* effect of IGF-I in the presence of pepstatin A, a cell membrane-permeable inhibitor. A concentration of 0.1 mg/ml pepstatin A in the culture medium did not result in any toxicity to the THP-1 cells according to a morphological assessment; furthermore, it did not affect the cell number or lactic dehydrogenase activity. We initially thought that this nontoxic concentration of pepstatin A would enhance aromatase, but it did not increase either the activity or the protein level in the microsomal fraction (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org).

We subsequently compared the subcellular localization of aromatase. Immunogenic aromatase (54 kDa) was barely detectable in lysosomal fractions under normal conditions (Fig. 3A). However, in the presence of pepstatin A, DEX alone caused a profound increase in the expression of immunogenic aromatase (54 kDa) in the lysosomal fraction, whereas IGF-I attenuated the DEX-induced accumulation of lysosomal aromatase (Fig. 3B). Conversely, IGF-I enhanced the DEX-induced accumulation of immunogenic aromatase in the microsomal fraction (Fig. 3, A and B, *second vs. third line*). This reciprocal action of IGF-I in the lysosomal and microsomal fractions suggests that DEX simultaneously increases the synthesis of aromatase and also increases the transport of the newly synthesized aromatase into lysosomes; furthermore, IGF-I inhibits this transport of aromatase into lysosomes and consequently enhances the accumulation of aromatase in the microsomes.

The addition of serum markedly reduced the levels of aromatase in the lysosomal fractions and enhanced the aromatase level in microsomal fractions (Fig. 3C). Furthermore, the addition of serum completely abolished the IGF-I-induced changes in the aromatase levels by enhancing the concentration in the microsomal fractions and attenuating the expression in the lysosomal fractions.

## IGF-I inhibits the lysosomal transport of aromatase

Subcellular transport of aromatase was morphologically traced using KW cells that expressed pcDNA3.1D/V5-arom. V5-tagged aromatase was localized in the endoplasmic reticulum (Fig. 4, A–C). After 24 h of treatment with IGF-I, the color of the lysosomes changed to a reddish orange (Fig. 4I) from a yellowish orange in the merged figures (Fig. 4F), indicating that IGF-I reduced the concentration of aromatase in the lysosomes. An IGF-I-induced color change of the lysosome was observed even in

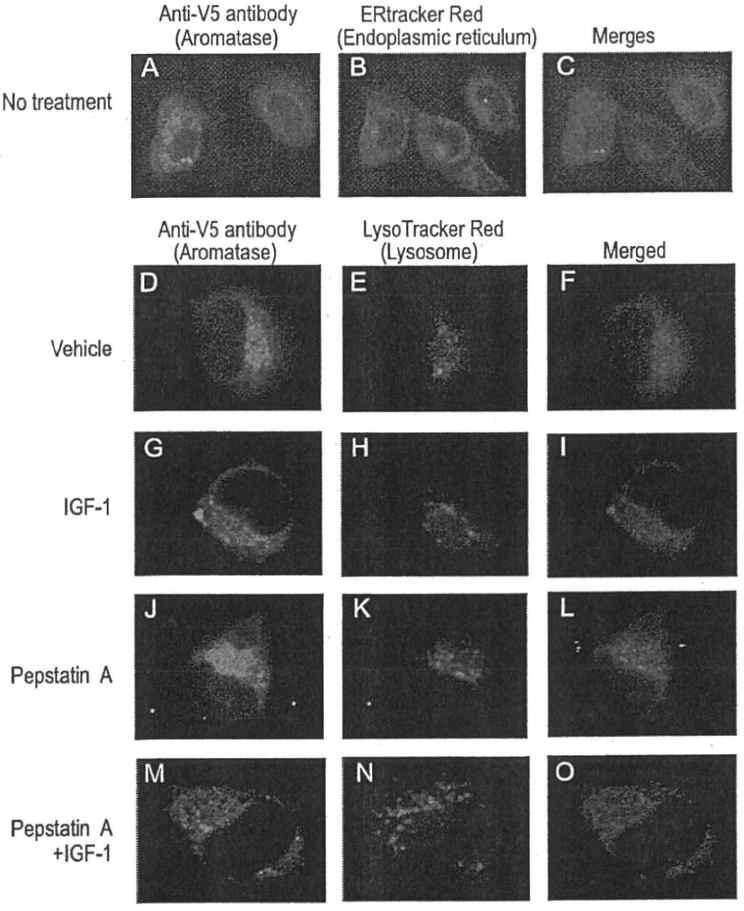


**FIG. 4.** Fluorescent detection of the subcellular localization of the recombinant aromatase in KW cells. A, Antibodies against His tag of aromatase; B, ER-Tracker RED for endoplasmic reticulum; C, a merged image of A and B; D–O, aromatase (D, G, J, and M) was stained *green*, and lysosomes were stained *red* (E, H, K, and N). KW cells were cultured in the presence of vehicle only (D–F), IGF-I (5 ng/ml, G–I), pepstatin A (0.1 mg/ml, J–L), or pepstatin A plus IGF-I (M–O). A representative figure is shown for each treatment. Note that the exogenous aromatase was overexpressed in the transfected KW cells without DEX, despite lower dilutions of the vector. Thus, it might be possible to suppose that autophagy occurred in these cells without DEX.

the presence of pepstatin A (Fig. 4, O *vs.* L). As shown in Fig. 4L, pepstatin A alone induced no or a minimal change in the lysosomal color. The results after a 1-h treatment with IGF-I were essentially the same, thus demonstrating that the activity occurs rapidly after treatment with IGF-I. These findings are consistent with the notion that IGF-I inhibits the transport of aromatase into lysosomes and that pepstatin A inhibits the degradation of aromatase in lysosomes.

### The action of IGF-I on aromatase activity is rapid, rapamycin dependent, and serum starvation dependent

Based on the results reported above, we hypothesized that IGF-I enhances aromatase activity by inhibiting autophagy. To support this hypothesis, we performed two complementary experiments.

To show that the enhancement in expression and activity by IGF-I is a rapid process, THP-1 cells, which were preincubated with DEX in serum-free medium to induce aromatase, were challenged with IGF-I in serum-free medium, and the aromatase activity was measured every 15 min. As expected, the aromatase activity decreased in a time-dependent fashion (Fig. 5A). IGF-I overcame the reduction in aromatase and reversed the trend, resulting in an increase in expression by approximately 50% *vs.* a time-matched control as early as 60 min after the initiation of treatment. The increase in activity observed with IGF-I treatment was most likely due to the temporal continuation of protein synthesis from preexisting mRNA in the absence of protein degradation after the removal of DEX. Conversely, the rate of protein degradation probably exceeded the rate of protein synthesis in the control groups, thus resulting in the rapid decrease in activity over time. Therefore, IGF-I's action on aromatase is a rapid process, comparable to the rapid regulation of the autophagic process through the sequential activation of the kinase cascade (34, 35).

We next examined the effect of inhibiting autophagy on the expression and activity of aromatase. The addition of 1% serum and 1% ethanol eliminated the acute IGF-I-induced increase in aromatase activity at 60 min (data not shown). Similarly, the addition of rapamycin, which directly inhibits mTOR and induces autophagy, abolished the IGF-I-induced enhancement of aromatase activity at 60 min after treatment (Fig. 5B).

