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Steroidogenic factor 1/adrenal 4 binding protein transforms human bone marrow mesenchymal cells into steroidogenic cells

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Abstract

Steroidogenic factor 1/adrenal 4 binding protein (SF-1/Ad4BP) is an essential nuclear receptor for steroidogenesis as well as for adrenal and gonadal gland development. Mesenchymal bone marrow cells (BMCs) contain pluripotent progenitor cells, which differentiate into multiple lineages. In a previous study, we reported that adenovirus-mediated forced expression of SF-1 could transform mouse primary long-term cultured BMCs into steroidogenic cells. For future clinical application, trials using human BMCs would be indispensable. In this study, we examined whether SF-1 could transform human BMCs into steroidogenic cells and compared the steroid profile of these cellswith that of mouse steroidogenic BMCs. Primary cultured human BMCs infected with adenovirus containing bovine SF-1 cDNA could produce progesterone, corticosterone, cortisol, dehydroepiandrosterone, testosterone, and estradiol. Such a mixed character of adrenal and gonadal steroid production in human BMCs was supported by the expressions of P450scc, 3β-hydroxysteroid dehydrogenase (3β-HSD), P450c21, P450c11, P450c17, 17β-HSD, and P450arom mRNAs. Unlike mouse steroidogenic BMCs, introduction of SF-1 into human BMCs caused dramatic inductions of both ACTH and LH receptors, thus leading to good responsiveness of the cells to ACTH and LH respectively. Importantly, among several factors that are known to be closely associated with adrenal and/or gonadal development, introduction of only SF-1 enabled the human BMCs to express P450scc and to produce cortisol and testosterone, suggesting that SF-1 is truly a master regulator for the production of steroidogenic cells from human BMCs.

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Introduction

Steroidogenic factor 1 (SF-1) was initially identified as a ubiquitous transcription factor for steroidogenic genes (Yanase *et al.* 1991, Honda *et al.* 1993, Omura & Morohashi 1995, Parker & Schimmer 1997). SF-1 binds as a monomer to its responsive element located in the promoter regions of steroidogenic genes and enhances their transcriptional levels (Honda *et al.* 1993, Omura & Morohashi 1995, Parker & Schimmer 1997). In addition, the cAMP-protein kinase A signal pathway can strongly potentiate SF-1 transactivation activity (Fan *et al.* 2004). Since knockout mice for SF-1 show agenesis of both the adrenal glands and the gonads, as well as decreased expression levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the pituitary gonadotroph (Ingraham *et al.* 1994, Luo *et al.* 1994, Morohashi & Omura 1996), SF-1 has been considered to be an essential factor for differentiation of the pituitary–adrenal or pituitary–gonadal axis. Dosage-sensitive sex reversal, adrenal hypoplasia congenital, critical region on the X chromosome gene 1 (DAX-1) is also an important factor for the development of this axis since patients with DAX-1 mutations show X-linked adrenal hypoplasia congenital (AHC) and hypogonadotropic hypogonadism (Muscatelli *et al.* 1994, Yanase *et al.* 1996). Thus, DAX-1 is considered to be involved in the differentiation process of the fetal adrenal gland. A tumor suppressor gene, Wilms' tumor 1 (WT1), was also proven to be related to urogenital development, including the development of the adrenal gland, from the findings of phenotypes in patients with mutations (Little & Wells 1997) and knockout mice (Wagner *et al.* 2003). Although their exact roles in humans are unknown, many other factors, such as pre-B-cell leukemia homeobox 1 (PBX-1; Schnabel *et al.* 2003), CBP/p300-interacting transactivator with Glu/Asp-rich C-terminal domain 2 (CITED2; Bamforth *et al.* 2001), wingless type MMTV integration site family member 4 (WNT4; Vainio *et al.* 1999), M33 (Katoh-Fukui *et al.* 1998), fibroblast growth factor 9 (Colvin *et al.* 2001), and GATA binding protein 4 (Tevosian *et al.* 2002), are also known to be involved in

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adrenal or gonadal development since disruption or overexpression of these genes in mice causes sex reversal or delayed sexual differentiation (Katoh-Fukui *et al.* 1998, Vainio *et al.* 1999, Bamforth *et al.* 2001, Colvin *et al.* 2001, Schnabel *et al.* 2003, Hammer *et al.* 2005).

In animal experiments, adrenocortical tissue has been successfully regenerated through xenotransplantation of cloned adrenocortical cells (Thomas *et al.* 1997), suggesting that the intraadrenal stem cells required for such tissue formation may be present in the adrenal cortex. Stable expression of SF-1 has been shown to direct embryonic stem cells toward the steroidogenic lineage. However, this steroidogenic capacity was very limited since progesterone was the only steroid produced in the presence of an exogenous substrate, 20α-hydroxycholesterol (Crawford *et al.* 1997).

Bone marrow cells (BMCs) may contribute to the regeneration of hematopoietic or mesenchymal lineages in multiple organs (Pittenger *et al.* 1999, Song & Tuan 2004). Therefore, we tested whether the introduction of SF-1 into mouse BMCs could produce steroidogenic cells (Gondo *et al.* 2004). We expanded a relatively pure BMC population by culturing the cells for 120–180 days, and then demonstrated that adenovirus-mediated forced expression of SF-1 in long-term cultured BMCs can produce steroidogenic cells with the capacity for *de novo* synthesis of various steroid hormones in response to adrenocorticotropic hormone (ACTH). Similar to our data, a recent report demonstrated that cAMP stimulation of a cloned mouse BMC line, KUM9, and human BMC line, hMSC-hTERT-E6/E7, stably expressing SF-1 induced cell differentiation into cells with a testis- and adrenal-type steroidogenic capacity respectively (Yazawa *et al.* 2006). These results suggest a promising utility of BMCs as a regenerative source of steroidogenic cells.

For the future clinical application of SF-1 in autologous cell transplantation therapy for patients with steroid hormone deficiency, it is very important to examine its effect on human BMCs. In this regard, we applied this technique to human BMCs and compared their steroidogenic profiles with those of mouse BMCs. In addition, we investigated the impacts of several known factors, WT1, DAX-1, PBX-1, CITED2, and WNT4, which are involved in adrenal and/or gonadal development, to determine whether the single introduction of each factor may also be sufficient to transform human BMCs into steroidogenic cells.

Materials and methods

Construction of the adenovirus and the lentivirus vectors

A recombinant adenovirus vector derived from the human type 5 adenovirus using a commercially available Adenovirus Expression Vector Kit (TakaraBio Ltd, Shiga, Japan), containing bovine SF-1 cDNA (Adx-bSF-1) or LacZ cDNA (Adx-LacZ) as a control, and was prepared as previously described (Gondo *et al.* 2004). Full-length human cDNAs for SF-1, WT1, DAX-1, PBX-1, CITED2, and WNT4 were cloned by reverse transcriptase-polymerase chain reaction (RT-PCR) using appropriate tissue total RNA with primers based on the GenBank database, subcloned into pCR-Blunt II-TOPO vector (Invitrogen) and then the entire sequences of these constructs were verified by sequencing. Each human cDNA was inserted into lentiviral vector (CS-CDF-CG-PRE, RIKEN BioResource Center, Tsukuba, Japan) and the preparation of each recombinant lentivirus was performed according to the manufacturer's protocol.

Culture of human mesenchymal BMCs and treatment with adenovirus or lentivirus

Human bone marrow mononuclear cells purchased from Cambrex (East Rutherford, NJ, USA) were cultured in nonhematopoietic (NH) Expansion medium (Miltenyi Biotec, Bergisch Gladbach, Germany) at 37 °C in a 5% CO_2 incubator for 4 weeks. Only adherent cells were used in the experiments. To test the capability for osteoblastic differentiation, $4{\cdot}5\times10^4$ cells were plated on 35 mm dish, cultured in NH OsteoDiff medium (Miltenyi Biotec) for 10 days and stained with alkaline phosphatase (Gondo *et al.* 2004). Adipogenic differentiation was confirmed at a cell density of $7{\cdot}5\times10^4$ cells/35 mm dish following treatment with NH AdipoDiff medium (Miltenyi Biotec) for 21 days and stained with Oil red O to test the capacity of cells to differentiate into mature adipocytes (Song & Tuan 2004).

Human BMCs (10^4 cells/well) were cultured on a collagen type I-coated 24-well plate, incubated in Adx-bSF-1 or Adx-LacZ medium at 37 °C for 1 h, rinsed with PBS, and cultured for 7 days. Culture medium was then changed and BMCs were cultured for another 4 days for measurements of steroid contents. In addition, human BMCs (10^4 cells/well) were cultured on a 24-well plate, incubated in NH Expansion medium containing lentivirus vector at 37 °C overnight, rinsed with PBS, and then cultured for another 10 days before being subjected to experiments.

