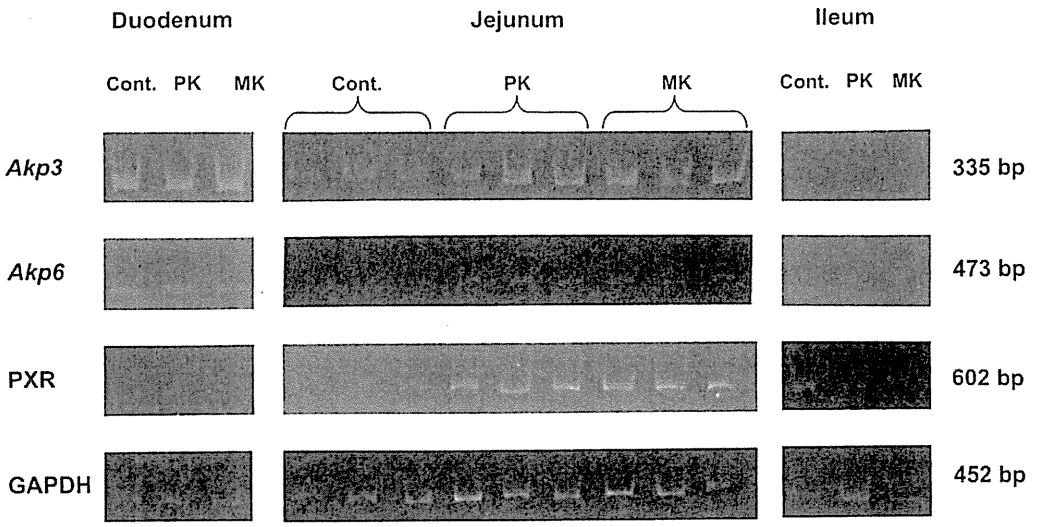

Fig. 2. Detection by RT-PCR of mRNAs for *Akp3*, *Akp6*, PXR, and GAPDH in the duodenum, jejunum, and ileum. PCR products were electrophoresed in a 5.25% polyacrylamide gel. Cont.: control, PK: phylloquinone, MK: menaquinone-4.

difference in the relative activities among these groups. We confirmed that these jejunum ALP preparations were effectively inhibited by L-Phe but not by Lev, and were heat-stable (60°C, 10 min), corresponding to the property of other mammalian intestinal type ALPs.

RT-PCR analysis of ALP mRNA expression

RT-PCR-based detections of mRNA for *Akp3*, *Akp6*, and PXR in the duodenum, jejunum, and ileum are shown in Fig. 2. The PCR products of *Akp3* (335 bp) were detected in the duodenum and jejunum. The PCR products of *Akp6* (473 bp) were detected in the duodenum, jejunum, and ileum. The PCR products of PXR (602 bp) were detected in the duodenum, jejunum, and ileum. The intensities of mRNA expression of *Akp3* and *Akp6* were very similar both in the duodenum and ileum among the three groups (Cont., PK, and MK groups). The intensities of mRNA expression of *Akp3*, *Akp6*, and PXR were enhanced in both the PK and MK groups compared with the control group in the jejunum.

PCR products of *Akp2* (198 bp) and *Akp5* (500 bp) were not detected in any of these intestinal samples.

In order to compare these intensities of mRNA expression in the jejunum, we determined the relative density of the PCR products of the mouse jejunum. As shown in Fig. 3A, the intensities of *Akp3* expression increased significantly in the PK group compared with the control group ($p<0.01$). Furthermore, the intensities of *Akp6* expression were also increased in both the PK and MK groups compared with the control group ($p<0.01$, $p<0.05$, respectively) (Fig. 3B). Interestingly, the intensities of PXR expression in both the PK and MK groups were significantly higher than in the control group ($p<0.05$, $p<0.01$, respectively) (Fig. 3C).

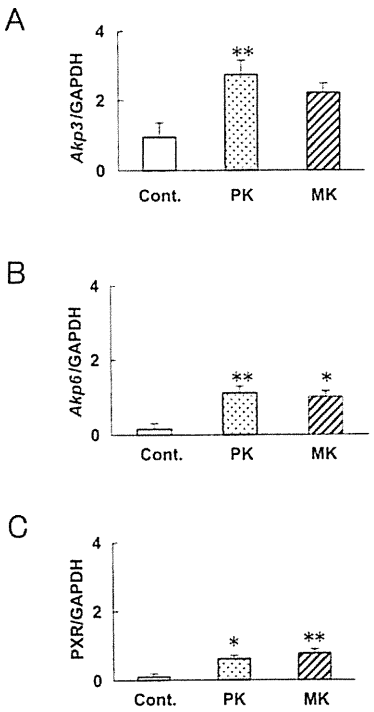

Fig. 3. The relative density of PCR products in the mouse jejunum. The diagrams show the relative density of the PCR products. A: The relative density of PCR products for *Akp3*. B: The relative density of PCR products for *Akp6*. C: The relative density of PCR products for PXR. Results are the mean±SE of 4 animals. Significant difference between the PK or MK and control groups ($^{*}p<0.05$, $^{**}p<0.01$). Cont.: control, PK: phylloquinone, MK: menaquinone-4.

DISCUSSION

Previously, we reported that several dietary factors such as fat-feeding, vitamin K, and lactose increased IAP activities in rats (*12*, *15*, *24*). The high-level activity of IAP, which localizes at the brush border of intestinal epithelium cells, suggests the participation of this enzyme in the transport of nutrients.