## Discussion

Aromatase is a short-lived protein, and its expression is regulated primarily at the transcriptional level (12, 36–39). Regulation through protein phosphorylation and degradation by the proteasome have also been reported (37, 39–42). The present study demonstrated a previously unknown mechanism of aromatase regulation, namely autophagy. Autophagy down-regulated aromatase, and conversely, the inhibition of autophagy by IGF-I caused an acute and profound increase in aromatase activity. This autophagy-dependent mechanism well explains the serum dependence of the effects of IGF-I, and does demonstrate the importance of the serum concentration in culture medium for the measurement of aromatase activity in cell-based assays.

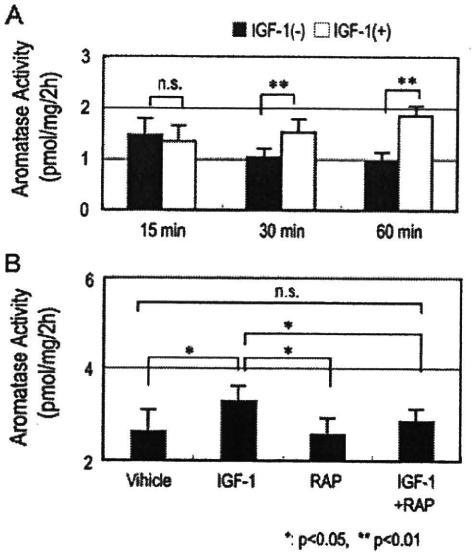


**FIG. 5.** Acute effects of IGF-I and rapamycin on aromatase activity. A, THP-1 cells were maintained to fully induce aromatase for 42 h in complete medium containing 10% FBS and 25 nM DEX. After 6 h of starvation in serum-free medium containing 25 nM DEX, the cells were washed three times with PBS and fed with serum-free and DEX-free medium containing IGF-I (5 ng/ml). Aromatase activity was measured by every 15-min incubation with 1β-[$^3$H]androstenedione. Data are presented as the mean ± SEM of four independent experiments. B, THP-1 cells were pretreated as above and were challenged with IGF-I (5 ng/ml), rapamycin (1 μg/ml), or both for 60 min. Aromatase activity was determined by a 15-min incubation with 1β-[$^3$H]androstenedione at the end of the culture. Data are from five independent experiments.

It has previously been reported that the addition of serum in culture media potentiates the expression of aromatase in many different cell types (12, 28, 43). This action has been ascribed to the effects of serum-derived factors on the transcription of the protein (9, 15). In fact, several humoral factors (including cytokines, growth factors, prostaglandins, and some hormones) in the serum activate aromatase promoters and therefore activate its transcription. In addition to transcriptional up-regulation, our results revealed the contribution of the proteolytic process to the acute control of aromatase activity. Increased aromatase activity was also observed after the addition of albumin to serum-starved breast cancer cells in the mid 1990s, but the underlying mechanism of this phenomenon had never been determined (15, 44). The suppression of autophagy by albumin would, at least in part, explain the enhancement of aromatase activity. In fact, it has already been shown by the same author that IGF-I increases the cellular uptake of iodo-labeled albumin (44). Ethanol also increases the basal activity of aromatase, and transcriptional up-regulation by ethanol has been reported in breast cancer cells (45). This enhancement of aromatase activity could also be attributed, at least in part, to autophagy, because ethanol suppresses autophagy (46).

Our Western blot analysis of cellular fractions demonstrated that IGF-I reduced aromatase in the lysosomes and concurrently increased aromatase in the microsomes (Fig. 3B). In concert with the change in the aromatase activity, the addition of serum completely abrogated the IGF-I-induced changes in aromatase expression levels seen in the lysosomes and microsomes (Fig. 3C). This close coupling between microsomal and lysosomal aromatase supports the notion that IGF-I enhances the functional aromatase residing in microsomes by inhibiting its translocation into lysosomes. This type of subcellular translocation can occur through autophagy.

Autophagy is a degradation pathway for the disposal of cellular components, by which eukaryotic cells can renew their own components and restore the metabolic balance during conditions of nutrient starvation. There are three major mechanisms for autophagy: chaperone-mediated autophagy (degradation of specific cytosolic proteins), macroautophagy (bulk degradation of cytosol and organelles), and microautophagy (engulfing portions of cytosolic material and organelles) (47). Autophagy is induced by amino acid deficiency and is inhibited by IGF-I through the activation of mTOR kinase complexes (34, 35). Serum abolished the aromatase-enhancing activity of IGF-I in THP-1 cells. Rapamycin, an inhibitor of mTOR, also repressed the IGF-I-induced enhancement of aromatase activity, thus supporting the role of autophagy. The effect of IGF-I on aromatase is observed in nearly all types of aromatase-expressing cells that were examined under serum-free conditions, including the intrinsic aromatase-negative KW cells that were transfected to express aromatase driven by a viral promoter. This suggests that the mechanism for disposing of aromatase was not specifically developed for aromatase-expressing cells. The kinetics of aromatase degradation shown by our pulse-chase experiment were different from that of GAPDH, a well known target of chaperone-mediated autophagy (48, 49). Collectively, these results are consistent with the notion that autophagy, specifically macroautophagy, is the mechanism responsible for the translocation of aromatase. We are currently using electron microscopic analysis to directly assess the process of macroautophagy under these conditions.

During macroautophagy, a small double-membrane structure is initially induced in the cytosol. This structure subsequently expands around and sequesters an organelle to form an autophagosome. The autophagosome eventually fuses with the lysosomal membrane, thus allowing the contents to be digested (in the autophagolysosome) (47). The lysosomal fraction that we prepared by density gradient centrifugation included both autophagosomes and autophagolysosomes (50, 51), whereas the microsome fraction contained the endoplasmic reticulum, where aromatase resides as a functional protein. Therefore, the re-

duction in lysosomal aromatase signifies that IGF-I inhibited the step before the completion of autophagosome formation, thus keeping enzymes active. In contrast, pepstatin A prevented just the final step of autophagy, leading to conservation of the aromatase protein in the autophagolysosome, which had already been sequestrated from substrates in the cytosol, so that the protein would no longer function. This explains why IGF-I, but not pepstatin A, enhanced aromatase activity in cells, whereas both suppressed autophagy.

To confirm the effect of IGF-I on autophagy, we conducted a morphological examination using KW cells that expressed cytomegalovirus promoter-driven aromatase. KW cells are derived from myometrial smooth muscle cells that express low basal levels and high induced levels of aromatase under pathological conditions, such as the generation of a leiomyoma. KW cells, despite being aromatase negative, are therefore potentially equipped with a system to express and dispose of aromatase, and this system would be affected by IGF-I in the same way as cells with higher basal expression levels. We used the KW cells to examine the fate of recombinant aromatase, without the potential interference of endogenous aromatase. Our results demonstrated that IGF-I reduced the aromatase signal within the lysosomes, even in the presence of pepstatin A (Fig. 4, L *vs.* O). The use of pepstatin A, an inhibitor of lysosomal proteases, would intensify the aromatase signal within the lysosomes if formation of aromatase-containing autophagolysosomes continued in the presence of IGF-I. Our observation that this did not occur indicates that the formation of aromatase-engulfing autophagosomes had been virtually stopped by treatment with IGF-I.