Measurements of the steroid content in the medium secreted from human BMCs

Basal secretion levels of progesterone (P4), corticosterone (B), cortisol (F), aldosterone, testosterone (T), and estradiol (E_2) secreted into the culture medium were measured using an EIA kit (Cayman Chemical, Ann

Arbor, MI, USA). Dehydroepiandrosterone (DHEA) was measured using another EIA kit (Assay Designs, Ann Arbor, MI, USA). The detection limits of P4, B, F, aldosterone, T, E_2, and DHEA were 10, 38, 17, 21, 6, 8, and 15 pg/ml respectively. The secretions of F and T into the medium were also confirmed in the presence or absence of synthetic 1–24 ACTH (Shionogi Co., Osaka, Japan) or human chorionic gonadotropin (hCG; Aska Co. Ltd, Tokyo, Japan). Human BMCs were infected with Adx-bSF-1 (multiplicity of infection, MOI=20) and cultured for 7 days. Culture medium was then collected at day 11, and the cells were stimulated with 2·4 μM ACTH or 10 mU/ml hCG. Every 3–4 days, culture medium was collected for measurement of F and T concentrations and cells were retreated with ACTH or hCG.

Quantitative real-time PCR

We performed quantitative analysis of the mRNA expression levels of ACTH receptor (ACTH-R), LH receptor (LH-R), and various steroidogenic enzymes including P450scc, 3β-hydroxysteroid dehydrogenase (3β-HSD), P450c21, P450c11, P450c17, P450ald, 17β-HSD type 3, and P450arom, by real-time PCR using a LightCycler (Roche Diagnostics GmbH) as described previously (Gondo *et al.* 2004). We isolated total RNA from cultured human BMCs using an RNeasy Mini kit (Qiagen, GmbH). We synthesized first-strand complimentary DNA using 1 μg total RNA as a template with QuantiTect RT kit (Qiagen) and carried out PCR in a LightCycler according to the manufacturer's instructions. We used the carefully designed sense/antisense primers of P450scc, 3β-HSD, P450c21, P450c11, P450ald, 17β-HSD type 3, ACTH-R, LH-R, P450arom, and β-actin (TakaraBio). PCR primers and conditions are available on request. Threshold values were obtained where fluorescent intensity was in the geometric phase of amplification, as determined with LightCycler Software Ver. 3.5. Products were verified on 2% agarose gels. We verified the nucleotide sequences of each PCR product by direct sequencing using the appropriate primers. Relative expression levels of the mRNAs were calibrated to those of β-actin.

Flowcytometry

The protocol essentially followed a previously described method (Hirase *et al.* 2000). Briefly, 1×10^5 BMCs were incubated with either phycoerythin (PE)-conjugated anti-human c-kit, CD11b, CD31, CD34, CD44, CD45, and CD105 monoclonal antibodies (BD Biosciences, Franklin Lakes, NJ, USA) or an isotype-matched PE-conjugated mouse IgG (BD Biosciences) for 30 min at 4 °C. The cells were finally analyzed on a FACScan flowcytometer (BD Biosciences).

Immunofluorescence cell staining

We conducted immunofluorescence cell staining of BMCs using goat anti-ACTH-R and anti-LH-R antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), rabbit antibody against bovine SF-1 (kindly provided by Prof. Morohashi, Kyushu University, Japan), Alexa Fluor 488 donkey anti-rabbit IgG and Alexa Fluor 594 rabbit anti-goat IgG (Molecular Probes, Eugene, OR, USA). Inoculated cells were plated onto CC2-treated chamber slides (Nalge Nunc International Co., Naperville, IL, USA), cultured for 3 days and fixed with 4% paraformaldehyde at 4 °C for 1 h. Immunofluorescence cell staining was then performed according to the manufacturer's protocol. The fluorescence was observed using fluorescence microscopy Biozero (Keyence, Tokyo, Japan).

Immunoblotting

Human BMCs were washed with PBS and lysed in CelLytic M cell lysis reagent (Sigma). A total of 20 μg protein was subjected to SDS-PAGE and transferred onto a PVDF membrane (Bio-Rad Laboratories). Western blotting was performed according to the manufacturer's protocol. Protein levels were visualized using an ECL Plus kit (GE Healthcare, Buckinghamshire, UK) and a LAS3000 detector (Fuji Film, Tokyo, Japan).

Statistical analysis

One-factor ANOVA was used for statistical evaluation. $P<0·05$ was considered to be statistically significant.

Results

Flowcytometric analyses revealed that the human BMCs prepared for steroidogenic transformation were negative for the surface markers including CD45 (leukocyte marker), CD11b (monocyte/macrophage marker), CD34, c-kit (hematopoietic stem cell markers), and CD31 (endothelial cell marker) but positive for CD44 and CD105 (potential marker for mouse mesenchymal cells; data not shown). Although the BMCs in our experiment still constitute a heterogeneous population, the analysis of cell surface markers strongly suggested the possibility that the steroid-producing cells originate from multipotent and immature stem cells. Importantly, human BMCs differentiated into an osteoblastic phenotype and adipocyte phenotype following respective proper treatment (data not shown), suggesting that the character of the human BMCs may be much closer to that of mesenchymal BMC lineages. These results suggest that

steroid-producing cells originate from multipotent and immature stem cells.

The above human mesenchymal BMCs were inoculated with Adx-bSF-1 (MOI=0, 10, 50, 100, and 200) and cultured for 3, 7, and 11 days. We analyzed the expression level of SF-1 using antibody against SF-1. The bovine SF-1 expression was observed predominantly in the nucleus of most of the cultured human BMCs even by minimal infection at MOI of 10 (Fig. 1A). The expression level seems to be increased in a MOI-dependent manner as shown by immunofluorescence staining at day 3 (Fig. 1A) and by western blot analysis at day 11 (Fig. 1B). Thus, the following experiments were performed 10 days after infection. As in mouse cells (Gondo *et al.* 2004), throughout these experiments, induction of endogenous SF-1, namely the expression of human SF-1 was not observed by real-time PCR (data not shown).

First, to quantify the basal secretion levels of steroid hormones, cultured medium (day 11) of human BMCs infected with Adx-bSF-1 or Adx-LacZ was analyzed. Cells were infected with Adx-bSF-1 and cultured for 7 days. The steroid content accumulating in the medium over the next 4 days was then measured. Progesterone (P4) secretion was increased with a dependency on MOI of Adx-bSF-1, namely SF-1 expression level (data not shown). The secretions of P4, corticosterone (B), cortisol (F), aldosterone, DHEA, testosterone (T), and E_2 into the medium from human BMCs infected with Adx-bSF-1 (MOI=50) were increased when compared with non-infected cells (MOI=0) or cells infected with Adx-LacZ (MOI=50; Fig. 2A). To investigate whether steroid synthetic enzymes were upregulated in human BMCs infected with Adx-bSF-1, the mRNA levels of such enzymes were analyzed by real-time PCR (Fig. 2B). The expression levels of P450scc, 3β-HSD, P450c21, P450c11, P450c17, 17β-HSD type 3, and P450arom mRNAs relative to β-actin, expressed as the ratio to those of non-infected BMCs (MOI=0), were significantly increased with increasing SF-1 expression level; cells infected with Adx-LacZ did not show such a phenomenon (Fig. 2B). Despite the presence of aldosterone production, we could not detect P450ald by real-time PCR.

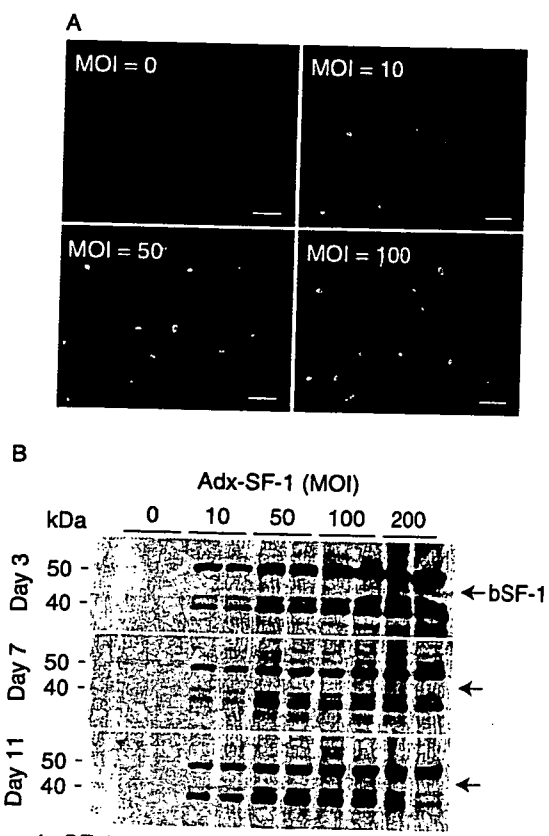

Figure 1 SF-1 expression in human BMCs infected with adenovirus vector. Human BMCs were inoculated with Adx-bSF-1 (MOI=0, 10, 50, 100, or 200) and cultured for 3, 7, and 11 days. To analyze the expression level of bSF-1, infected cells were subjected to (A) immunofluorescence staining and total cell lysate was subjected to (B) immunoblot analysis using an antibody against SF-1 (please see Materials and methods). The bar indicates a 100 μm scale.