Recently, we reported that both long-term dietary PK and MK-4 supplementations enhance IAP activity in rats (*12*). After 3 mo of feeding, we measured IAP activity by dividing it into five segments. In each segment, both PK and MK-4 increased IAP, and the level of IAP activity in the proximal jejunum was significantly

higher than that in the control group ($p<0.05$) (*12*). To examine whether the enhancing effect of PK or MK-4 on IAP activity occurs via the intestinal mucosa directly, we performed an oral administration of PK or MK-4 using mice. In the present study, we discovered that the levels of mouse jejunum ALP activity were also significantly increased by the oral administration of PK or MK compared with the control group (Fig. 1B). In addition, we confirmed that the increased ALP isozymes induced by the oral administration of PK or MK showed similar biochemical properties to the typical intestinal type ALP, with no significant differences among these groups (Table 1).

By SDS-PAGE analysis, we detected a 110-kDa ALP enzyme in the duodenum and 90-kDa ALP enzyme in the ileum (Fig. 1D). Both the 110- and 90-kDa ALP enzymes were detected in the jejunum, and the enzymatic activities of these bands were enhanced by the oral administration of PK or MK. The product of the *Akp3* gene was expressed specifically in the duodenum, and the product of *Akp6* was expressed through the small intestine (*5*). Therefore, we considered that the 110- and 90-kDa ALP enzymes may correspond to the IAPs encoding *Akp3* and *Akp6*, respectively.

We then performed RT-PCR analysis in order to examine the expression of IAPs (*Akp3* and *Akp6*) in the mouse jejunum. PCR products for *Akp3* and *Akp6* mRNAs in the jejunum were detected, and a significant increase in the PCR products of *Akp3* due to the oral administration of PK was observed (Fig. 3A). Moreover, a significant increase in the PCR products of *Akp6* due to the oral administration of PK or MK-4 was also observed (Fig. 3B). These results suggest that the induction of *Akp3* and *Akp6* may be regulated by PK or MK-4.

As the results of RT-PCR, the expression of mRNA for PXR was detected in the duodenum, jejunum and ileum, and it was enhanced significantly in both the PK and MK groups in the jejunum compared with the control group (Fig. 3C). Interestingly, the intestinal segment where the expression of mRNA for PXR by vitamin K had been enhanced corresponded to a similar segment where the expression of mRNA for IAP was enhanced.

Recent studies have revealed that vitamin K functions as a ligand for nuclear steroid and xenobiotic receptor (SXR), as well as a cofactor for γ-carboxylase (*25*). SXR is expressed predominantly in the liver and intestine, and it regulates transcription such as of cytochrome P450 (CYP) 3A4, which is an enzyme involved in drug metabolism, and MDR1 (multidrug resistance protein 1) which is activated by a diverse array of pharmaceutical agents including taxol, rifampisin, and clotrimazole (*26*, *27*). Ichikawa et al. identified novel SXR target bone-related genes that were regulated by MK-4 in osteoblastic cells using microarray analysis (*28*). Among extracellular matrix-related genes, they demonstrated that a small leucin-rich repeat proteoglycan, tsukushi, contributes to collagen accumulation (*28*).

We demonstrated for the first time that the oral administration of vitamin K (both PK and MK-4) enhanced the level of IAP mRNA expression in the mouse intestine, and PXR mRNA expression also increased. Further studies on the physiological functions of ALP and transcriptional regulation of ALP induction will provide useful data on the novel effect of vitamin K.

Acknowledgments

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Association of CYP19 *Gene Polymorphism with Vertebral Fractures in Japanese Postmenopausal Women*

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Association of *CYP19* Gene Polymorphism with Vertebral Fractures in Japanese Postmenopausal Women

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Abstract This study investigates aromatase gene polymorphism, which might influence bone strength in terms of mineral density and quality. We explored the relationship between *CYP19* polymorphisms and vertebral fractures in postmenopausal Japanese women. In addition, we compared estrogen and testosterone levels in Japanese postmenopausal women with and without fractures. Osteoporotic postmenopausal women showed higher incidences of vertebral fractures than osteopenic women or women with normal lumbar bone mineral density (L2-4 BMD). Estrogen concentrations in postmenopausal women were associated with BMD; however, no association was found between sex hormone levels and the presence of fractures. The C allele rs2470152 was significantly associated with increased risk of vertebral fractures ($P = 0.04$), whereas none of the *CYP19* polymorphisms showed differences in sex steroid levels between subjects with and without fractures. Allelic variants of aromatase genes appear to interact to influence the risk of vertebral fractures in postmenopausal Japanese women.

Keywords Aromatase gene polymorphisms · Vertebral fractures · Postmenopausal women

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Introduction

Osteoporosis is caused by multiple factors, including environmental factors (such as calcium intake), exercise, and estrogen levels. The main source of estrogen in postmenopausal women is the aromatization of androgenic precursors, a reaction catalyzed by the cytochrome P450 (CYP) aromatase enzyme, encoded by *CYP19* located on chromosome 15q21.1. It has recently been reported that estrogen levels are genetically determined by aromatase activity (Olson et al. 2007; Haiman et al. 2007; Sowers et al. 2006). In addition, allelic variants of the aromatase gene have been associated with bone mineral density (BMD) and bone fractures (Hong et al. 2007; Masi et al. 2001; Somner et al. 2004). A/G polymorphisms in the 3′ untranslated region (UTR) and the I.2 promoter (rs10046 and rs1062033; Rinancho et al. 2005) and an A/G polymorphism in the I.6 promoter rs4775936 (Enjuanes et al. 2006) of the aromatase gene have been studied in relation to osteoporosis and BMD, but the results remain controversial. In addition, an rs2470152 polymorphism in the aromatase gene has been shown to affect serum estrogen levels in Swedish men (Eriksson et al. 2009). Therefore, in order to analyze the association with the risk of vertebral fractures in postmenopausal women, we conducted a cross-sectional study of the interaction between *CYP19* gene polymorphisms and sex steroid hormone levels or risk of vertebral fractures in Japanese postmenopausal women. In this study, we focused on four markers (rs2470152, rs4775936, rs1062033, and rs10046) to clarify the association between polymorphisms in aromatase genes and vertebral fractures.

