Changes in Uterine Microvessels as a Possible Pathogenic Factor in the Development of Adenomyosis Induced by Pituitary Grafting in Mice

Ying-Fang Zhou, Manabu Matsuda, Shinobu Sakamoto* and Takao Mori

Department of Biological Sciences, Graduate School of Science, University of Tokyo, Bunkyo, Tokyo 113–0033 and *Department of Endocrinology, Medical Research Institute, Tokyo Medical and Dental University, Bunkyo, Tokyo 113–8519

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Changes in uterine blood vessels were evaluated quantitatively by an immuno-histochemical study using an antibody to von Willebrand factor (vWF) during the development of experimentally induced adenomyosis in mice. In all mice treated with pituitary grafting, which is a useful method of inducing adenomyosis, slight adenomyosis was found near the mesometrium 4 weeks after the operation. In the uteri with adenomyosis, the mean surface area and minor axis of blood vessels increased significantly in the endometrium and showed a tendency to increase in the myometrium, while the mean number of blood vessels in both endometrium and myometrium decreased significantly compared with those in the normal uteri. The mean percentage of total surface area of blood vessels to mean total tissue area of either endometrium or myometrium was not different in the two groups. Remarkably dilated venules in the endometrium were frequently found in pituitary-grafted mice. In the myometrium, the mean percentage of surface area and mean number of blood vessels were greater in the mesometrial side than in the antimesometrial side in both the control and pituitary-grafted mice. Thus, pituitary grafting significantly increased the number of dilated venules and decreased the number of microvessels in the uterus, suggesting that vascular changes may be one of the important etiological factors for the development of adenomyosis.

Key words: Adenomyosis, Uterus, Von Willebrand factor, Immunostaining, Mice

I. Introduction

Uterine adenomyosis is defined as the presence of endometrial components, such as glands and in stroma, in the myometrium. It occurs spontaneously in humans and in some experimental animals, although the precise pathogenesis of the disease remains unknown [8]. The SHN strain of mice has a high incidence of developing uterine adenomyosis spontaneously, and develops the disorder very soon after pituitary grafting [8, 9, 13]. Mori and Nagasawa [7] suggested that an early step of the development of uterine adenomyosis is marked invasion of stromal fibroblasts into the myometrium along the branches of blood vessels.

Von Willebrand factor (vWF) is an important element of the blood coagulation system [3], and has traditionally been used as a broad-based endothelial cell marker. In the present experiments, quantitative changes in endometrial blood vessels were investigated by using immunohistochemical techniques in order to evaluate the contribution of the vascular system to the development of adenomyosis.

II. Materials and Methods

Animals and treatments

Virgin female SHN mice maintained in our laboratory were used. Mice were housed in a temperature- and light-controlled room with a 12/12-hr light/dark cycle in accordance with the principles outlined in the Guide for Animal Care and Use of the Committee of the Graduate School of Science, University of Tokyo, and were fed laboratory chow (CE-7; Japan CLEA) and given tap water ad libitum. All experiments conformed to the regulations described in the National Institute of Health Guide to the Care and Use of Laboratory Animals.

Seven-week-old mice were implanted with a single pituitary each under the capsule of the right kidney and
were killed 4 weeks postoperatively. Females receiving no pituitary grafts were killed at a comparable age and served as controls. Both groups consisted of six mice showing diestrous vaginal smear at autopsy.

**Immunohistochemical and histological preparation**

At autopsy, uteri were weighed, fixed in neutral buffer 10% formalin solution. Uterine samples embedded in paraffin were cut transversely at 4μm thickness, deparaffinized, and rehydrated through graded ethanol for immunohistochemical staining using the alkaline phosphatase avidin-biotin-complex (ABC-AP) method [15, 17]. To improve the staining intensity, antigen retrieval was performed by treating sections in 0.1% trypsin in 0.1% CaCl₂, pH 7.8, at 37°C for 15 min. Non-specific background staining was reduced by treating the sections with non-immune 10% goat serum in Dulbecco’s phosphate buffer saline (DPBS) solution. Rabbit anti-human vWF (A-0082; Glostrup, Denmark, 1: 600 dilution) was applied to the sections overnight at 4°C in a moisture chamber. After washing them in DPBS, the sections were incubated with the biotinylated second antibody (Vectastain ABC-AP kit, Vector Laboratories, USA) for 2 hr at room temperature and again washed in DPBS. The avidin-biotin-complex was layered on the slides for 30 min and washed. The sections were rinsed in alkaline phosphatase buffer (100 mM Tris-HCl, 100 mM NaCl and 50 mM MgCl₂, pH 9.5), and then stained with ABC-AP substrate solution with 1 mM levamisole at final concentration. Finally, the sections were mounted with glycerol gelatin. Negative controls for immunostaining were prepared by substituting the first antibody with normal rabbit serum. In addition, serial 7μm thickness sections of paraffin blocks were cut and stained with hematoxylin and eosin according to ordinary procedure.

**Quantitative evaluation of staining**

Tissue sections were examined under a light microscope (Olympus, Tokyo, Japan). Images of five sections from each uterus were randomly captured by an Argas 20 system (HAMAMATSU Image Processor, Hamamatsu, Japan) after contrast enhancement for later analysis with NIH image software (version 1.55; Wayne Rasband, NIH, USA) and the blood vessels with area > 60μm² were measured. The parameters evaluated in blood vessels included the surface area of blood vessels and uterine tissues, minor axis (the shorter diameter) of blood vessels and number of blood vessels per mm² of uterine tissues. In addition, the mean percentage of the total surface area of blood vessels to the total tissue area was calculated. The endometrium and myometrium of the uteri were evaluated separately. The morphometric analysis of each specimen was done by one observer and confirmed by a second observer blinded as to the specimen source.