In Adx-bSF-1-infected human BMCs, the mRNA expression levels of both ACTH-R and LH-R relative to β-actin were increased in a MOI-dependent manner (Fig. 3A), while those in cells infected with Adx-LacZ was undetectable. Next, human BMCs were infected with Adx-bSF-1 or Adx-LacZ (MOI=200), and 3 days later they were stained with specific antibodies against the ACTH-R, LH-R, or SF-1. As a result, both receptors were detected in SF-1-positive cells, suggesting actual inductions of both receptors by SF-1 (Fig. 3B). At 3 day intervals after infection of the cells with Adx-bSF-1 (MOI=20), the cells were stimulated with 2·4 μM ACTH or 10 mU/ml hCG. After stimulation the medium was collected for the measurement of steroid content. The secretion of cortisol into the medium was increased in response to both 2·4 μM ACTH and 10 mU/ml hCG stimulation (Fig. 3C). The maximum response of cortisol to ACTH or hCG was observed on day 21, when cortisol production was increased by 9·5- and 6-fold in response to ACTH and hCG respectively. Likewise, the secretion of testosterone was increased in response to both hCG and ACTH stimulation, and the maximum response was obtained at day 18 (Fig. 3C). Together, these data indicate a capability of human mesenchymal BMCs to be transformed into steroidogenic cells, which are capable of responding to ACTH and LH.

Finally, we tested the effects of several factors that are reported to be associated with adrenal and/or gonadal development, mainly based on the results of human or mouse phenotypes due to a complete or partial lack of each factor (Muscatelli *et al.* 1994, Yanase *et al.* 1996, Vainio *et al.* 1999, Bamforth *et al.* 2001, Schnabel *et al.*

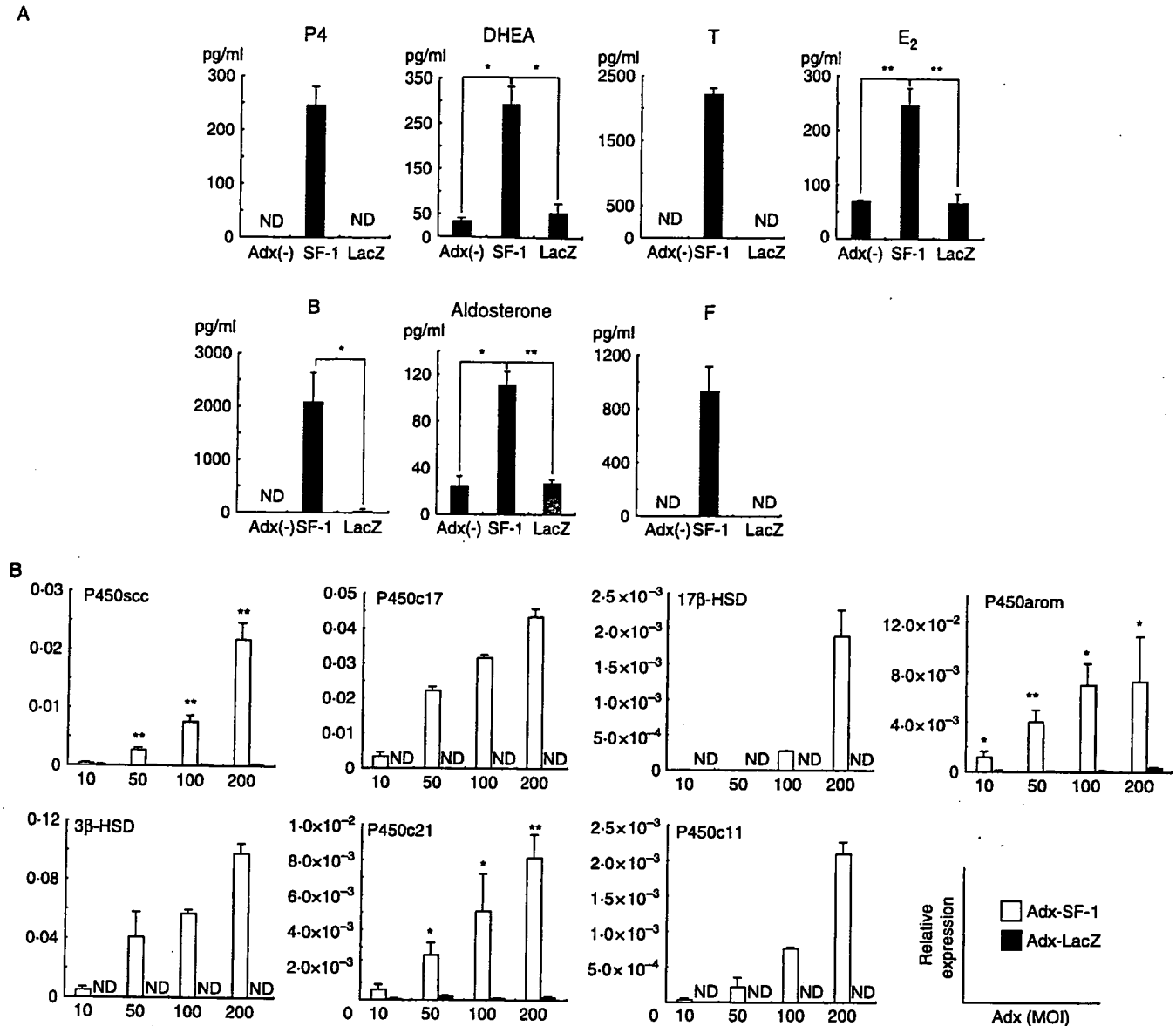

Figure 2 Basal steroid production and mRNA expression levels of steroidogenic enzymes in human BMCs infected with Adx-bSF-1. Cells were infected with Adx-bSF-1 and cultured for 7 days. The steroid content accumulated in the medium over the next 4 days was then measured. (A) Basal secretion of progesterone (P4), corticosterone (B), aldosterone, cortisol (F), DHEA, testosterone (T), and estradiol (E_2) into the medium from the human BMCs infected with Adx-bSF-1 (MOI=0, 50) or Adx-LacZ (MOI=50). (B) Real-time PCR analysis of the expression levels of P450scc, 3β-HSD, P450c11, P450c21, P450c17, 17β-HSD type 3, and P450arom mRNAs in human BMC cells infected with Adx-bSF-1 or Adx-LacZ. The mRNA expression levels relative to the levels of β-actin are expressed. Values represent the means±S.D. (n=3). $^{*}P<0.05$, $^{**}P<0.01$.

2003). The tested factors were SF-1 as a positive control, GFP as a negative control, WT1, DAX-1, PBX-1, CITED2, and WNT4. WT1 encodes a zinc finger transcription factor. Alternative splicing of exon 5 results in a 17 amino acid insertion, and alternative splicing of exon 9 produces an insertion three amino acid Lys-Thr-Ser (KTS). Alternative splicing these two sites gives rise to four different protein isoforms designated A, B, C, and D; or (−/−), (+/−), (−/+), and (+/+), representing the presence or absence of exon 5 and KTS respectively (Haber *et al.* 1991). In this experiment, for more convenience in the preparation or infection of cells with vector, cells were infected with lentivirus containing each factor. In a preliminary experiment, the expression of each factor was confirmed by real-time PCR or western blotting of the respective infected cells, 10 days after infection (data not shown). Therefore, 10 days after infection, the medium was changed and the F and T content in the medium on each of the next 4 days (day 11–14 days) were measured; the expression levels of P450scc in cells at day 14 were also determined by real-time PCR. Introduction