DEX induced an increase in lysosomal aromatase regardless of whether IGF-I existed (Fig. 3B, compare the second lane to the first lane and the third lane to the fourth lane to observe the action of DEX in the absence and presence of IGF-I, respectively). When there is increased protein synthesis, more mis- or unfolded proteins accumulate as more new protein is formed, and this dysfunctional protein is tracked into the lysosome for disposal (34). Because DEX strongly induces aromatase transcription (12), it is reasonable to assume that DEX activates the transcription (and therefore the translation) of aromatase, which consequently increases the number of dysfunctional aromatase molecules translocated into the lysosomes. Under conditions of relative malnutrition, there may be more chances of mis-incorporation of amino acids and therefore much production of dysfunctional aromatase protein. However, this quality-control mechanism may not fully explain all of the DEX-induced increase in the amount of aromatase in lysosomal fractions. When the cells were treated with IGF-I, it led to the preservation of the DEX-induced aromatase that had been synthesized during the malnutrition, and this enzyme was still functional. This means that even normally folded, and thus functional, protein had been routed into the lysosomes under serum-free conditions. Based on these findings, we reasoned that DEX induces autophagy in nutrient-poor cells, which consequently reduces the amount of aromatase protein. Our preliminary experiment using electron microscopy demonstrated that autophagosome is increased in DEX-treated THP-1 cells. Recently, similar DEX-induced autophagy was reported for the mouse lymphoma cell line WEHI7.2 (52, 53). Therefore, DEX appears to have a dual action on aromatase activity in serum-starved THP-1 cells; it increases the aromatase activity by transcriptional activation and decreases its activity by enhancing autophagy.

It is interesting to note that the rapid control observed in our study, including the down-regulation by autophagy, may play an important role in the brain, where rapid changes in estrogen action through rapid changes in aromatase activity occur in physiologically relevant situations (54). Such activity may also be important in the ovary, where rapid control of aromatase activity may be responsible for rapid fluctuations in the serum estradiol concentration (55). However, the precise pathophysiological significance remains to be determined. New drugs targeting the mTOR complex, a gatekeeper for cell proliferation, apoptosis, and autophagy, are currently being developed for various types of cancer, including breast, colon, and esophageal cancer. Because aromatase is overexpressed in all of these cancers, it is expected that these mTOR inhibitors may be especially effective. It may also be worthwhile to consider the potential modification of aromatase when these drugs are examined in clinical trials.

In summary, we have demonstrated that IGF-I enhances aromatase activity by inhibiting the induction of autophagy in serum-starved cells treated with DEX. This finding reveals a novel mechanism for the rapid and profound clearance of aromatase activity. Moreover, our findings emphasize the need to pay attention to the nutritional condition of cells when aromatase expression is experimentally assessed.