Materials and Methods

Study Subjects

Three hundred sets of genomic DNA and serum samples were provided from the collected samples of the Institute of Medical Sciences, Tokyo University, obtained for tailor-made medicine realization projects. These samples were collected from the various institutions that were members of these projects following the approval of the individual ethics committees. Ethical approval was obtained from the Ethics Committee of the Leading Project for Personalized Medicine in the Institute of Medical Science, University of Tokyo, and the Tokyo Metropolitan Geriatric Hospital. Another 300 DNA samples were collected from women for the purpose of analyzing the relationship between polymorphisms and the etiology of disease in the Japanese population. The samples were provided by the Leading Project for Personalized Medicine of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

The samples were divided into three categories according to the *T* score of the measurement of lumbar spine BMD (L2-4 BMD) by dual energy X-ray absorptiometry (DXA) as defined by the World Health Organization: *T* scores of −1.0 and above were classified as normal BMD, scores of −2.5 to −1.0 were considered osteopenia, and scores below −2.5 were considered osteoporotic.

For assessment of vertebral fractures, anteroposterior and lateral X-ray examinations of the thoracic and lumbar spine were performed. Morphometrically,

a vertebral fracture was defined in terms of the ratio of the anterior height of vertebral body to the posterior height (below 0.75) or the ratio of the center height to the anterior or posterior height (below 0.8). In all cases, the vertebral fractures were evaluated by two groups of radiologists and geriatricians in each institute.

Sex Steroid Assay

The serum levels of testosterone (T) and estradiol (E_2) were measured by mass spectrophotometry (LC–MS/MS). Bioavailable testosterone and estrogen, which includes the free form and the albumin-binding form, were measured by LC–MS/MS (Arai et al. 2010). Serum samples were stored at −70°C until analyzed. For statistical analysis, the values were transformed into logarithmic form, since the values are exponential and the distributions of T and E_2 levels were skewed using the raw data.

Genotype Analysis

We examined four polymorphisms of *CYP19*: rs1062033, a G/C SNP located at around exon 1.2 (at position chromosome 15, 49335230); rs10046, a T/C SNP located in the 3′ UTR (at position chr.15, 49290276); rs4775936, a C/T SNP located in the vicinity of exon 1.6 (at position chr.15, 49323314); and rs2470152, a T/C SNP located in intron 1 (at position chr.15, 49382254). These SNPs were identified by searching the National Center for Biotechnology Information (NCBI) database because they are analyzable by the readily available TaqMan assays used for disease association studies (Applied Biosystems). Polymorphisms in genomic DNA were measured by the TaqMan assay. Age, body mass index, and years since menopause were examined in three SNP genotypes among four *CYP19* markers.

Statistical Analyses

Chi-square analysis was used to compare the numbers of osteoporosis, osteopenia, and normal patients by T scores of L2-4 BMD with and without fractures. Similarly, each parameter was compared among the three genotypes in four *CYP19* markers using ANOVA. The correlation between estradiol levels and L2-4 BMD was shown using Pearson's coefficients. The associations between aromatase gene polymorphisms and vertebral fracture risk were compared by Chi-square analysis using SPSS software.

Results

Bone Density Data

There were significantly more women with fractures than without among patients with osteoporosis ($T < -2.5$; $P < 0.05$), and there was no significant increase in fractures among normal patients or those with osteopenia. There were no differences

in the log estradiol (Log E_2) or log testosterone (Log T) values between women with fractures and those without fractures (Table 1).

Relationship Between L2-4 BMD and Estrogen level

Log E_2 levels in postmenopausal women were significantly associated with L2-4 BMD ($r = 0.21$, $p = 0.03$; Fig. 1), whereas log T levels showed no association (data not shown).

Genotype Analysis

When we examined the correlation between the four polymorphisms (rs2470152, rs1062033, rs4775963, and rs10046) and vertebral fractures in postmenopausal women, we found a significant correlation for rs2470152 ($P = 0.04$) but not for the

Table 1 Bone mineral density of postmenopausal women with and without fractures

T score[a] & sex steroids	Women without fractures (137)	Women with fractures (138)	P
$T < -2.5$	18	37	0.015
$-2.5 \leq T < 1.0$	12	13	NS
$-1.0 \leq T$	4	5	NS
Log E_2 (pg/ml)	0.335 ± 0.383	0.327 ± 0.330	NS
Log T (ng/dl)	2.033 ± 0.367	2.067 ± 0.247	NS

[a] Bone mineral density was measured in 89 of the 275 subjects

NS not significant

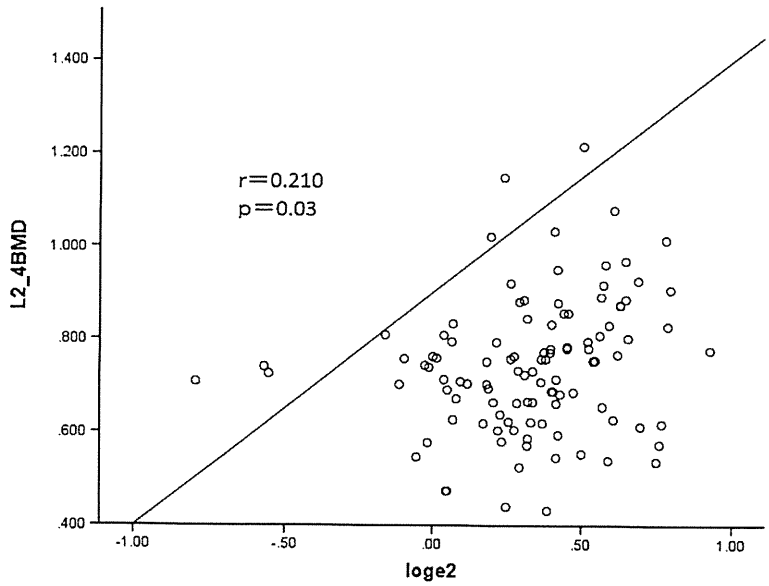

Fig. 1 Correlation between log E_2 and L2-4 BMD in postmenopausal women. Estrogen levels were significantly correlated with L2-4 BMD ($r = 0.21$, $p = 0.03$)