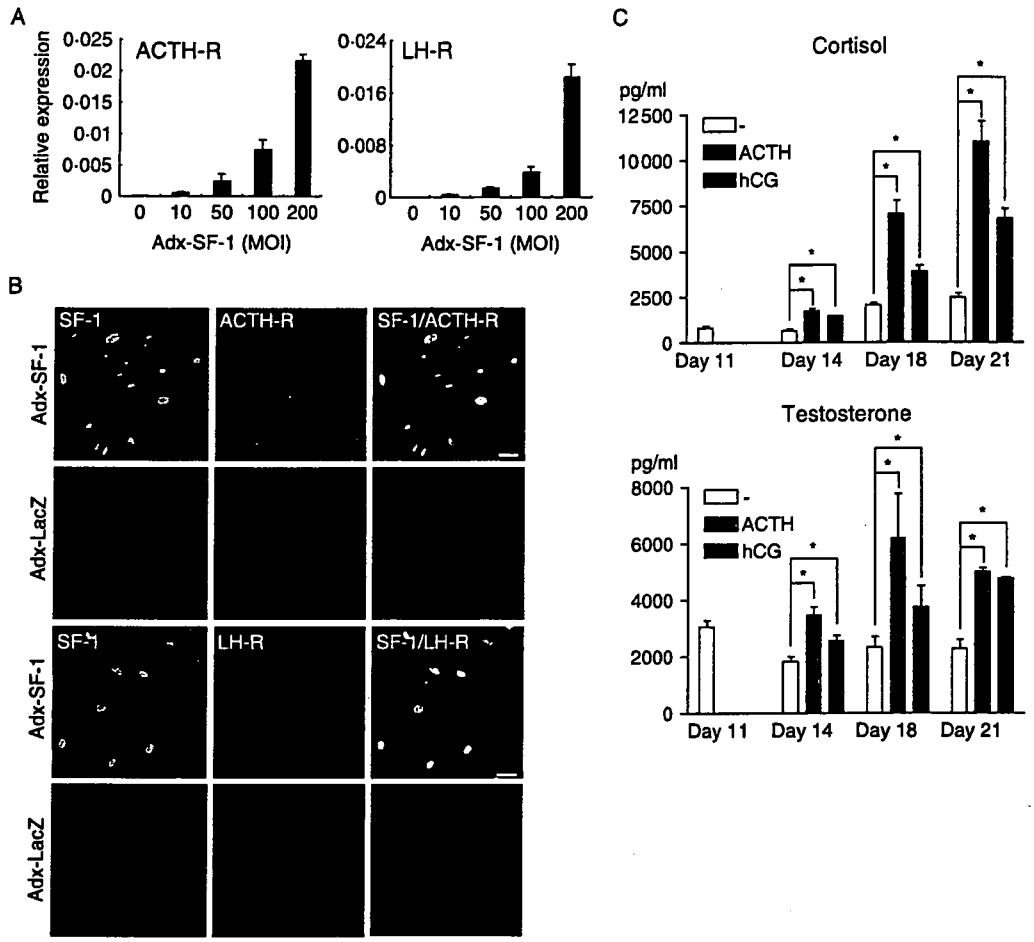

Figure 3 Expression of ACTH-R and LH-R and responsiveness to ATCH and LH in cultured human BMCs. (A) Human BMCs infected with Adx-bSF-1 or Adx-LacZ were cultured for 11 days and then the expression levels of ACTH-R and LH-R were determined by real-time PCR. (B) Human BMCs infected with Adx-bSF-1 or Adx-LacZ (MOI=200) were cultured for 3 days, and then the expression levels of ACTH-R and LH-R were determined by immunofluorescence staining using antibodies against SF-1 and ACTH-R or LH-R. The bar indicates a 50 μm scale. (C) The effect of ACTH and LH on the secretion of cortisol and testosterone respectively, from cultured human BMCs. Human BMCs were infected with Adx-bSF-1 (MOI=20) and cultured for 7 days. Culture medium was then collected at day 11 for measurement of basal secretions of steroids, and the cells were stimulated with 2·4 μM ACTH or 10 mU/ml hCG. Every 3–4 days, culture medium was collected for measurement of cortisol and testosterone concentrations and cells were treated with ACTH or hCG. The secretion of cortisol or testosterone into the medium was increased in response to ACTH and hCG stimulation respectively. Values represent the means±S.D. (n=3). $P<0·05$ against the control (absence of ACTH or hCG).

of SF-1 only into human BMCs induced a 4·7-fold induction of P450scc expression when compared with GFP, and caused 11·3- and 12·5-fold increase in the secretion of F and T into the medium respectively. Single introductions of WT1 (−/−, −/+, +/−, and +/+), DAX-1, PBX-1, CITED2, and WNT4 did not produce any such effects (data not shown).

Discussion

Although the character of human mesenchymal stem cells has not been fully clarified and remains controversial, steroid-producing cells may originate from multipotent and immature stem cells. Importantly, human cultured BMCs were able to differentiate into an osteoblastic or adipocyte phenotype following appropriate treatments, suggesting that the character of the prepared human BMCs may be much closer to mesenchymal BMC lineages. Under such conditions, we demonstrated, for the first time, that human BMCs infected with Adx-bSF-1 can produce significant amounts of progesterone, corticosterone, cortisol, DHEA, testosterone, and estradiol, owing to the expression of mRNAs for P450scc, 3β-HSD, P450c11, P450c21 P450c17, 17β-HSD type 3, and P450arom, as detected by RT-PCR. Most of the profiles of steroidogenic enzymes were quite similar to those in mouse

BMCs (Gondo *et al.* 2004), except for the finding that P450c21 is expressed in human cells but not in mouse cells. Although we observed a significant amount of aldosterone production, we could not show the expression of P450ald by real-time PCR (data not shown). Thus, we cannot completely conclude a capability of human BMCs for aldosterone production. Further studies are needed on this point including the optimization of PCR conditions. As in mouse cells (Gondo *et al.* 2004), the steroid profile of cultured human BMCs showed a mixed pattern of adrenal and gonadal steroidogenesis, namely the simultaneous production of cortisol and sex steroids. This finding matches well an established concept that common precursor cells divide into two distinct characteristic cell types, designated adrenal precursor cells and bipotential gonadal precursor cells, and finally develop into the adrenal cortex or testes and ovaries, respectively, under the regulation of many transcriptional factors, including SF-1 (Yanase *et al.* 2006).

A feature of human steroidogenic cells that makes them remarkably distinct from mouse cells is the clear induction of ACTH-R and LH-R following the introduction of SF-1. Mouse BMCs originally expressed ACTH-R without introduction of SF-1, namely predominant expression of an adipose tissue type of ACTH-R (Kubo *et al.* 2004), rather than an adrenal tissue type of ACTH-R (Cammas *et al.* 1997). The introduction of SF-1 itself did not increase ACTH-R expression in mouse BMCs, since the promoter region of the adipose tissue type ACTH-R gene does not contain SF-1 sites, unlike the promoter of the adrenal type ACTH-R gene (Cammas *et al.* 1997). On the other hand, the gene encoding the human ACTH receptor does not seem to produce extra-adrenal type isoform from its gene structure (Kubo *et al.* 2004) and produces only adrenal type receptor, which can be responsive to SF-1; mRNA for this receptor was expressed in human BMCs (data not shown). Similarly, LH-R was dramatically induced in human BMCs, but not in mouse BMCs. The human LH-R gene promoter also contains a potential SF-1 site, although binding SF-1 to this site is not proven (Geng *et al.* 1999). There may be an unknown SF-1 induced factor that promotes the expression of LH-R. Anyway, the findings of good induction of ACTH-R and LH-R in human BMCs and responsiveness of these cells to ACTH and LH are physiologically important when we consider a future clinical application for autologous BMC transplantation into patients with steroid insufficiency.

In humans, sex-determining region Y (SRY) initiates the testis-determining pathway by inducing the expression of a series of downstream factors, including SF-1, WT1, DAX-1, SRY-related genes HMG-box 9, and several other factors, thus resulting in a male phenotype (Yanase *et al.* 2006). In this pathway, SF-1 has been considered to be a master regulator that controls the overall stream of the developmental mechanism. WT1 seems to be of interest because, in contrast to the adrenal agenesis seen in mice lacking all Wt1 transcript (Wagner *et al.* 2003), analyses of mice with isoform-specific disruption of Wt1 have shown different roles for two alternatively spliced transcripts the −KTS and +KTS isoforms (Hammes *et al.* 2001). Namely, selective inactivation of the +KTS isoform did not impair ovary development, but rather impaired testis and male sex differentiations. PBX-1 is a homeodomain protein that contributes to adrenal development since, PBX-1 KO mice, which die *in utero* due to defects in multiple organs, completely lacked adrenal glands and had impaired testis development associated with decreased proliferation in the urogenital ridges (Schnabel *et al.* 2003). A coactivator CITED2 coregulates genes that are essential for adrenal development since its KO mice were found to have adrenal agenesis (Bamforth *et al.* 2001). Wnt proteins act via the frizzled receptor family to initiate a canonical cascade of intracellular signals leading to β-catenin accumulation in the nucleus and subsequent transactivation of downstream target genes (Tolwinski & Wieschaus 2004). Disruption of WNT4 in mice causes a marked masculinization of XX females with absence of the female Mullerian duct and persistence of the male Wolffian duct derivatives due to excess gonadal testosterone synthesis, and abnormal differentiation of the definitive zone in the adrenal gland (Vainio *et al.* 1999).

While, admittedly, the present experiment was preliminary in that we have not dealt with all known factors, we tested whether the above factors, which are closely associated with sexual differentiation including adrenal and/or gonadal development, could transform human BMCs into steroidogenic cells. Our finding that only SF-1 among SF-1, WT1, DAX-1, PBX-1, CITED2, and WNT4 could transform human BMCs into steroidogenic cells suggested that SF-1 is truly a master regulator, even for the production of steroidogenic cells from human mesenchymal BMCs. However, it is also important to test the functional relationship between SF-1 and other factors involved in steroidogenesis by multiple transfections, because sexual differentiation takes place in a series of cascades of various factors (Yanase *et al.* 2006). Such detailed experiments are currently underway in our laboratory.