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## References


1. Santen RJ, Brodie H, Simpson ER, Siiteri PK, Brodie A 2009 History of aromatase: saga of an important biological mediator and therapeutic target. Endocr Rev 30:343–375
2. Bulun SE, Imir G, Utsunomiya H, Thung S, Gurates B, Tamura M, Lin Z 2005 Aromatase in endometriosis and uterine leiomyomata. J Steroid Biochem Mol Biol 95:57–62
3. Shozu M, Murakami K, Inoue M 2004 Aromatase and leiomyoma of the uterus. Semin Reprod Med 22:51–60
4. Shippen ER, West Jr WJ 2004 Successful treatment of severe endometriosis in two premenopausal women with an aromatase inhibitor. Fertil Steril 81:1395–1398
5. Shozu M, Murakami K, Segawa T, Kasai T, Inoue M 2003 Successful treatment of symptomatic uterine leiomyoma with a nonsteroidal aromatase inhibitor in a perimenopausal woman. Fertil Steril 79: 628–631
6. Bulun SE, Lin Z, Zhao H, Lu M, Amin S, Reierstad S, Chen D 2009 Regulation of aromatase expression in breast cancer tissue. Ann NY Acad Sci 1155:121–131
7. Zhao Y, Mendelson CR, Simpson ER 1995 Characterization of the sequences of the human CYP19 (aromatase) gene that mediate regulation by glucocorticoids in adipose stromal cells and fetal hepatocytes. Mol Endocrinol 9:340–349
8. Zhao Y, Nichols JE, Valdez R, Mendelson CR, Simpson ER 1996 Tumor necrosis factor-α stimulates aromatase gene expression in human adipose stromal cells through use of an activating protein-1 binding site upstream of promoter 1.4. Mol Endocrinol 10:1350–1357
9. Zhao Y, Nichols JE, Bulun SE, Mendelson CR, Simpson ER 1995 Aromatase P450 gene expression in human adipose tissue. Role of a Jak/STAT pathway in regulation of the adipose-specific promoter. J Biol Chem 270:16449–16457
10. Shozu M, Zhao Y, Simpson ER 2000 TGF-beta1 stimulates expression of the aromatase (CYP19) gene in human osteoblast-like cells and THP-1 cells. Mol Cell Endocrinol 160:123–133
11. Shozu M, Sumitani H, Segawa T, Yang HJ, Murakami K, Kasai T, Inoue M 2002 Overexpression of aromatase P-450 in leiomyoma tissues is driven through the promoter I. 4 of aromatase P-450. J Clin Endocrinol Metab 87:2540–2548
12. Shozu M, Zhao Y, Simpson ER 1997 Estrogen biosynthesis in THP1 cells is regulated by promoter switching of the aromatase (CYP19) gene. Endocrinology 138:5125–5135
13. Meng L, Zhou J, Sasano H, Suzuki T, Zeitoun KM, Bulun SE 2001 Tumor necrosis factor α and interleukin 11 secreted by malignant breast epithelial cells inhibit adipocyte differentiation by selectively down-regulating CCAAT/enhancer binding protein α and peroxisome proliferator-activated receptor γ: mechanism of desmoplastic reaction. Cancer Res 61:2250–2255
14. Pollak MN, Schernhammer ES, Hankinson SE 2004 Insulin-like growth factors and neoplasia. Nat Rev Cancer 4:505–518
15. Reed MJ, Topping L, Coldham NG, Purohit A, Ghilchik MW, James VH 1993 Control of aromatase activity in breast cancer cells: the role of cytokines and growth factors. J Steroid Biochem Mol Biol 44:589–596
16. Steinkampf MP, Mendelson CR, Simpson ER 1988 Effects of epidermal growth factor and insulin-like growth factor I on the levels of mRNA encoding aromatase cytochrome P-450 of human ovarian granulosa cells. Mol Cell Endocrinol 59:93–99
17. Emoto N, Ling N, Baird A 1991 Growth factor-mediated regulation of aromatase activity in human skin fibroblasts. Proc Soc Exp Biol Med 196:351–358
18. Chabrolle C, Tosca L, Ramé C, Lecomte P, Royère D, Dupont J 2009 Adiponectin increases insulin-like growth factor I-induced progesterone and estradiol secretion in human granulosa cells. Fertil Steril 92:1988–1996
19. Randolph Jr JF, Kipersztok S, Ayers JW, Ansbacher R, Peegel H, Menon KM 1987 The effect of insulin on aromatase activity in isolated human endometrial glands and stroma. Am J Obstet Gynecol 157:1534–1539
20. Sirianni R, Chimento A, Malivindi R, Mazzitelli I, Andò S, Pezzi V 2007 Insulin-like growth factor-I, regulating aromatase expression through steroidogenic factor 1, supports estrogen-dependent tumor Leydig cell proliferation. Cancer Res 67:8368–8377
21. Ginther OJ, Bergfelt DR, Beg MA, Meira C, Kot K 2004 In vivo effects of an intrafollicular injection of insulin-like growth factor 1 on the mechanism of follicle deviation in heifers and mares. Biol Reprod 70:99–105
22. Spicer LJ, Chamberlain CS, Maciel SM 2002 Influence of gonadotropins on insulin- and insulin-like growth factor-I (IGF-I)-induced steroid production by bovine granulosa cells. Domest Anim Endocrinol 22:237–254
23. Thierry van Dessel HJ, Lee PD, Faessen G, Fauser BC, Giudice LC 1999 Elevated serum levels of free insulin-like growth factor I in polycystic ovary syndrome. J Clin Endocrinol Metab 84:3030–3035
24. Ayabe T, Tsutsumi O, Sakai H, Yoshikawa H, Yano T, Kurimoto F, Taketani Y 1997 Increased circulating levels of insulin-like growth factor-I and decreased circulating levels of insulin-like growth factor binding protein-1 in postmenopausal women with endometrial cancer. Endocr J 44:419–424
25. Matsuo O, Fukao H, Izaki S, Matsuo C, Ueshima S 1989 Production and characterization of single-chain tissue-type plasminogen activator produced by an established cell line from human uterine muscle. Cell Struct Funct 14:45–60
26. Sourla A, Reyes-Moreno C, Koutsilieris M 1994 Characterization of KW smooth muscle-like human myometrial cells. Anticancer Research 14:1887–1892
27. Sumitani H, Shozu M, Segawa T, Murakami K, Yang HJ, Shimada K, Inoue M 2000 *In situ* estrogen synthesized by aromatase P450 in uterine leiomyoma cells promotes cell growth probably via an autocrine/intracrine mechanism. Endocrinology 141:3852–3861
28. Shozu M, Simpson ER 1998 Aromatase expression of human osteoblast-like cells. Mol Cell Endocrinol 139:117–129
29. Harada N 1988 Novel properties of human placental aromatase as cytochrome P-450: purification and characterization of a unique form of aromatase. J Biochem 103:106–113
30. Ishikawa H, Shozu M, Okada M, Inukai M, Zhang B, Kato K, Kasai T, Inoue M 2007 Early growth response gene-1 plays a pivotal role in down-regulation of a cohort of genes in uterine leiomyoma. J Mol Endocrinol 39:333–341
31. Shozu M, Zhao Y, Bulun SE, Simpson ER 1998 Multiple splicing events involved in regulation of human aromatase expression by a novel promoter, I. 6. Endocrinology 139:1610–1617
32. Corbin CJ, Graham-Lorence S, McPhaul M, Mason JI, Mendelson CR, Simpson ER 1988 Isolation of a full-length cDNA insert encoding human aromatase system cytochrome P-450 and its expression in nonsteroidogenic cells. Proc Natl Acad Sci USA 85:8948–8952
33. Shozu M, Murakami K, Segawa T, Kasai T, Ishikawa H, Shinohara K, Okada M, Inoue M 2004 Decreased expression of early growth response-1 and its role in uterine leiomyoma growth. Cancer Res 64:4677–4684
34. Klionsky DJ, Emr SD 2000 Autophagy as a regulated pathway of cellular degradation. Science 290:1717–1721
35. Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, Tavaré S, Arakawa S, Shimizu S, Watt FM, Narita M 2009 Autophagy mediates the mitotic senescence transition. Genes Dev 23: 798–803
36. Bulun SE, Sebastian S, Takayama K, Suzuki T, Sasano H, Shozu M 2003 The human CYP19 (aromatase P450) gene: update on phys-

iologic roles and genomic organization of promoters. J Steroid Biochem Mol Biol 86:219–224
37. Shozu M, Sumitani H, Murakami K, Segawa T, Yang HJ, Inoue M 2001 Regulation of aromatase activity in bone-derived cells: possible role of mitogen-activated protein kinase. J Steroid Biochem Mol Biol 79:61–65
38. Chen S, Ye J, Kijima I, Kinoshita Y, Zhou D 2005 Positive and negative transcriptional regulation of aromatase expression in human breast cancer tissue. J Steroid Biochem Mol Biol 95:17–23
39. Catalano S, Barone I, Giordano C, Rizza P, Qi H, Gu G, Malivindi R, Bonofiglio D, Andò S 2009 Rapid estradiol/ERα signaling enhances aromatase enzymatic activity in breast cancer cells. Mol Endocrinol 23:1634–1645
40. Balthazart J, Baillien M, Charlier TD, Cornil CA, Ball GF 2003 Multiple mechanisms control brain aromatase activity at the genomic and non-genomic level. J Steroid Biochem Mol Biol 86: 367–379
41. Wang X, Chen S 2006 Aromatase destabilizer: novel action of exemestane, a food and drug administration-approved aromatase inhibitor. Cancer Res 66:10281–10286
42. Miller TW, Shin I, Kagawa N, Evans DB, Waterman MR, Arteaga CL 2008 Aromatase is phosphorylated in situ at serine-118. J Steroid Biochem Mol Biol 112:95–101
43. Lanoux MJ, Cleland WH, Mendelson CR, Carr BR, Simpson ER 1985 Factors affecting the conversion of androstenedione to estrogens by human fetal hepatocytes in monolayer culture. Endocrinology 117:361–368
44. Singh A, Blench I, Morris HR, Savoy LA, Reed MJ 1992 Synergistic interaction of growth factors and albumin in regulating estradiol synthesis in breast cancer cells. Mol Cell Endocrinol 85:165–173
45. Etique N, Chardard D, Chesnel A, Merlin JL, Flament S, Grillier-Vuissoz I 2004 Ethanol stimulates proliferation, ERα and aromatase expression in MCF-7 human breast cancer cells. Int J Mol Med 13:149–155
46. Donohue Jr TM 2009 Autophagy and ethanol-induced liver injury. World J Gastroenterol 15:1178–1185
47. Todde V, Veenhuis M, van der Klei IJ 2009 Autophagy: principles and significance in health and disease. Biochim Biophys Acta 1792:3–13
48. Franch HA, Sooparb S, Du J, Brown NS 2001 A mechanism regulating proteolysis of specific proteins during renal tubular cell growth. J Biol Chem 276:19126–19131
49. Shen W, Brown NS, Finn PF, Dice JF, Franch HA 2006 Akt and Mammalian target of rapamycin regulate separate systems of proteolysis in renal tubular cells. J Am Soc Nephrol 17:2414–2423
50. Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM 2005 Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol 64:113–122
51. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ 2009 Autophagy regulates lipid metabolism. Nature 458:1131–1135
52. Swerdlow S, McColl K, Rong Y, Lam M, Gupta A, Distelhorst CW 2008 Apoptosis inhibition by Bcl-2 gives way to autophagy in glucocorticoid-treated lymphocytes. Autophagy 4:612–620
53. Laane E, Tamm KP, Buentke E, Ito K, Kharaziha P, Khahariza P, Oscarsson J, Corcoran M, Björklund AC, Hultenby K, Lundin J, Heyman M, Söderhäll S, Mazur J, Porwit A, Pandolfi PP, Zhivotovsky B, Panaretakis T, Grandér D 2009 Cell death induced by dexamethasone in lymphoid leukemia is mediated through initiation of autophagy. Cell Death Differ 16:1018–1029
54. Cornil CA, Ball GF, Balthazart J 2006 Functional significance of the rapid regulation of brain estrogen action: where do the estrogens come from? Brain Res 1126:2–26
55. Lephart ED, Simpson ER, McPhaul MJ 1992 Ovarian aromatase cytochrome P-450 mRNA levels correlate with enzyme activity and serum estradiol levels in anestrous, pregnant and lactating rats. Mol Cell Endocrinol 85:205–214