**Statistical analysis**

All parameters are expressed as mean±SE. Statistical analysis was performed by the unpaired t-test, and p<0.05 was considered significant.
III. Results

All mice with pituitary grafting developed slight adenomyosis as defined by Mori and Nagasawa [8], but no control mice developed adenomyosis. There was no significant difference in uterine weight between the control (135.7±10.2 mg/30 g body weight) and pituitary-grafted mice (148.9±5.0 mg/30 g body weight).

In pituitary-grafted mice, the mean surface area and minor axis of blood vessels increased significantly in the endometrium, and showed a tendency to increase in the myometrium compared with those in control mice (Fig. 1A, B). In contrast, the mean number of blood vessels in both endometrium and myometrium decreased significantly compared with those in the controls (Fig. 1C). The mean percentage of the total surface area of blood vessels to the total tissue area of either endometrium or myometrium was similar in the two groups (Fig. 1D). Markedly dilated venules in the endometrium were frequently found in mice with pituitary grafting (Fig. 2A, B) when compared with normal controls (Fig. 2C).

In all six mice with adenomyosis, the penetrating endometrial tissues were always found near the mesometrium of the uterus, although in some mice they were also detected in other regions of the uterus. The ectopic endometrial tissues in the muscular layer were usually accompanied by blood vessels. When blood vessels were not found, the ectopic endometrial tissues invaded,
in general, towards the mesometrium. To ascertain the anatomic localization of the pathogenesis of the disease, uterine sections were divided into two even parts, i.e. the mesometrial side (half of the uterus near the mesometrium, M-side) and the antimesometrial side (the other half of the uterus, A-side) and the changes in the vascular system were evaluated separately. In both endometrium and myometrium, the mean surface area and minor axis were similar in the M-side and A-side in both control and pituitary-grafted mice. In the endometrium, the mean percentage and mean number were also similar in either control or pituitary-grafted mice (data not shown). In the myometrium, the mean percentage of surface area and number of blood vessels were greater in the M-side than in the A-side in both control and pituitary-grafted mice, although the difference of mean percentage of surface area in pituitary-grafted mice was not significant (Fig. 3).

IV. Discussion

The present study demonstrated that pituitary grafting significantly increased the number of dilated venules and decreased the number of microvessels in the endometrium. Recent studies have provided evidence that estrogen and progesterone receptors are present in the endothelial and smooth muscle cells of the blood vessel wall in both humans and animals [1, 18, 19]. It has been reported that, in women treated with progesterone, the dilated venules increased in number and the density of microvessels decreased in the endometrium when compared with those in controls [4, 14, 16].

In mice, pituitary grafting results in a significant increase of circulating prolactin levels [8]. The 16-kilodalton N-terminal fragment of prolactin (16K PRL) inhibits the growth of capillary endothelial cells, and the receptors for 16K PRL have been detected in endothelial cells of bovine brain capillaries [2]. Along with hyperprolactinemia, the serum progesterone levels also increased [5]. It seems reasonable that the higher levels of progesterone and 16K PRL might be responsible for the increase in the dilated venules and decrease in the number of microvessels in the present study.

As the mice were killed shortly after pituitary grafting and showed an early stage of adenomyosis, the vascular changes in the uterus seem unlikely to result from the progression of the disease. Thus, the increase in the number of dilated venules may be one of the important etiological factors for uterine adenomyosis. Mori and Nagasawa [7, 10] described in detail early signs of adenomyosis, i.e., at first the appearance of highly developed and markedly dilated blood vessels running straight across the inner myometrium, followed by invasions of stromal fibroblasts into the myometrium along the branches of blood vessels. The present immunohistochemical study of vWF quantitatively confirmed that uterine microvessels increased in diameter after pituitary grafting. The dilated venules provide a convenient channel for the invasion of endometrial stromal cells. The increase in cell death of the inner muscle layer may facilitate this invasion process [20].

As the main blood vessel reaches the uterine body through the mesometrium, the vascular network is abundant near the mesometrium. In the present experiments, adenomyosis was commonly observed near the mesometrium, which is consistent with the previous findings [11]. Furthermore, it was found that the penetrating endometrial tissues in the muscular layer are always accompanied by blood vessels when the vessels can be detected. In cases where a blood vessel can not clearly be seen, the ectopic endometrial tissues penetrate, in general, in the direction of the mesometrium. All these findings suggest that uterine blood vessels may provide a favorable avenue for the development of adenomyosis in mice.

Until now, little has been known about the pathogenesis of uterine adenomyosis in women. Few publications refer to uterine blood vessels in relation to the pathogenesis of the disease. It is well known that pregnancy, delivery and deep curettage of the uterus are the main risk factors of the disease [6]. It is of interest to note that either marked vascularization and/or weakness of the myometrium always accompany these risk factors. It has been reported, indeed, that in adenomyosis patients, endometrial vascularization increased markedly, and dilated microvessels were seen frequently in adenomyosis [12]. The change in blood vessels is suggested to be an important etiological factor for adenomyosis in women, and treatment by suppressing the growth of the vascular endothelium might be worth considering.

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VI. References

Uterine Adenomyosis and Microvessels