In summary, we showed that SF-1 could transform human bone marrow mesenchymal cells into steroidogenic cells. This finding is expected to develop extensively in the near future, possibly leading to the development of therapies to relieve the many patients who are forced to take hormone replacement therapy.

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肥満・高脂血症

～産婦人科の立場から～

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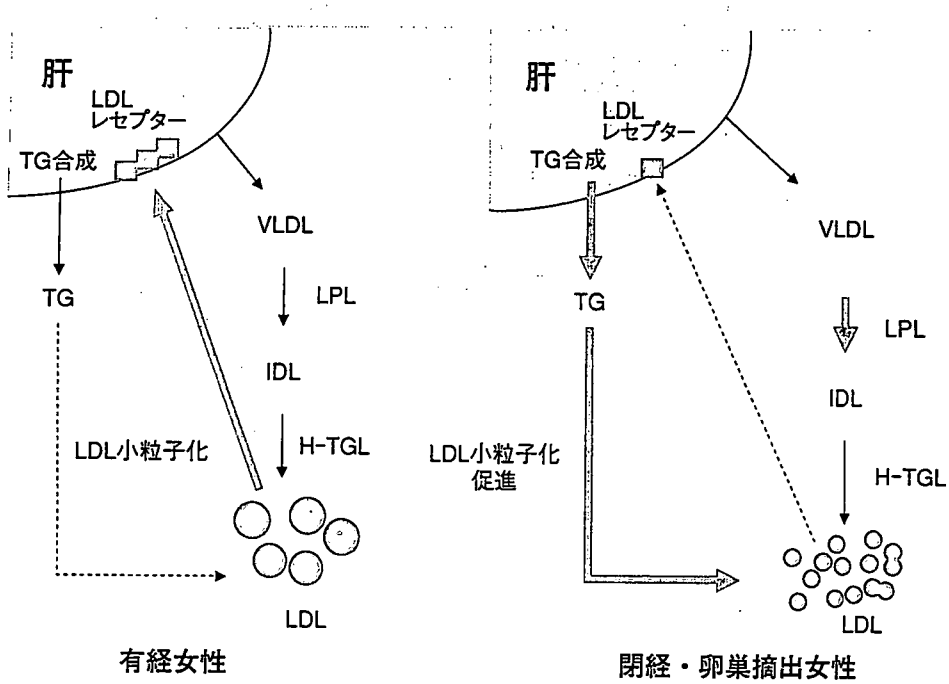
はじめに

Framingham Studyによると心血管系疾患(CVD)の発症頻度は，50歳以前では男性が女性の3～4倍高率であるのに対し，50歳以降，女性の頻度が急激に増加し，70歳代でほとんど男女差がなくなる。高脂血症の頻度も50歳以前では，男性が高率で推移するのに対し，50歳以降では逆に女性が高率となり，50％以上の割合で高脂血症と診断される。女性の平均閉経年齢は50歳といわれており，閉経後のエストロゲン濃度の低下が脂質代謝異常を惹起するものと考えられる。

一方，ライフスタイルの欧米化に伴い，日本人の肥満人口は増加の一途をたどっている。肥満の年次推移をみると，その頻度は男女ともに増加の傾向が示されており，男女間の肥満の頻度を比較すると，50歳未満では男性が高率であるが女性の場合は年齢とともに増加し，50歳以降では逆に男性より高率となる。肥満の分類としては，1940年代には単に男性肥満と女性肥満という概念が提唱されていたが，1980年代になるとウエスト・ヒップ比(W/H比)を用いた上半身肥満と下半身肥満が，さらに近年，肥

図1．有経および低エストロゲン環境女性の脂質代謝動態（文献1より）



満を皮下脂肪と内臓脂肪に分別するようにもなってきた。女性における肥満も閉経を契機にその頻度が増加するといわれており，エストロゲン濃度が肥満に関与する可能性が考えられる。

閉経後高脂血症の発症機序

1. エストロゲン欠乏（図1）

閉経後，エストロゲン濃度が低下すると，肝のLDL受容体からのLDLの取り込みが低下するため，血中にLDLが停滞する。また，閉経後や両側卵巣摘除後，LDLの律速酵素の一つであるリポプロテインリパーゼ（LPL）活性が亢進し，LDL合成の亢進につながることも明らかになっている。したがって，エストロゲン濃度の低下が，LDLの合成系を亢進し，異化系を低下させるため，LDLは血中に蓄積する。HDL-コレステロール（HDL-C）は閉経後，若干低下あるいは変化無しなどと報告されている。

一方，中性脂肪（TG）もLDLと同様，閉経後に上昇する。単独のCVDの危険因子とみなされている高TG血症は，超悪玉といわれる小型サイズのLDL粒子を産生することが最近注目されている。低エストロゲン環境の女性のLDL粒子サイズと血中脂質濃度との関連性を検討した結果，閉経後に上昇するTGがLDLを小粒子化させることが明らかにされている[2]。小型LDLは，血中ではLDL受容体との親和性に乏しいため，肝内へ取り込まれにくく，血中に停滞しやすい。さらに血管内皮下では活性酸素に容易に酸化変性される。

以上の2点が，小型LDLが超悪玉といわれる理由である。したがって，低エストロゲン環境になると血中に蓄積した小型LDLは血管壁内に侵入し，容易に活性酸素に酸化される。この酸化されたLDLはマクロファージに一方的に取り込まれ，最終的に泡沫細胞から粥状硬化へと進展する。このように，閉経後女性のLDLの蓄積は，量的にも質的にもCVDの最大の危険因子になる。

2. 肥満

内臓脂肪型肥満とは，内臓脂肪/皮下脂肪比（v/s比）が0.4以上の場合をいい，高脂血症のみならずインスリン抵抗性の存在，アディポサイトカインの産生，内皮機能低下など様々な病態を惹起する（表1）[3,4]。脂質代謝では総コレステロール（TC），TGが高値でIIb，IV型の高脂血症が多い。内臓脂肪型肥満には脂質代謝異常を併発することが多いといわれているが，その機序として，まず蓄積した内臓脂肪が分解され，遊離脂肪酸（FFA）が放出される。次にFFAは門脈を介して肝臓に運ばれ，肝でのTG合成が高まり，VLDL合成亢進につながる。また，肝へのFFAの過剰流入はインスリン感受性を低下させ，インスリン抵抗性のさらなる増悪にもつながる。

表1．皮下，内臓脂肪型肥満分類（文献3，4より）

〈皮下脂肪型肥満〉
内臓脂肪面積/皮下脂肪面積の比が0.4未満
〈内臓脂肪型肥満〉
内臓脂肪面積/皮下脂肪面積の比が0.4以上
1. インスリン抵抗性の存在 2. 総コレステロールと中性脂肪高値，HDLコレステロール低値 3. アディポサイトカイン産生 4. 血管内皮機能低下

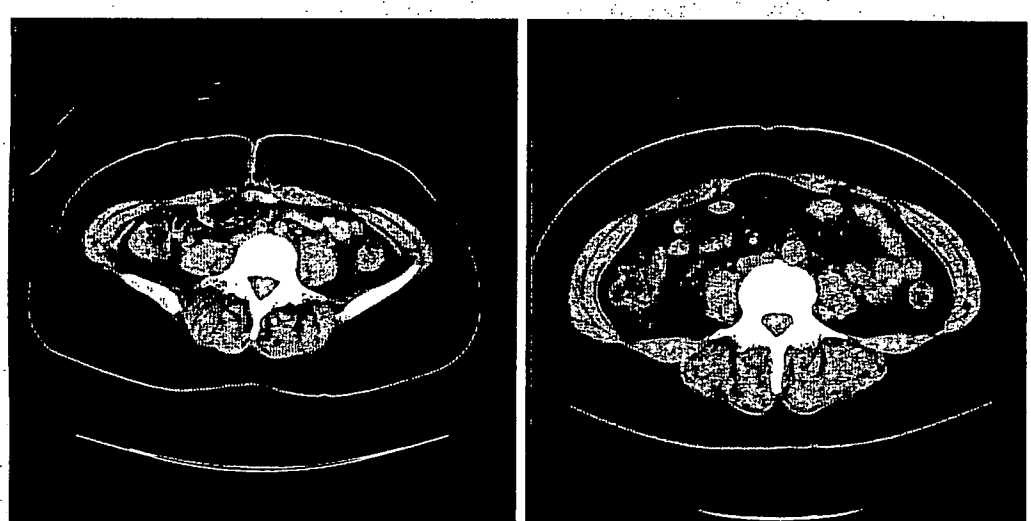
内臓脂肪型肥満を増加させる因子として遺伝，加齢，食事，生活習慣などがあるが，女性では閉経後に内臓脂肪型肥満が急速に増加するといわれており，性ステロイドとの関連性が考えられる。

図2に有経，閉経後女性の内臓脂肪/皮下脂肪比の典型例を示したが，明らかに閉経後女性の内臓脂肪が多いことがわかる。このように，閉経後のTGの上昇は，内臓脂肪型肥満の増加が関与している可能性がある。