特集 子宮内膜症合併不妊の治療法 [各論] 妊孕性向上のための内膜症治療

# 10. 子宮内膜症治療とアロマターゼ阻害剤

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要旨

子宮内膜症組織にはアロマターゼが過剰に発現しており，局所で産生されるエストロゲンが内膜症の発育・進展に関与している可能性がある．アロマターゼ阻害剤による内膜症治療は，この局所アロマターゼを標的分子としている．単独もしくは黄体ホルモン剤やGnRHアゴニスト等との併用投与で，疼痛の軽減に有効である可能性が示唆されている．妊孕性の改善効果については不明である．

Key Words aromatase inhibitor, endometriosis

子宮内膜症は黄体ホルモン受容体陽性であり，従来から黄体ホルモン剤が治療に用いられてきた．子宮内膜症はエストロゲン受容体も陽性であり，エストロゲン依存性の発育を示すことから，エストロゲンもまた治療標的となっている．GnRHアゴニストは，卵巣からのエストロゲン分泌を低下させる目的で使用されている．黄体ホルモン剤やダナゾールにもゴナドトロピン抑制作用があり，治療効果の少なくとも一部は，卵巣性エストロゲンの低下による．

この10年ほどの間の研究から，子宮内膜症組織自体がエストロゲン合成酵素であるアロマターゼを発現しており，血中のアンドロゲンを原料として組織内でエストロゲンを産生していることが明らかとなった．

この内膜症組織内で産生されたエストロゲンが内膜症細胞自身の増殖を促し，その病態形成に関与している可能性があり，局所のアロマターゼを内膜症治療の標的とする臨床研究も行われている．本稿では，アロマターゼ阻害剤による治療の理論的背景とこれまでの臨床報告を概説する．

## 子宮内膜症組織におけるアロマターゼの発現

子宮内膜症組織では，おもに間質細胞でアロマターゼが過剰に発現しており，エストロゲンが過剰に産生されている（**図1**）．このエストロゲンは，内膜上皮に作用して増殖を促進するとともに，シクロオキシゲナーゼ2型酵素（cycloxygenase type 2, COX2）の発現を誘導しプロスタグランジンE2等の産生を促す．プロスタグランジンE2は炎症性反応のメディエーターとなり，内膜症病変の進行や疼痛の発生を

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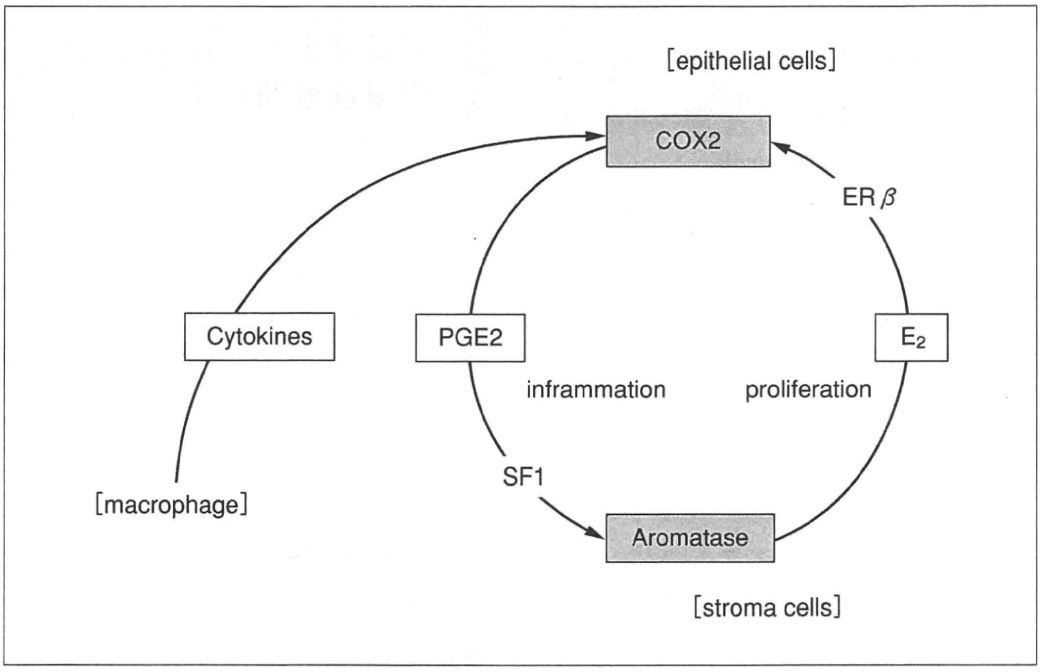


**図1　内膜症組織におけるエストロゲンとプロスタグランジン産生系と内膜症進展への寄与（positive feed forward loop）**

PGE2：プロスタグランジンE2，$E_2$：エストラジオール

促進する．さらに，プロスタグランジンE2には，間質細胞のアロマターゼ発現を誘導する作用がある．亢進したアロマターゼはエストロゲンを産生し，エストロゲンがさらにCOX2の発現を促進する．すなわち，間質のアロマターゼと上皮のCOX2がpositive feed forward loopを作っていて，それぞれの生産物であるエストロゲンとプロスタグランジンE2を介して，内膜症の増殖・進展に関わると推測される．

ゲノムの脱メチル化やメチル化などのエピゲノム変化が，子宮内膜症におけるアロマターゼとその発現調節因子，特にsteroidogenic factor 1の発現亢進に関わっているとの推測がある[1]．しかし，ゲノムの脱メチル化などのエピゲノム変化は，炎症性メディエーターが長期に作用した結果である可能性もあり，アロマターゼ発現異常の真の原因はよくわかっていない．