Table 2 Correlation of four *CYP19* SNPs with vertebral fractures

SNP	Genotype	Premenopause		*P*	Postmenopause		*P*
		Control ($n = 19$)	Case ($n = 6$)		Control ($n = 136$)	Case ($n = 138$)	
rs2470152	CC	9	1	0.408	44	47	0.040[a]
	TC	8	4		56	71	
	TT	2	1		36	20	
rs4775936	CC	5	1	0.712	48	59	0.416
	CT	9	4		68	59	
	TT	5	1		20	20	
rs1062033	CC	4	1	0.693	47	56	0.507
	CG	9	4		64	62	
	GG	6	1		25	20	
rs10046	TT	7	1	0.550	31	26	0.324
	TC	8	4		67	62	
	CC	4	1		38	50	

[a] Only rs2740152 polymorphisms of the aromatase gene showed a significant correlation with vertebral fractures ($P = 0.04$)

Table 3 Characteristics of postmenopausal Japanese women and three SNPs of rs2470152

Characteristic	Genotype			*P*
	CC	CT	TT	
Age (years)	72.8 ± 9.3	73.6 ± 8.3	74.3 ± 6.8	NS
Body mass index (kg/m^2)	22.4 ± 4.6	21.8 ± 4.5	21.1 ± 4.3	NS
Years since menopause	24.2 ± 9.5	24.3 ± 10.3	25.4 ± 11.6	NS
Log E_2 (pg/ml)	0.302 ± 0.319	0.330 ± 0.280	0.307 ± 0.357	NS
Log T (ng/dl)	2.001 ± 0.347	2.067 ± 0.269	2.050 ± 0.313	NS
L2-4BMD (110)	0.753 ± 0.135	0.739 ± 0.157	0.729 ± 0.118	NS
LT score (89)	−2.6 ± 1.2	−2.6 ± 1.3	−3.3 ± 0.8	NS

NS not significant

other three polymorphisms (Table 2). There were no differences in age, body mass index, or years since menopause among the three SNP types in the four *CYP19* markers (Table 3).

Discussion

We examined the relationship between aromatase-related genes and vertebral fractures by analyzing *CYP19* gene polymorphisms in Japanese women. Among four markers, no differences were found in serum T and E_2 concentrations in the Japanese postmenopausal women. It is possible that local E_2 concentrations are

more important in local tissues rather than serum levels. Bone cells are able to express aromatase and other enzymes required for estrogen synthesis locally (Janssen et al. 1999; Shouzu and Simpson 1998; Watanabe et al. 2004), and aromatase activity in cultured osteoblasts is quantitatively similar to that in adipose stromal cells (Shouzu and Simpson 1998). Thus, estrogen synthesized in bone cells might be important in postmenopausal bone metabolism.

Eriksson et al. (2009) found that genetic variants of rs2470152 in aromatase are associated with E_2 levels, showing that G alleles were correlated with higher serum E_2 levels and BMD in Swedish men than other alleles. Our results, however, showed that the C allele of rs2470152 is associated with vertebral fractures, a finding that suggests that ethnicity, race, and sex differences might influence the results of SNP studies in osteoporosis. The SNP rs2470152 is located in the region of the I.4 promoter (Bulun and Simpson 1994), and it is interesting that the G→A transition of rs2470152 is likely to alter a potential binding site for the binding protein of the transcription factor cAMP response element. The major reason for the discrepancy between our results and those of the Swedish study may be gender differences. The Swedish study focused only on male cohorts. We could not detect any disequilibrium between rs2470152 and the other three markers viewed in HapMap.

CYP19 SNPs (rs10046) were found to be associated with differences in E_2 levels in the European Prospective Investigation of Cancer-Norfolk (EPIC-Norfolk) cohort study (Dunning et al. 2004). SNP rs10046 explains 1.6% of the variance in the E_2:T ratio; however, this SNP is not associated with breast cancer risk (Dunning et al. 2004). The rs10046, a T/C SNP located in the 3′ UTR, 19 nucleotides downstream from the translation terminus, has been reported to be associated with increased levels of aromatase mRNA expression in tumors (Gruber et al. 2002). In our study, rs10046 was correlated with neither serum E_2 levels nor vertebral fractures. The *CYP19* genotypes demonstrated higher mRNA levels at the rs1062033 locus in postmenopausal osteoporosis. *CYP19* is regulated in a different manner and in different tissues by a hormonally controlled promoter or adipose stromal cell promoter (Mahendroo et al. 1993; Harada et al. 1993). Genetic polymorphisms of *CYP19* might be involved in other processes, such as mRNA stabilization, transcription enhancement, or the post-translational regulation of expression. Neither SNP 1062033 nor rs4775936 was significantly correlated with either serum E_2 levels or vertebral fractures.