閉経後ホルモン補充療法と脂質代謝

経口エストロゲンを投与すると，TCとLDLコレステロール(LDL-C)は低下し，HDL-Cは増加する。経口エストロゲンは閉経後に減少した肝のLDL受容体を再度増加させ，さらにIDLからLDLへの変換をつかさどる肝性リパーゼ(H-TGL)活性を抑制することから，LDLの合成系を抑制し，異化系を亢進させることで血中LDL濃度を低下させる。

図2. 有経および閉経後女性の腹部CT写真



V/S比：0.079
有経女性

V/S比：0.91
閉経後女性

表2. 経口と経皮エストロゲンの差異

	経口エストロゲン		経皮エストロゲン
結合型エストロゲン	0.625mg	0.3125mg	
脂質			
TC	減少	減少	不変
TG	上昇	不変	減少
HDL-C	上昇	不変	不変
LDL-C	減少	減少	不変
LDLサイズ	減少	不変	増加
LDL酸化	不変	減少	減少

好影響
悪影響

また，H－TGLはHDLの肝内への移行を促すので，H－TGL活性の抑制が血中HDLの増加にもつながる。このように経口エストロゲンは脂質代謝改善作用を有する反面，TGを増加させる。従来，経口エストロゲンによるTG上昇は動脈硬化の発症には関与しないと考えられてきたが，経口エストロゲンによるTGの上昇がLDLを小粒子化することが明らかになっている。したがって，経口エストロゲンによるTG増加はエストロゲンの抗酸化作用を相殺し，逆に酸化されやすい小型LDLに変化させることが証明されている[1]。

一方，エストロゲンを経皮的に投与した場合，TC，LDL－C，HDL－Cへの明らかな影響はなく，脂質代謝改善作用という面からみると経口エストロゲンが優れているといえる。しかし，経皮エストロゲンは逆にTGを減少するためLDL粒子径はむしろ大型化し，酸化されにくいLDLに変化することもわかっている[5]。これはおそらく経皮投与の場合，初回通過効果がないためと考えられる。また，経口エストロゲンを減量することでもこれらの悪影響を回避できることもわかっており[6]，これからのHRTは経口よりも経皮を，あるいは低用量の経口エストロゲンを選択すべきである(表2)。

おわりに

エストロゲン補充はCVDのリスクを低下させるとの数多くの疫学研究結果から，欧米では約1,000万人の閉経後女性がHRTを受けていた。しかし，2002年に報告されたWomen's Health Initiative (WHI)により[7]，今までとは全く逆にHRTはCVDリスクを増加すると結論された。なぜ，このような矛盾した成績になったのか，最近の研究により明らかになりつつある。

経口エストロゲンはLDLを低下，HDLを増加する脂質代謝改善作用を有するが，TGを増加させ，LDLを小型化する。さらに，経口エストロゲンは血管炎症を促進させる作用があることなどがわかってきた。これらの悪影響は投与ルートを経口から経皮に変更したり，経口投与量を減量することで，回避できることも証明されている。しかし，WHIの報道以来，残念ながら現段階ではHRTは高脂血症の治療としての適応にはならない。したがって，TCやTGが高値の場合にはスタチンやフィブラート製剤の投与が原則である。

しかし，女性の高脂血症は治療する必要があるのか否か，あるいは脂質をどのレベルまで低下すべきかについては，いまだ明確な結論が出ておらず，現在進められている大規模臨床試験の結果に期待したい。

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シンポジウム ホルモン補充療法—その基礎と臨床—

心血管疾患の発症予防を目的とした新しいホルモン補充療法の開発

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Hormone Replacement Therapy for Reducing the Risk of Cardiovascular Disease

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Key words : Postmenopausal women・Estrogen・Cardiovascular disease

はじめに

閉経後のエストロゲン濃度の低下は，心血管疾患(CVD)のリスクを増加することが知られている．Framingham Studyでは平均閉経年齢である50歳以後にCVDの発症率が上昇することが報告されており[1]，わが国でも同様に50歳を越えると女性のリスクが急増し，男性のリスクに近づく傾向にある．したがって，エストロゲン濃度がCVD発症と密接に関連することが窺える．一方，ホルモン補充療法(HRT)はLDLコレステロール(LDL-C)やLp(a)を低下し，HDLコレステロール(HDL-C)を増加する脂質代謝改善作用，抗酸化作用，血管拡張作用，エンドセリン1抑制作用，接着因子低下作用，降圧作用など多くの動脈硬化抑制作用を有し，CVDの発症や死亡率を減少するといわれてきた．しかし，最近行われた大規模臨床試験により，HRTはCVDリスクを逆に増加すると結論され，HRTを断念する症例が増加している．しかしHRTを受けていたほとんどの症例は更年期障害の治療目的で，閉経後女性のQOLに多大なる悪影響を及ぼしている．米国ではCVDによる死亡率が高率であるため，その予防目的も含めて多数の閉経後女性がHRTを受けていたが，最近のHRTの報道以来減少傾向にあるといわれている．このように現在，HRT施行について世界中が混乱した状態にある．これらの大規模臨床試験では対象者の年齢が高齢でCVDの程度が重度であったことや，肥満，高血圧，喫煙者などのCVDの危険因子を有する患者が多数存在したなどの欠点はあるものの，従来のHRTは動脈硬化に抑制的な作用を有する一方，促進的作用もあると考えるべきである．したがって，従来のHRTの短所を明確にし，今後HRTをどう改善すべきかを検討する必要がある．

1. HRTとCVDリスクに関する大規模臨床試験

(1) CVDリスクに肯定的な臨床試験

① Nurses Health Study[2]

米国の看護師を対象に行ったアンケート調査である．この試験ではHRTによる冠動脈疾患と脳卒中の死亡に関する相対危険度は各々0.47と0.68と低値で，HRTが動脈硬化に抑制的に作用することが示されている．

これらの結果を基に，1992年にAmerican College of Physiciansは「すべての閉経後女性にHRTを考慮すべき」と勧告したため，米国でHRTが広く普及するようになり，本邦でもこの頃からHRTについての理解が徐々に深まりはじめた．

② Estrogen/Progestin Interventions Trial(PEPI)試験[3]

HRTを施行する際，子宮を有する女性には子宮内膜癌の発症予防目的でプロゲスチン製剤を併用するが，PEPI試験はエストロゲンのみならずプロゲスチン製剤が脂質代謝などに与える影響を検討したものである．HRTの方法は結合型エストロゲン(CEE)0.625mg単独群，CEE＋酢酸メドロキシプロゲステロン(MPA)併用群，CEE＋micronizedプロゲステロン(MP)併用群，プラセボ群に分類され，CEEはLDL-Cを低下し，HDL-Cを増加することが示された．しかし，MPAはCEEのHDL-Cの増加作用に相殺する一方，MPには相

殺作用がないことも示された．また，MPAのみならずMPでも子宮内膜保護作用が認められたので，脂質代謝という観点からみると，MPの使用が適切と結論している．PEPI試験の結果から，HRTは脂質代謝の改善作用を有し，CVDの発症予防に有効であろうと結論され，American Heart Association(AHA)は1995年に「すべての虚血性心疾患を有する閉経後女性にHRTを考慮すべき」とした．さらに1997年には「虚血性心疾患の1次予防に関し，高LDL血症のような危険因子を多数有する閉経後女性にはHRTを考慮すべき」という内容の勧告を出したので，米国ではHRTがさらに普及していった．

しかし，PEPI試験以外の大規模臨床試験の多くは観察試験によるもので，信頼性に関しては未だ疑問点が残された．そこで，無作為化した前向きの2重盲検試験での結果が必要とされ，この頃すでに開始されていた後述するWomen's Health Initiative (WHI) やHeart and Estrogen/Progestin Study(HERS)の結果に期待がよせられた．

(2) CVDリスクに否定的な臨床試験

① HERS[4]

冠動脈疾患を有する女性を対象とし，CEE 0.625 mg＋MPA 2.5mgの連続投与がCVDのイベント発生にどのような影響を与えるかを前向きの無作為化・2重盲検法を用いて検討した試験である．その結果，PEPI試験の結果と同様に，HRT群はLDL-Cを低下し，HDL-Cを増加させることが示されたが，最初の数年間に限ってイベント発生の増加を認めた．HERSでは対象者の年齢が高齢であり，冠動脈疾患の程度が重度の患者が多かったことなど，試験デザインに欠点はあるものの，従来の報告とは全く異なる成績であったため，AHAは2001年に「虚血性心疾患を有する閉経後女性に対し，CVDの2次予防としての使用は推奨しない」と従来とは全く逆の勧告を出した．

② WHI(CEE＋MPA投与群)[5]