## 阻害剤の作用機序

アロマターゼ阻害剤の治療効果は，内膜症組織内のエストロゲンレベルの低下によってもたらされると考えられる．

アロマターゼ阻害剤が卵巣のアロマターゼを抑制する可能性はあるが，有経者に通常量を投与した場合にもたらされる血中エストラジオール濃度の低下はほんのわずかで，投与前値とほとんど変わらないかもしくは1/2～1/5程度の低下と考えられる[2)~4)]（アロマターゼ阻害剤の投与量は，閉経後乳癌患者を対象にして設定されている．すなわち，卵巣周期の閉止した女性で乳癌局所でのエストロゲン産生が低下する投与量に設定されている）．したがって，卵巣におけるエストロゲン産生の低下が，治療効果の本体とは考えにくい．

これに対して，子宮内膜症組織内でのエストロゲン産生は，アロマターゼ阻害剤によりほぼ

完全に抑制されると考えられる[5]．アロマターゼ阻害剤は拮抗型阻害剤であるために基質（アンドロゲン）濃度（2 μM 程度）の高い卵巣（卵胞）でのエストロゲン産生を抑制することはできない．一方，血中から供給される低濃度のアンドロゲン（数 nM）を基質としてエストロゲンを合成する子宮内膜症組織では阻害剤が基質である血中アンドロゲン濃度を凌駕してアロマターゼに結合しエストロゲン合成が阻害される．したがって，子宮内膜症患者に対するアロマターゼ阻害剤の投与は，内膜症組織内で産生され内膜症組織内で作用するエストロゲンをおもな標的としている．

アロマターゼ阻害剤を閉経前の女性に投与する場合，考慮しなければならない大切な点がある．それは，アロマターゼ阻害剤が血中エストロゲンレベルを下げることで，ゴナドトロピン分泌を促進し，卵胞発育を促進する可能性がある点である（これが，アロマターゼ阻害剤を排卵誘発に応用する根拠となっている）[6][7]．したがって，閉経前の女性への投与では，複数卵胞の発育が生じることや過排卵が誘発されることがある．そこで，アロマターゼ阻害剤を内膜症患者に投与する場合には，閉経もしくは閉経期に近い患者では単独投与するが，閉経前の患者では何らかの薬剤を併用して排卵を抑制することが多い．なお，内膜症にアロマターゼ阻害剤を投与した報告のうち，LH・FSH 値を検討したものについてみると，いずれも LH・FSH の上昇を検出していない．一方で，排卵誘発目的でアロマターゼ阻害剤が投与された報告では，アロマターゼ阻害投与中に FSH の上昇がみられ，卵胞発育促進効果が観察されている．この違いの原因ははっきりしないが，投与期間長や時期の違いなどが考えられる．FSH はパルス状に放出されているため振幅や頻度の変化を検出するには多数例でのより詳細な解析が必要であろう．

## アロマターゼ阻害剤の内膜症治療効果

### 1．閉経後の投与

閉経者ではゴナドトロピン上昇による卵巣活性化の心配がないので，下垂体抑制薬を併用せずアロマターゼ阻害剤の単独投与が行われる（**表1**）．これまでに 6 例の閉経後子宮内膜症患者への報告がある[8]~[13]．

いずれも再発性・難治性の子宮内膜症例で，長期にわたる内膜症治療歴を有する症例である．1 例を除いて根治的手術（子宮摘除と両側付属器切除）がすでに施行されていた．手術後黄体ホルモン療法や GnRH アゴニスト療法などの薬物治療が継続して行われており，これらの既存治療で奏功しなかったために，アロマターゼ阻害剤投与が選択された．すべての症例でアロマターゼ阻害剤投与により疼痛が改善している．しかし，Bohrer らの尿管の内膜症症例では，投与中に疼痛の軽減はみられたものの，水腎症は進行したという．後腹膜の繊維化が高度となっている場合には，アロマターゼ阻害剤による改善は望めないと著者らは考えている[12]．

血中エストラジオール濃度が測定されていた症例では，治療前値が 29~46 pg/mL と閉経後としてはやや高値で，アロマターゼ阻害剤投与後に低下傾向を示すものの必ずしも測定感度以下にならなかったことが示されている．これに対し，内膜症組織内のアロマターゼ活性が測定された症例では，治療前の活性亢進と治療後の検出感度以下への低下が示されている[8]．さらに，Sasson らの腹壁子宮内膜症腫の症例では，血液より高いレベルのエストラジオールが内膜症腫内容液中に検出されており，アロマターゼ阻害剤投与後にはこれが低下したことが確認されている[13]．

これらの観察は，閉経後の子宮内膜症組織が

表1 閉経後の子宮内膜症患者に対するアロマターゼ阻害剤の投与

| 報告者 | 報告年 | 年齢 | 既往手術など | 主訴・病変・診断 | 薬剤と投与期間 | 治療効果 | ホルモン値 |
|---|---|---|---|---|---|---|---|
| Takayama ら[8] | 1998 | 57 | 子宮摘除，両側付属器切除 | 疼痛，腟断端内膜症 | A 1 mg/d×9 M | 1 M で疼痛消失とアロマターゼ発現の低下． | 血中 $E_2$：46→33（2 M 後），血中 FSH 61→70（2 M 後） |
| Razzi ら[9] | 2004 | 31 | 26 歳内膜症手術，腎瘻，27 歳子宮亜全摘，両側付属器切除 | 直腸腟中隔内膜症 | L 2.5 mg/d×9 M | 3 M で疼痛軽減，9 M で疼痛ほぼ消失． | 血中 $E_2$：35→20 |
| Fatemi ら[10] | 2005 | 57 | 子宮摘除，両側付属器摘除 | イレウス，疼痛，骨盤内内膜症再発 | L 2.5 mg/d×18 M | 疼痛の消失，18 M で病巣ほぼ消失． | |
| Mousa ら[11] | 2007 | middle age | 子宮摘除，両側付属器切除 | 骨盤内内膜症，直腸腟中隔内膜症 | E（25 mg/d）×2 w<br>L（2.5 mg×3 回/w）×4 M | E は無効，L 1 M で疼痛著減． | |
| Bohrer ら[12] | 2008 | 47 | 37 歳子宮癌（IA 期，G1）のため子宮摘除，両側付属器切除 | 尿管内膜症，水腎症 | A 1 mg/d×15 M | 投与初期に疼痛が 90% 軽減，15 M で水腎症が進行し手術． | |
| Sasson ら[13] | 2009 | 61 | 30 歳卵管結紮，45 歳自然閉経，55 歳腹壁瘢痕ヘルニア，59 歳腹壁内膜症腫切除，60 歳腹壁内膜症腫再切除 | 腹壁の再発性内膜腫 | L 2.5 mg/d×2 w→<br>L 5 mg/d×1 w→<br>L 5 mg/d＋MPA 10 mg/d×1 w | 嚢胞内容の穿刺吸引後に再増大なく奏功． | 血中 $E_2$：39→24（2 w 後）<br>嚢胞内 $E_2$：89→28（2 w 後） |