We could not detect lower levels of bioavailable serum E_2 by LC–MS/MS in rs2470152; however, another group has shown differences in E_2 levels as measured by RIA according to *CYP19* genotype in a study that included both premenopausal and postmenopausal women (Somner et al. 2004). The discrepancy between the two studies seems to be due to the assay systems used. Bioavailable estrogen levels in postmenopausal women are more relevant than total estrogen levels, which include E_2 bound by sex hormone-binding globulin (SHBG), for bone metabolism. Despite the absence of differences in estrogen levels among the various genotypes, we found that vertebral fracture rates are associated with the *CYP19* genotype in postmenopausal Japanese women in this study. There is much evidence for the role of aromatase activity in bone homeostasis (Miyaura et al. 2001; Oz et al. 2000), and, as previously described, the pharmacological inhibition of aromatase is also associated

with a decrease in BMD and increased risk of fractures (Eastell and Hannon 2005). This indicates that aromatase in local tissues plays roles, both physiologically and pathologically, in bone metabolism.

In conclusion, we provide statistical evidence that the C allele in rs2470152 of the *CYP19* gene is associated with an increased risk of vertebral fractures in postmenopausal Japanese women. Further studies are necessary to detect functional SNPs that induce differences in bone metabolism. Furthermore, we need more participants to detect differences in E_2 levels based on the *CYP19* SNPs of aromatase genes.

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Ⅱ. 歯

2. 歯周病と炎症

Periodontitis and inflammation

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歯周病の病態形成には，細菌感染による炎症反応と非感染性の慢性炎症が複雑に絡み合っている．歯周ポケットに存在する各種の歯周病関連細菌は，Toll様受容体を活性化し，歯周組織の慢性炎症形成に寄与している．加えて，HMGB1などの内因性リガンドが恒常的な炎症反応を誘導する．また，歯周病関連細菌 *P. gingivalis* は血管内皮との相互作用により，血管炎症を進展させる．

歯周病は細菌感染を伴った慢性炎症性疾患である

歯周病は感染症であり，歯周病関連細菌によって惹起される炎症性疾患である．また，その発症と進行にはそれらの細菌の病原性だけではなく，歯周組織における宿主側の各種細胞の応答が深く関与する[1]．歯周ポケットには，グラム陰性桿菌をはじめとした数多くの細菌が生息し，競合・共生関係を維持しながら細菌叢を形成している．そのなかで，特定の細菌が歯周病の発症や進行に関与しており，特に関連性が高いと考えている細菌として，*Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Tanneralla forsythia, Treponema Denticola* などが知られている[2]．それらの菌は，内毒素，蛋白分解酵素，線毛などの病原因子を有しており，それらの菌が歯周組織に感染する際の重要な因子となる．また，同因子は歯周組織において宿主細胞を活性化し，強力に炎症反応および免疫応答を惹起する．歯周ポケットに形成される細菌叢は時にグリコカリックス様の構造物で被覆され，バイオフィルムの形態を呈する．このようなバイオフィルムを形成した歯周病関連細菌は，抗体，食細胞，抗生物質に対して抵抗性を示すことから，歯周組織に細菌が持続感染しやすい状態となる[3]．このような細菌と宿主の相互作用が持続することと，さまざまな環境因子や遺伝因子が複雑に絡み合って，歯周病の慢性炎症病態が形成されるものと考えられる(図1)．

生活習慣病は慢性炎症性疾患である

近年，肥満，糖尿病，動脈硬化性疾患などの生活習慣病，アルツハイマー病，パーキンソン病などの神経変性疾患，関節リウマチなどの自己免疫疾患，癌などに共通する分子基盤として慢性炎症が注目されている．慢性炎症では長期にわたるストレス応答によって炎症反応が遷延化するとともに，宿主の恒常性が十分維持されず破綻をきたし，不可逆的な組織のリモデリングを生じ，組織・臓器の機能不全をきたすようになる[4]．たとえば，動脈硬化では血管壁に対して，物理的，科学的刺激により内皮細胞や血管平滑筋細胞の機能が変化する．この内皮細胞の機能障害は脂質などの血管壁への浸透・蓄積，血

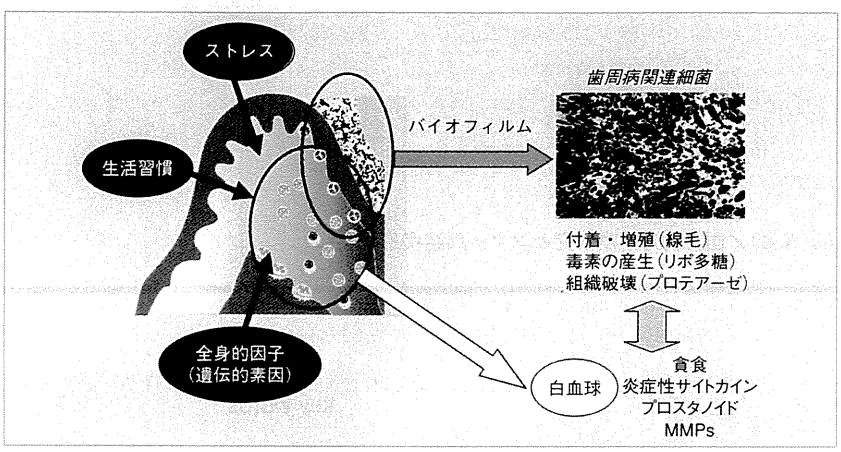

図1　歯周病はバイオフィルム感染症である

歯周病においては，歯周ポケットに生息する細菌群が歯周組織を直接傷害するとともに，各種菌体毒素によって宿主細胞を活性化し，炎症反応が惹起される．加えて，それらの細菌はバイオフィルムを形成し，宿主あるいは抗菌薬に対して抵抗性を示す．

管内皮への白血球の浸潤を引き起こす．このような細胞応答が長期に持続することにより，血管壁の破壊とそれに伴う修復反応が同時に進行し，その結果，組織構築が改変されることになる[5]．以上のような，複雑な細胞・組織応答が他の慢性炎症性疾患にも共通して認められる現象である．近年，歯周病と動脈硬化性疾患や糖尿病などの生活習慣病との関連性が注目されているが，両者の間には単に相関関係が認められるというだけではなく，その発症機序に共通の分子基盤が存在していることが考えられる．歯周病を生活習慣病として捉え，生活習慣病の共通分子基盤である慢性炎症といった視点から考えることは，両者の関係をより深く理解するために極めて重要である．