米国のNational Institute of Health(NIH)は更年期以後の米国女性のQOLに関する疾患の研究を目的としてWHIを企画した．試験は健康女性を対象とし，前向きに，無作為・2重盲検法で行われた．HRT群はCEE 0.625mg＋MPA 2.5mgの合剤を1錠/日の連続投与で行った．その結果，HRT群において乳癌の発症が前もって設定してあった数値を超えたため，試験は2002年に急遽，中止された．しかし，後述するCEE単独群では悪影響が認められず，試験を継続することとなった．HRT群における解析結果では，全死亡率や全ての癌の発症はHRT群とプラセボ群間で差がなかったが，HRT群で全心臓疾患は22%増加し，骨折は24%低下した．さらにこの結果を詳細にみてみると，HRT群で心筋梗塞が29%，脳卒中が41%，肺塞栓症が113%，乳癌が26%増加する一方で，大腸癌は37%，大腿骨骨折は34%減少しているが，総合的にはHRTによるリスクがベネフィットを上回ると判定された．したがって，HRTのCVDに対する1次予防についても否定的な結果となり，AHAは「HRTはCVDの1次および2次予防の目的で施行すべきでない」と勧告するに至った．このWHIの成績は本邦ではマスメディアによりかなりの偏りをもって報道されたため，HRT施行中の女性のみならず更年期医療に携わっている医療従事者にも強い衝撃が与えられ，HRTの可否について現在，極めて混乱した状態となっている．

④ WHI(CEE投与群)[6]

CEE単独投与による結果が2004年に報告された．内容はCEE＋MPA群とは異なり，冠動脈疾患と乳癌のリスクは増加せず，脳卒中はHRT群と同様に増加することが示された．したがって，CVDリスクにMPAが悪影響を与えている可能性があるが，エストロゲン投与方法も悪影響していると考えられた．

2. HRTがCVDリスクを増加した要因

(1) 経口エストロゲンによる中性脂肪(TG)の増加がLDLを小粒子化する．

経口エストロゲンはLDL-Cを低下し，HDL-Cを増加する脂質代謝改善作用を有するが，TGも増加させる．このTG増加の臨床的意義は従来ないとされてきたが，我々の検討により，LDLを超悪玉の小型LDLに変化させることを明らかにした[7][8]．小型LDLが超悪玉である理由としては，肝のLDL受容体との親和性に乏しいため血中にLDLが停滞しやすく[9]，血管壁内では容易に活性酸素に酸化変性され，マクロファージに取り込まれ，粥状硬化に進展しやすいことがある[10]．

LDLの小粒子化の機序には3つのステップがあることを明らかにしている．まず最初は経口エストロゲンによるTGの増加である．次にTG増加がリポ蛋白間の脂質転送能を亢進させ，深層脂質であるTGとコレステロールエステル(CE)成分に変化を与え，TG-rich，CE-poorなLDL粒子に変化させる[11]．この状態

ではまだLDLサイズに変化はないが，最終的に少量のリパーゼが存在するとTGを加水分解し，LDLは小粒子化する[12]．

(2) 経口エストロゲンが血管炎症に促進的に作用する．

動脈硬化の発症，進展には血管の炎症が密接に関与することが注目されている．急性冠症候群の発症機序としてはまず，血管プラーク内の炎症が活発になり，さらに蛋白分解酵素のmatrix metalloproteinase (MMP)が活性化され，プラークの線維性被膜が脆弱化し，最終的に破綻する．破綻後は血管内に血栓が急速に充満するため，急性症状を呈するようになる．このように血管炎症はCVDのイベントを増加させる重大な危険因子である．我々は経口エストロゲンの炎症マーカーに対する影響を検討し，高感度CRPや血清アミロイド蛋白A(SAA)やinterleukin-6(IL-6)などの急性炎症マーカーを上昇し，MMP-3を上昇，MMPに抑制的に作用するtissue inhibitor of MMP(TIMP)-1を低下させることを明らかにした[13][14]．したがって，HERSにおけるHRTのCVDイベントの増加は，経口エストロゲンの血管炎症促進作用がプラークを不安定にさせた可能性が考えられる．

(3) エストロゲンに併用する酢酸メドロキシプロゲステロン(MPA)がエストロゲンの抗動脈硬化作用に相殺する．

①脂質：経口の結合型エストロゲン(CEE)に併用するMPAの量を0，2.5，5.0mgの3群に分別して3カ月間連続投与するとLDL-Cはいずれの群も同様に低下するが，HDL-CはCEE単独群で増加するが，MPAはその併用量と用量依存的に低下させることが示された[15]．したがって，MPAはLDL-Cへの影響は否定的であるが，HDL-Cを低下させることが明らかになった．

②血管内皮機能

超音波プローベで上腕動脈径を計測し，内皮依存性の血管拡張反応をflow mediated vasodilation(FMD)とし，内皮非依存性の血管拡張反応をnitroglycerin induced vasodilation(NID)とした．いずれの群もNIDには変化なかったが，FMDはCEE単独投与で上昇し，MPA併用群でMPAの量と用量依存的に低下した．したがって，MPAはエストロゲンによる血管内皮改善作用に相殺的に作用することが明らかになった．一方，エストロゲンやMPAの血管平滑筋への作用は否定的であると考えられる[15]．

3. 悪影響を回避した新しいHRT

(1) 低用量の経口エストロゲンあるいは経皮的エストロゲン投与

これまでHRTのほとんどは経口投与であったが，この場合，初回肝通過効果が悪影響していると考え，経口エストロゲン量の減量する，あるいはHRTの投与ルートを経口から経皮へ変更する方法を考案した．実際には従来のCEE 0.625mgと0.3125mgの連日投与さらには経皮エストロゲン0.72mgの隔日投与を3カ月間行い，下記のパラメーターを比較検討した．

① 脂質およびLDL粒子サイズ

CEE 0.625 mgの場合，総コレステロール(TC)とLDL-Cは低下し，HDL-Cは増加する．CEE 0.3125 mgの場合は，HDL-Cには変化がないが，TCとLDL-Cは同様に低下した．したがって，低用量にしてもエストロゲンのLDL-Cの低下は温存されることが明らかになった．一方，経皮エストロゲンではいずれも変化なく，脂質に関しては経口の方が有利と考えられる．しかし，CEE 0.625mgではTGが増加し，LDLは小粒子化する．このため，LDLの被酸化性の指標であるTBARSやlag timeは変化せず，LDLの小粒子化がエストロゲンの抗酸化作用に相殺的に作用した可能性が示唆された．CEE 0.3125mgではTGの増加やLDLの小粒子化はなく[16]，経皮エストロゲンでは逆にTGは低下し，LDLは大型化した[17]．このため，両群ともにLDLの小粒子化がないため，TBARSはいずれも低下し[16][17]，エストロゲンの抗酸化作用が発揮でき(表1, 2)，従来の経口エストロゲンの悪影響を回避できることが示された．

②血管炎症

CEE 0.625mgでは血管炎症マーカーであるCRPやSAAさらにはIL-6などを上昇させ，MMP-3も増加し，TIMP-1は低下する．したがって，血管炎症に促進的に作用し，プラークの破綻に促進的に作用する可能性が示唆された．CEE 0.3125mgでは血管炎症マーカーやMMP，TIMPに影響せず[18]，経皮エストロゲンではCRPを低下させ，TIMP-1を上昇させることが示された[19]．したがって，低用量の経口エストロゲンでは従来の経口のような血管炎症促進作用はなく，経皮では逆に炎症に抑制的に作用し，プラークを安定化させる可能性が考えられた(図1～3)．

血中の単球は血管内皮上をローリング後，接着因子

表1　低用量エストロゲンがLDLの粒子径と被酸化性に与える影響

	コントロール		CEE			
			0.3125 mg		0.625 mg	
	投与前	投与後	投与前	投与後	投与前	投与後
LDL粒子径（nm）	25.46 ± 1.13	25.44 ± 0.90	25.50 ± 0.89	25.41 ± 0.74	25.99 ± 1.00	25.46 ± 0.96 **
LDL-derived TBARS (nmol/200μg)	21.6 ± 9.4	23.3 ± 10.9	20.0 ± 3.7	17.2 ± 2.8 *	19.0 ± 9.3	20.5 ± 9.8
LDL-lag time（min）	—	—	74.8 ± 11.6	84.7 ± 12.8 **	82.9 ± 11.1	84.6 ± 6.1

CEE, conjugated equine estrogen; LDL, low-density lipoprotein;
TBARS, thiobarbituric acid reactive substances;
* $P < 0.05$；** $P < 0.01$ vs 投与前

表2　経皮エストロゲンがLDLの粒子径と被酸化性に与える影響

	コントロール		経口投与		経皮投与	
	投与前	投与後	投与前	投与後	投与前	投与後
LDL粒子径（nm）	25.46 ± 1.13	25.44 ± 0.90	26.08 ± 0.96	25.59 ± 0.91 ***	25.42 ± 0.72	25.70 ± 0.71 **
LDL subclass pattern A/B (no. of subjects)	7/5	7/5	11/5	6/10 *	11/5	11/5
LDL-derived TBARS (nM/200μM)	21.6 ± 9.4	23.3 ± 10.9	16.3 ± 9.1	16.3 ± 10.2	17.3 ± 4.8	14.9 ± 4.6 **