A：アナストロゾール，L：レトロゾール，E：エキセメスタン，$E_2$：pg/mL, FSH：IU/L

エストロゲンを産生していること，これが血中エストロゲンを軽度に上昇させているとの仮説を支持するものである[13]．重症の子宮内膜症で癒着が高度であった症例では，外科的に両側卵巣が切除された後にも腸管の漿膜面などの剝離面に卵巣の一部が残存していることがあり，このような症例ではアロマターゼ阻害剤の効果が残存卵巣を介している可能性が否定できない．しかし，報告された6症例のうち少なくとも3例は57歳以上と高齢で，残存した卵巣が機能していたとは考えにくい．

このように，症例数は少ないものの，閉経後の再発性難治性の子宮内膜症の疼痛管理にアロマターゼ阻害剤は有用である可能性がある．しかし，アロマターゼ阻害剤の長期投与では骨量の低下や関節炎などの副作用に注意が必要である．

### 2．閉経前の投与

月経周期がある患者に対するアロマターゼ阻害剤投与の報告としては，現在までに10報[2)~4)14)~20)]があり，169症例への投与が報告されている（**表2**）．このうち8報は，対照のない観察研究であり，治療前後での症状改善が観察項目となっている．いずれの報告でもアロマターゼ阻害剤投与開始後1カ月以内に疼痛の改善が認められている．また，6カ月間の投与後

表2 閉経前の子宮内膜症患者に対するアロマターゼ阻害剤の投与

| 報告者 | 報告年 | 研究デザイン | 症例数 | 投与期間 | 対象 | アロマターゼ阻害剤と併用薬 | 治療効果 | ホルモン値 |
|---|---|---|---|---|---|---|---|---|
| Ailawadiら[2] | 2004 | Non-randomized | 10 | 6M | GnRHアゴニスト投与後の再発例で周期当たり2週間以上の疼痛持続例 | L (2.5 mg/d)＋N (2.5 mg/d) | 疼痛軽減，病巣サイズ縮小 | 血中$E_2$，LH，FSH不変 |
| Soysalら[3] | 2004 | RCT | 80 | 6M | 重症の内膜症で，保存手術後，rASRM score＞40 | A (1 mg/d)＋G vs. G | 治療終了後24Mでも再発率が低下 | 血中$E_2$低下(50→23 pg/mL) |
| Shippenら[14] | 2004 | Case report | 2 | 6M | 難治性内膜症，1例は直腸腟中隔内膜症を含む | A(1 mg/d)＋経口プロゲステロン (200 mg/d) | 疼痛軽減．1例で病変の消失，腹腔鏡で確認 | |
| Amsterdamら[15] | 2005 | Prospective, non-randomized | 15 | 6M | 難治性再発例，慢性骨盤痛 | A (1 mg/d)＋ethinyl estradiol 20 μg/日 levonorgestrel 0.1 mg/日 | 1M目より疼痛スコアが低下，6Mで55%低下．1例は投与中に妊娠 | 血中$E_2$低下 |
| Heflerら[16] | 2005 | Non-randomized | 10 | 6M | 難治性の直腸腟中隔内膜症 | A (0.25 mg/d)，経腟投与 | 月経困難症は改善し，骨盤痛と性交痛は改善せず | 血中$E_2$，LH，FSH不変 |
| Remorgidaら[17] | 2007 | Prospective, non-randomized | 12 | 6M | 直腸腟中隔内膜症 | L (2.5 mg/d)＋N (2.5 mg/d) | 疼痛が改善したが，投与終了後疼痛再発．投与後，組織学的に内膜症の残存確認 | 血中$E_2$不変 |
| Remorgidaら[18] | 2007 | Prospective, non-randomized | 12 | 2-4M** | 難治性内膜症 | L (2.5 mg/d)＋desogestrel 75 μg/日 | 疼痛は改善したが，全例に卵巣嚢腫が発生したため投与中止．投与中止後，3カ月で疼痛再発 | |
| Vermaら[4] | 2009 | Case Report | 4 | 6M | 難治性症例 | A (1 mg/d)，またはL (2.5 mg/d) | 疼痛の改善 | 血中$E_2$，LH，FSH不変 |
| Losslら[19] | 2009 | Prospective, non-randomized | 20 | 69d | 2～7 cmのチョコレート嚢胞を合併する不妊症 | A (1 mg/d)＋G | IVF/ICSIの前に投与．投与中にチョコレート嚢胞の縮小とCA125の低下がみられた．45%が妊娠したが，流産率が高かった | |
| Ferreroら[20] | 2009 | Prospective, non-randomized | 82 | 6M | 直腸質中隔内膜症 | L (2.5 mg/d)＋N (2.5 mg/d) vs. N (2.5 mg/d) | 投与中には疼痛改善に上乗せ効果がみられたが，投与終了後には上乗せ効果は消失 | |

A：アナストロゾール，L：レトロゾール，E：エキセメスタン，G：ゴゼレリン，N：norethindrone

の腹腔内の観察で，病変の縮小または消失を認めたとの報告もある[2]．しかしながら，2報を除きプロゲストーゲンまたはピル，GnRHアゴニストの同時投与が行われていることから，投与前後での症状改善が，これらの排卵抑制性薬剤の効果によるものかアロマターゼ阻害剤併用投与による上乗せ効果が加わったものであるのかは明らかではない．

排卵抑制が行われていないVermaら[4]およびHeflerら[16]の報告でも疼痛の軽減が観察されていることから，アロマターゼ阻害剤自体にに治療効果があることが示唆される．Heflerらの報告は，直腸膣中隔の内膜症10例に対してのアロマターゼ阻害剤（アナストロゾール，アリミデックス®，0.25 mg/日）を経膣的に6カ月間投与して疼痛の改善効果を認めたとするものである．血中エストラジオール・LH・FSHに変化はなく，アロマターゼ阻害剤の治療効果が卵巣性（内分泌性）エストロゲンの低下によるものではないことが示唆されている．Vermaらもアロマターゼ阻害剤の単独（経口）投与で，血中ホルモン値に有意の変化が認められなかったが疼痛の改善効果が認められたことを確認している．したがって，アロマターゼ阻害剤は閉経前の内膜症患者の疼痛の軽減に有用であることが示唆される．両報告ともに，血中ホルモン値の変化はほとんどみられないことから，閉経前の女性においても内分泌性エストロゲンに比し内膜症組織内で産生される局所エストロゲンの貢献が比較的大きいことが示唆される．

排卵抑制剤と併用投与された8報のうち，対照群のある研究デザインとなっているのは，Ferreroらの前向き非ランダム化試験とSyosalらのランダム化試験のみである．Ferreroら[20]は，直腸膣中隔の子宮内膜症の患者にノルエチステロン単独またはノルエチステロン＋レトロゾール，フェマーラ®（2.5 mg/d）併用投与を行い，投与中の疼痛改善についてレトロゾールの上乗せ効果を認めたが，投与終了後に疼痛は速やかに再発し，投与後6カ月後の時点でキャリーオーバー効果は認められられなかったとしている．

Soysalの報告[3]は唯一のランダム化比較試験である．保存手術後の重症の子宮内膜症に対し，GnRHアゴニスト単独もしくはアナストロゾール併用で6カ月間の投与が行われた．投与治療終了後の無疼痛期間が有意に延長し，疼痛再発率も有意に低下した．また，治療直後には骨密度の低下が認められた．治療終了後2年の時点で，両群に骨密度の差を認めなかった．したがって，アナストロゾールの投与が有効であったと結論している．この研究は80例と症例数も比較的多く，前向きランダム化比較試験として行われている点で最も評価が高い[21)22)]．