慢性炎症には臓器特異性が認められる一方，臓器を超えた共通の分子機構が存在する可能性が高い．慢性炎症は，微生物感染により誘導されるものと感染が関連しないものとに大別される．歯周病は前者に属し，糖尿病や動脈硬化性疾患などはおおむね後者に属するものと考えられる．ただし，歯周病の病態形成には細菌感染による炎症反応と非感染性の慢性炎症が複雑に絡み合っているものと考えられる[6]（図2）．

自然炎症としての歯周病

感染症における免疫反応の機序における重要な分子として，Toll 様受容体（Toll-like receptor ： TLR）をはじめとする病原体センサーが重要な役割を担うことが知られている．免疫システムにおいて，病原体などの外来抗原をいかに認識するかは極めて重要であり，TLR はそれらを認識し，自然免疫応答を誘導して炎症反応を惹起する重要な分子である[7]．歯周ポケットに存在する各種の歯周病関連細菌は，これらの受容体を活性化し，歯周組織の慢性炎症形成に寄与しているものと考えられる．歯周病関連細菌の一種である *P.gingivalis* はこれらのリガンドを数多く有し，樹状細胞やマクロファージなどを活性化し，自然免疫応答とともに炎症反応を強力に惹起する．*P.gingivalis* のリポ多糖（LPS）は，他のグラム陰性菌のそれとは異なり，細胞壁のリポ蛋白，リポタイコ酸やペプチドグリカンと同様に TLR2のリガンドとなり得ることも報告されている．そして，同受容体の活性化を介して，マクロファージや単球の活性化に寄与する．また，同菌の菌体表層に存在する線毛は歯周局所への付着・定着に関与するだけでなく，同受容体を介して，炎症性サイトカイン産生を誘導することも報告されている[8][9]．TLR からのシグナルは，MyD88などのシグナル伝達物質を介して，NF-κB（nuclear factor-κB）や IRF（interferon regulatory factor）などの転写因子を活性化し，TNF-α や IL-6（interlenkin-6）といった炎症性サイトカインの産生を誘導する[10]．また，同菌の産生するシステインプロテアーゼ gingipain は，間質のマトリックス蛋白質を分解し，歯周組織を直接破壊するだけでなく，好中球受容体や補体を分解し宿主の免疫反応を抑制する．他方で，マトリックスメタロプロテアーゼ（MMP）や炎症性サイトカインの産生を誘導し，炎症反応を促進する（図3）．さらに，血液凝固因子である Factor X や プロトロンビンなどを活性化し，血液凝固反応

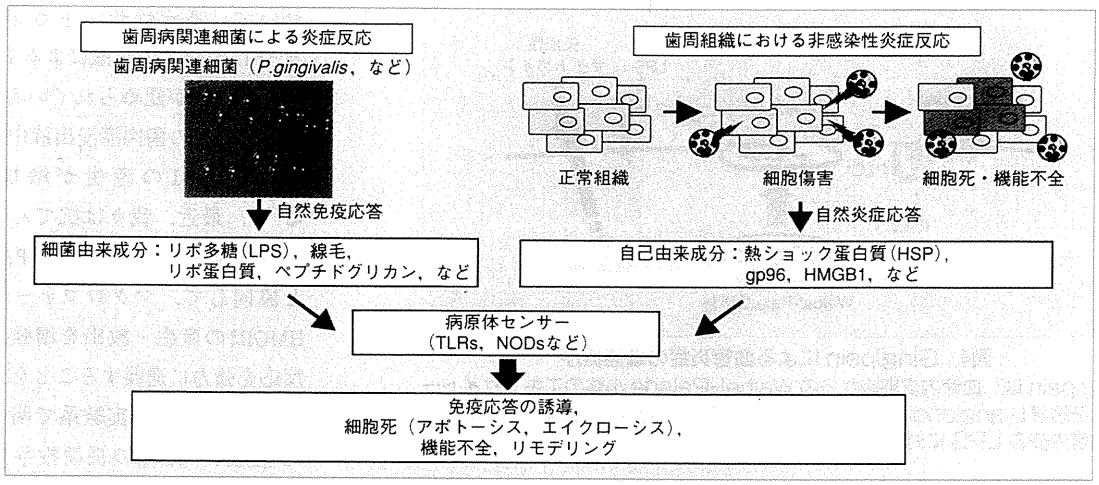

図2　慢性炎症としての歯周病の発症機序

歯周病関連細菌の毒素は，宿主の病原センサーに補足され，宿主細胞の免疫応答を促す．加えて，傷害を受けた細胞は細胞から放出される自己由来成分も同受容体を介して炎症反応を惹起する．これらが複合して慢性炎症としての歯周病の病態が形成される．NOD：nucleotide oligomerization domain

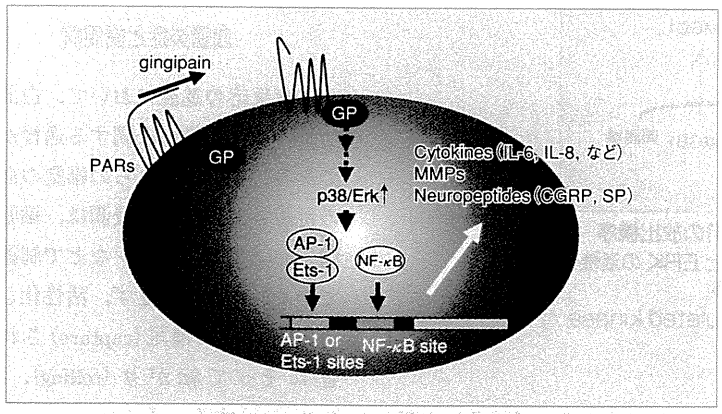

図3　Gingipainが炎症反応を惹起する仕組み

P.gingivalis が産生するトリプシン様システインプロテアーゼ gingipain は，PAR の活性化を介して，サイトカイン，マトリックスメタロプロテアーゼ，神経ペプチドなどの発現を誘導して炎症反応を惹起する．