LDL, low-density lipoprotein; TBARS, thiobarbituric acid reactive substances;
* $P < 0.1$；** $P < 0.05$；*** $P < 0.01$ versus 投与前

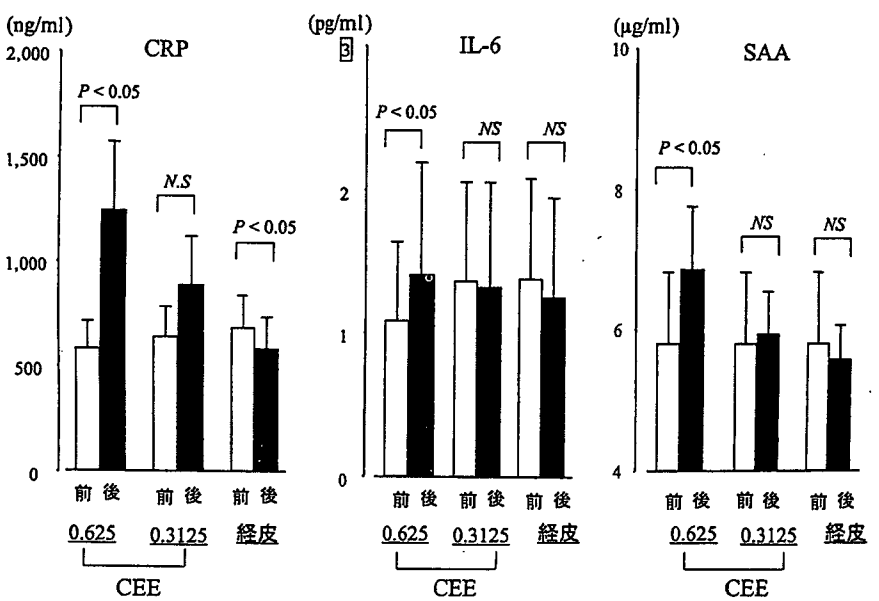

図1　血管炎症マーカー
(Wakatsuki A et al., Circulation, 2002)
(Wakatsuki A et al., Arterioscler Throm Vasc Biol, 2004)

により接着し，内皮下に侵入してマクロファージに変化する．CEE 0.625 mgではVCAM-1，ICAM-1に変化ないが，E-selectinは低下する．CEE 0.3125mgでも同様の変化を認めることから，低用量でもE-selectin低下作用は温存されることが示された[18]．一方，経皮投与の場合，全ての接着因子が低下した．近年，CRP

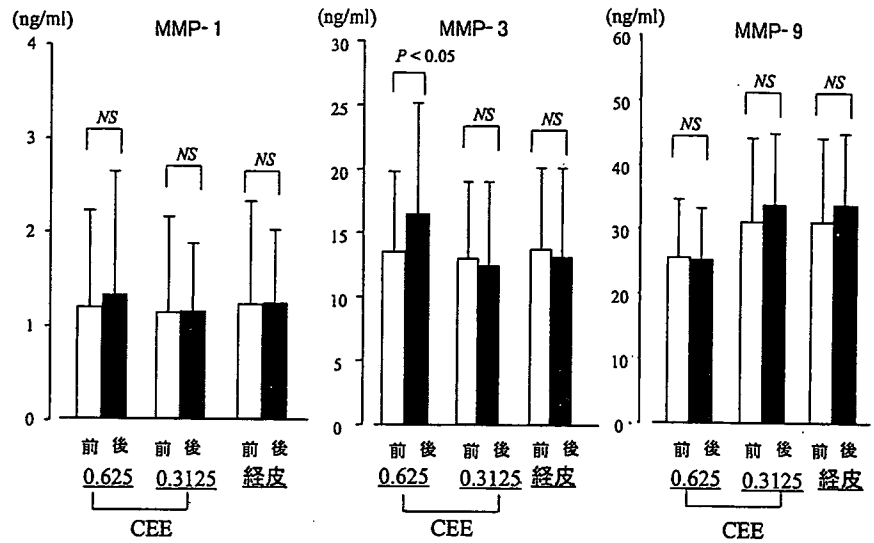

図2 Matrix Metalloproteinase
(Wakatsuki A et al., Arterioscler Throm Vasc Biol, 2003, 2004)

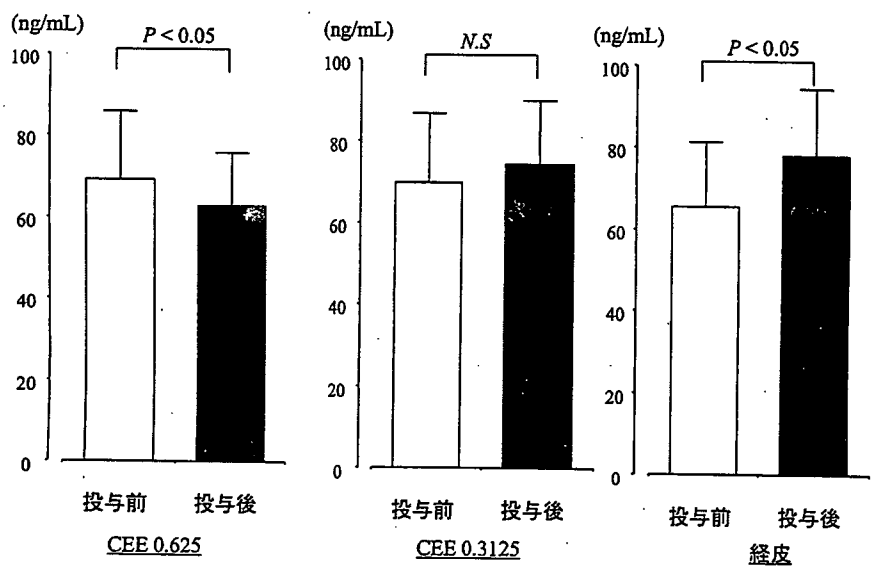

図3 Tissue Inhibitor of MMP-1
(Wakatsuki A et al., Arterioscler Throm Vasc Biol, 2003, 2004)

の上昇は接着因子の発現を亢進させることが知られており[20]，従来の経口エストロゲンの場合，CRPの上昇がエストロゲンの接着因子抑制作用に相殺するが，経皮ではCRPはむしろ低下するためエストロゲンの好影響が発揮できたものと考えられる．

③血管内皮機能

3群についてFMDとNIDを測定してみると，NIDはいずれの群も変化ないが，FMDは3群ともに上昇することが示された．したがって，いずれも血管平滑筋機能には影響しないが，エストロゲンの内皮機能改善作用は低用量投与や経皮投与でも温存されることが明らかになった．

(2) 併用するプロゲスチン製剤の選択

MPAのような合成型プロゲスチン製剤はアンドロゲン作用を有するため，HDL-Cや血管内皮機能に抑制的に作用するが，天然型プロゲスチン製剤にはアンドロゲン作用がないため，これらの悪影響は認めないと考えられる．脂質に関して検討したPEPI studyでは，

各HRT群でLDL-Cは同様に低下するが，HDL-CはCEE単独群で上昇し，MPA併用群で低下する．しかし，天然型プロゲスチンのMPではHDL-Cの低下はないことが示されている[3]．またエストロゲンの血管内皮改善作用も同様にMPを併用しても温存されることが示されている[21]．したがって，エストロゲンに併用するプロゲスチン製剤としては合成型プロゲスチン製剤に代わり，天然型プロゲスチンが考慮されるべきであるが，残念ながら日本では使用されておらず，MPAに頼らざるを得ない．我々はMPAの臨床的成績から，子宮を有する女性でMPAを連続併用投与する場合にはMPAは5.0mgより2.5mgを，また子宮摘出後の女性にはMPAを併用しないエストロゲン単独投与を推奨している．また，アンドロゲン作用の少ないdydrogesteroneも考慮すべきであるが，今後，臨床的に検討する必要がある．

おわりに

HERSやWHIの報告以来，現在HRTを受けている女性のみならず医療従事者までがHRTの開始，継続に不安を持っており，HRTを断念した症例も少なくない．しかし，これまでの多くの基礎的，臨床的データでHRTが抗動脈硬化作用を有することには疑いのないところである．それでは何故HRTでCVDリスクが増加したかを考えてみると，HRTには抗動脈硬化作用のみならず動脈硬化促進作用も存在し，HRTの短所が長所を相殺する症例があると考えるべきである．我々の最近の研究により，HRTのCVDに対する短所が明確になりつつあり，従来行ってきたHRTの改善策もかなり具体化してきた．最近の疫学成績によると，CVDリスクは経口エストロゲンの量と用量依存的に増加し，低用量では逆に低下することが報告されており[22][23]，低用量エストロゲンの有用性が示されている．また経皮投与については現在続行中の臨床試験の結果に期待がかかるところである．いずれにしても欧米人と日本人とでは生活習慣をはじめとし異なる点が多くあることから，今後は，日本人を対象としたさらなる臨床的検討を加え，将来CVDリスクの低下が可能なHRTの確立が望まれる．

文　献

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