## 直腸膣中隔内膜症に対する効果

現在までのところ，アロマターゼ阻害剤による内膜症治療は根治的手術療法や既存の薬物療法などで奏功しない難治性再発性の症例が中心である．直腸膣中隔の内膜症もその一つで，手術療法では侵襲性も高く，かつ再発例も多いため薬物療法が試みられることも多い．しかし，ダナゾールやレボノルゲストレル徐放性膣内リング・IUDなどの薬剤に抵抗する症例も少なくない[22]．

このような薬物療法抵抗性の症例や手術後再発症例に対して，アロマターゼ阻害の投与が試みられている[8)9)11)16)17)]．それらによると，他の部位の子宮内膜症と同様に，閉経前・閉経後にかかわらずアナストロゾールやレトロゾールの投与により速やかな疼痛軽減が観察されている．これらの報告のなかにはアロマターゼ阻害剤単独投与を行って直腸膣中隔内膜症の疼痛改善を確認しているものがあり，アロマターゼ阻害剤は直腸膣中隔内膜症に対しても有望と考え

られている．しかしながら，投与中止後に疼痛が速やかに再発した，月経困難症は軽減したが性交痛や骨盤痛は改善しなかったなど問題点も指摘されている．

## アロマターゼ投与の問題点

以上述べてきたように，難治性再発性の子宮内膜症にアロマターゼ阻害剤は有望とする報告が多い．しかし，質の高い研究はほとんどなく，対照群を置いた比較試験による検討が必要である．

アロマターゼ阻害剤の有経者への投与では，無排卵や無月経をきたさず，血中エストロゲン濃度の低下もわずかでありながら内膜症性の疼痛に改善効果を示すことから，アロマターゼ阻害剤のおもな作用は組織内エストロゲン産生を抑制することにあると推定される．今後組織内のエストロゲン濃度などを測定するなどの方法で，作用機序を明らかにする必要がある．

同じアロマターゼ阻害剤でもエキセメスタン，アロマシン®が無効，レトロゾールが著効であった症例が報告されており[11]，今後，薬剤ごとに作用機序や薬物代謝の特徴などを明らかにする必要がある．

アロマターゼ阻害剤の薬物としての副作用は，軽微な消化器症状程度のものが多く，重篤なものは少ない．しかし，長期投与では関節痛や肝炎などが報告されている．FSH上昇から卵巣の嚢胞性腫大をきたしたとする報告もあり，注意を要する[18]．また，アロマターゼ阻害剤とGnRHアゴニスト併用治療は，total estrogen blockageともいえる治療法であり，卵巣欠落症状や骨粗鬆症の進行なども懸念される．投与期間なども含めて十分配慮する必要がある．

## おわりに

アロマターゼ阻害剤による内膜症治療は，有望ではあるが質の高いエビデンスはほとんどなく[21]，これまでのところアロマターゼ阻害剤を内膜症治療薬として認めている国はない．

アロマターゼ阻害剤の投与は，アロマターゼ欠損症の患児に観察されているように，女胎児の男性化をきたすおそれがあり，妊娠中の投与は避けなければならない．実際に，投与中に妊娠したとする報告もあり，注意が必要である．

## 文　献


1) Bulun SE：Endometriosis. N Engl J Med 2009；360：268-279.
2) Ailawadi RK, et al：Treatment of endometriosis and chronic pelvic pain with letrozole and norethindrone acetate：a pilot study. Fertil Steril 2004；81：290-296.
3) Soysal S, et al：The effects of post-surgical administration of goserelin plus anastrozole compared to goserelin alone in patients with severe endometriosis：a prospective randomized trial. Hum Reprod 2004；19：160-167.
4) Verma A, et al：Successful treatment of refractory endometriosis-related chronic pelvic pain with aromatase inhibitors in premenopausal patients. Eur J Obstet Gynecol Reprod Biol 2009；143：112-115.
5) 生水真紀夫・他：アロマターゼ阻害剤による内膜症治療．産と婦 2008；33：33-38.
6) Fisher SA, et al：A randomized double-blind comparison of the effects of clomiphene citrate and the aromatase inhibitor letrozole on ovulatory function in normal women. Fertil Steril 2002；78：280-285.
7) 生水真紀夫・他：経口排卵誘発剤の新たな展開．産婦の実際 2010；59：53-60.
8) Takayama K, et al：Treatment of severe postmenopausal endometriosis with an aromatase inhibitor. Fertil Steril 1998；69：709-713.
9) Razzi S, et al：Treatment of severe recurrent endometriosis with an aromatase inhibitor in a young ovariectomised woman. Bjog 2004；111：182-184.
10) Fatemi HM, et al：Successful treatment of an aggressive recurrent post-menopausal endometriosis with an aromatase inhibitor. Reprod Biomed Online 2005；11：455-457.
11) Mousa NA, et al：Aromatase inhibitors in the treatment of severe endometriosis. Obstet Gynecol 2007；109：1421-1423.
12) Bohrer J, et al：Persistent bilateral ureteral obstruction secondary to endometriosis despite treatment with an aromatase inhibitor. Fertil Steril 2008；90：2004 e2007-2009.
13) Sasson IE, et al：Aromatase inhibitor for treatment of a recurrent abdominal wall endometrioma in a postmenopausal woman. Fertil Steril 2009；92：1170 e1171-1174.
14) Shippen ER, et al：Successful treatment of severe endometriosis in two premenopausal women with an aromatase inhibitor. Fertil Steril 2004；81：1395-1398.
15) Amsterdam LL, et al：Anastrazole and oral contraceptives：a novel treatment for endometriosis. Fertil Steril 2005；84：300-304.
16) Hefler LA, et al：Role of the vaginally administered aromatase inhibitor anastrozole in women with rectovaginal

endometriosis：a pilot study. Fertil Steril 2005；84：1033-1036.
17) Remorgida V, et al：Letrozole and norethisterone acetate in rectovaginal endometriosis. Fertil Steril 2007；88：724-726.
18) Remorgida V, et al：Letrozole and desogestrel-only contraceptive pill for the treatment of stage IV endometriosis. Aust N Z J Obstet Gynaecol 2007；47：222-225.
19) Lossl K, et al：Combined down-regulation by aromatase inhibitor and GnRH-agonist in IVF patients with endometriomas-A pilot study. Eur J Obstet Gynecol Reprod Biol 2009；144：48-53.
20) Ferrero S, et al：Letrozole combined with norethisterone acetate compared with norethisterone acetate alone in the treatment of pain symptoms caused by endometriosis. Hum Reprod 2009；24：3033-3041.
21) Nawathe A, et al：Systematic review of the effects of aromatase inhibitors on pain associated with endometriosis. Bjog 2008；115：818-822.
22) Vercellini P, et al：Medical treatment for rectovaginal endometriosis：what is the evidence? Hum Reprod 2009；24：2504-2514.

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