を誘導したりもする[11]．我々は，gingipain が血管内皮細胞に発現する PAR（protease-activated receptor）を活性化し，同細胞における血管形成調節因子 angiopoietin 2を含む分泌顆粒のエキソサイトーシスを誘導し，LPS に対する感受性を高めて血管の炎症応答を亢進することを明らかにした[12]（図4）．このように，歯周病関連細菌は歯周組織に侵入・定着し，さまざまな病原因子を産生して，歯周組織の炎症反応を惹起している．加えて，組織障害に伴って放出される内因性リガンドが TLR などの病原体センサーを活性化し，外因性リガンドの刺激と相まって感染症における慢性炎症の病態形成に寄与していることが考えられている．傷害を受けたり，壊死したりした細胞はそこに潜む危険を排除するために，免疫系の細胞の活性化を促すようなシグナル（danger signal）を発し，炎症反応を引き起こすことが知られている．このような分子は，DAMPs（danger-associated molecular patterns）とも呼ばれ，ATP，尿酸結晶，HSP（heat shock protein），HMGB1（high mobility group box protein-1）などが知られている．DAMPs は，宿主が自分自身を守り自らを再生させるために生ずると考えられるが，一方こうした

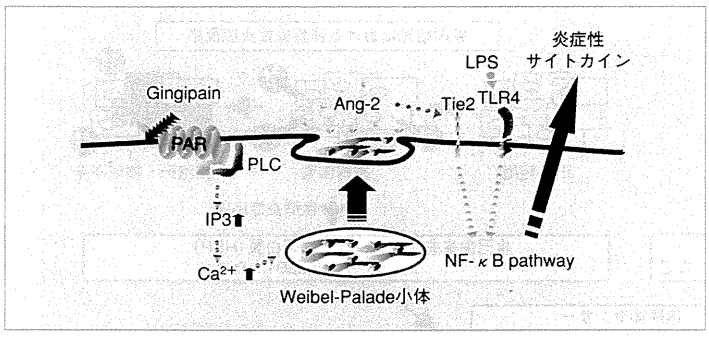

図4　Gingipainによる血管炎症の増強機序
gingipainは，血管内皮細胞からのWeibel-Palade小体のエキソサイトーシスを誘導しangiopoietin-2（Ang-2）の放出を促す．放出されたAng-2は血管内皮のLPSに対する感受性を高める．

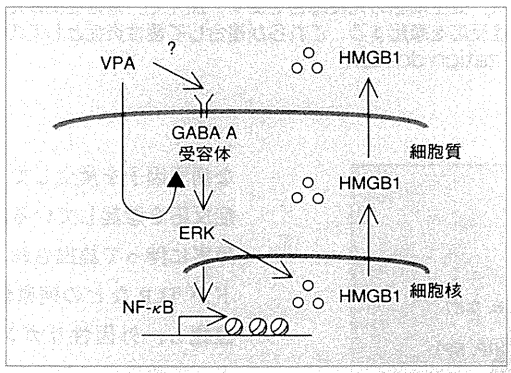

図5　バルプロ酸によるHMGB1の放出機序
バルプロ酸（VPA）はGABA受容体とERKの活性化を介して，HMGB1の放出を誘導する．
ERK：extracellular signal-regulated kinase

免疫応答が過剰に働き，炎症反応が必要以上に生ずるとさまざまな慢性疾患の発症や進行へとつながってゆく[13]．そのような分子の一つであるHMGB1は，細胞核に存在するDNA結合蛋白質の一種で遺伝子の転写を制御する因子である．HMGB1の受容体として，RAGE（receptor for advanced glycation end products），TLR2/4などが知られている[14]．同分子は細胞死によって細胞から漏出するが，活性化した単核球からもHMGB1は放出され炎症反応を惹起することが知られており，多くの炎症性疾患へのHMGB1の関与が示されている．具体的には敗血症の致死因子として，あるいは腎炎，肝炎，肺炎などの慢性炎症病態の形成に関与する因子として注目されている．歯周病との関与もいくつか報告されている．歯肉上皮細胞や歯肉由来線維芽細胞において，炎症性サイトカインTNF-αやLPSなどの刺激によるHMGB1の産生・放出が認められている[15]．また，慢性歯周炎の歯肉溝浸出液中や歯肉上皮にHMGB1の産生が増加している[16][17]．最近，我々は抗てんかん薬の一種であるバルプロ酸（VPA）がLPSと協同して，マクロファージからのHMGB1の産生・放出を増強し，炎症反応を強力に進展することを，*in vitro*および*in vivo*の実験系で明らかにした（図5）．VPAの長期投与の副作用として，歯肉増殖症と歯周炎の増悪が知られているが，VPAは歯周組織の炎症の増悪因子である可能性が示唆された．

血管炎症と歯周病

炎症反応の進展において，白血球が血管内から組織へ浸潤する過程が重要である．また，それらの細胞の血管への付着と血管外への浸潤は，細胞接着因子およびモノカインなどで制御されている．白血球はまず，活性化した血管内皮細胞に補足（capture）され，血管にそって転がり（rolling），付着（adhesion）する．その後，血管内皮細胞を通り抜け血管外へと移動し，組織へ浸潤する．特に，captureからrollingの過程で重要になってくる分子がselectinである[18]．血管内皮細胞には，E-selectinという同細胞に特異的に発現しているselectinが存在し白血球と同細胞との相互作用に寄与している．E-selectinは，TNF-α，IL-1β，LPSなどにより活性化した血管内皮細

胞に発現する．E-selectin は白血球上のリガンドである sialyl lewis X と相互作用し，白血球の rolling を誘発する．また，E-selectin の一部は，細胞外領域で切断され可溶性 E-selectin（sE-selectin）として血中に遊離される．敗血症や膠原病などの炎症性疾患，血栓性血小板減少性紫斑病のような微小血管障害および冠動脈疾患，脳血管障害，閉塞性末梢動脈疾患などの動脈硬化性疾患の患者において血中 sE-selectin 濃度が高値を示すことが知られている．重度の歯肉炎患者の血漿中あるいは歯肉溝浸出液中にも高濃度の sE-selectin が検出される．近年，閉塞性血栓性血管炎と歯周病との関連性が報告され，血管病変部に歯周病関連細菌が高頻度に検出されることが示されている．我々は，歯周病関連細菌，特に *P.gingivalis* が血管病変部に定着する機序の一つとして E-selectin に着目し，同分子と *P.gingivalis* との相互作用について検討した．その結果，TNF-α で刺激した血管内皮細胞上では，*P.gingivalis* の付着が著しく亢進すること，またそれは E-selectin 抗体および sialyl lewis X の添加によって有意に抑制されることを明らかにした（図6）．また従来，血管内皮への *P.gingivalis* の付着には，同菌の線毛が重要であることが報告されてきたが，E-selectin との相互作用には OmpA 様外膜蛋白質が重要であることが明らかになった．これらの結果は，炎症血管においては，*P.gingivalis* が付着しやすいこと，またその付着様式は上皮や正常血管内皮とのそれと異なる可能性が考えられた．このように血管炎症部位に付着した *P.gingivalis* は，その後，細胞あるいは組織に侵入し持続感染を成立させるとともに，血管炎症を増強する可能性が考えられる（図7）．

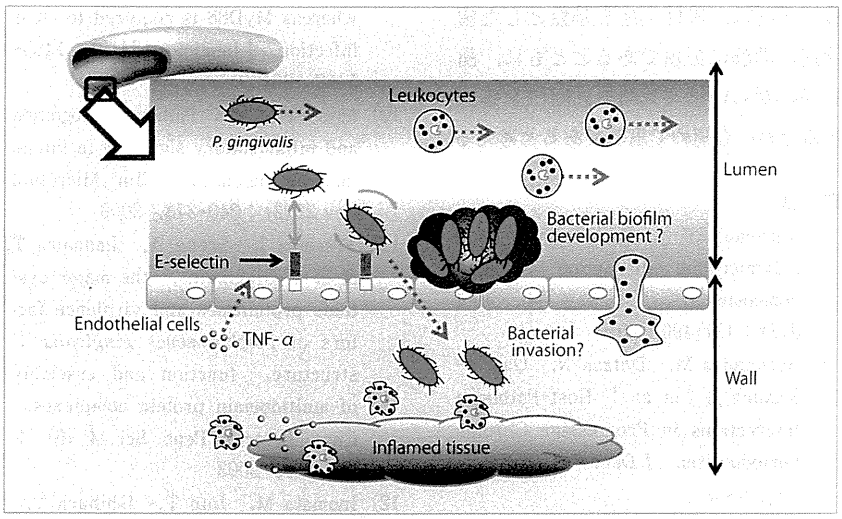

図6　E-selectin を介した *P.gingivalis* の血管付着

P.gingivalis は，E-selectin を介して活性化した血管内皮と付着する．その後，同菌は血管内皮に侵入，あるいは内皮上にバイオフィルムを形成し，血管炎症を増強する可能性がある．

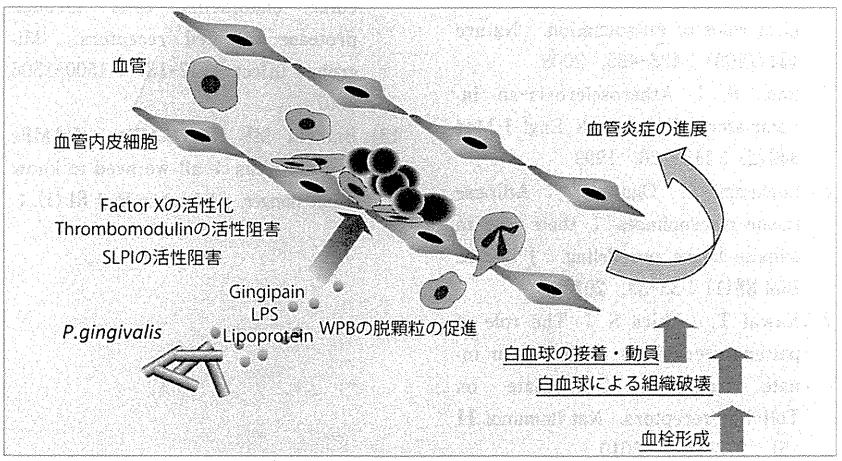

図7　*P.gingivalis* が血管炎症を増悪する機序

P.gingivalis が産生する種々の毒素は，白血球の活性化および血管内皮への接着・動員とそれによる血管傷害，さらには血管内における血栓形成を促進し，血管炎症を進展させる可能性がある．

歯周病関連細菌の血管炎症への関与は，歯周病の病態形成に極めて重要である．その分子機構と病態生理学的意

義の解明は，慢性炎症を基盤とした歯周病の理解に必須であるとともに，歯周病の新しい治療戦略や診断法を確立するためにも極めて重要であると考える．

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