Vascular system of intramural leiomyomata revealed by corrosion casting and scanning electron microscopy

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Background: The vascular system of leiomyomata, the most common benign tumours in women, is an important factor controlling development and growth of the tumour. It has not been, however, investigated morphologically using the best currently available technique, corrosion casting combined with scanning electron microscopy.

Methods: Myomatous uteri collected upon autopsy were perfused via afferent vessels with fixative followed by Mercox resin and corroded after polymerization of the resin. The obtained vascular casts visualizing all vessels including capillaries were examined using scanning electron microscopy.

Results: The smallest (1–3 mm) fibroids were avascular, in larger ones (<1 cm) a few small vessels invaded the lesion from the periphery. The largest tumours (>1 cm) contained irregular networks of blood vessels with density similar to or lower than that of normal myometrium. Such tumours were surrounded by an extremely dense vascular layer (‘vascular capsule’) which was the source of larger vessels supplying and draining the tumour.

Conclusions: During development of leiomyoma, the pre-existing blood vessels undergo regression and new vessels invade the tumour from the periphery, where intense angiogenesis, probably promoted by growth factors secreted by the tumour, leads to the formation of a ‘vascular capsule’ responsible for supply of blood to the growing tumour.

Key words: angiogenesis/blood vessels/corrosion casting/electron microscopy/leiomyoma

Introduction

The uterine leiomyomata (fibroids) are the most common benign tumours of the female genital system. Their high incidence, associated symptoms (pelvic pain, menorrhagia) and side-effects (infertility, loss of pregnancy) present a serious clinical problem.

In recent years, angiogenesis and vascularization have been regarded as significant factors controlling the growth of tumours, especially malignant ones. However, the information concerning fibroid vasculature is not abundant and to some extent controversial. Since the early paper of Sampson (1912), classical injection studies using colour or radio-opaque dyes demonstrated the arrangement of arteries and veins in the leiomyomata. There has been a consensus that small fibroids are significantly less vascular than the surrounding myometrium, but as far as large leiomyomata were concerned, some authors reported increased density of blood vessels (Sampson, 1912; Faulkner, 1944), whereas others observed the opposite (Farrer-Brown et al., 1970). More recent studies of blood flow in leiomyomata (Forssman, 1976a;b; Kurjak et al., 1992; Huang et al., 1996; Sosic et al., 1996), as well as quantitative assessment of the vascular density in the immunocytochemically stained fibroid sections (Casey et al., 2000; Hague et al., 2000), yielded ambiguous results.

The corrosion casting technique combined with scanning electron microscopy (SEM) is the best currently available method for morphological examination of the vascular networks (Lametschwandtner et al., 1990). The injected resin fills all blood vessels including capillaries, and SEM offers high resolution and quasi-three-dimensional image. Since this method has not been employed in the studies of fibroid vasculature, the present study was undertaken to examine the vascular architecture of leiomyomata.

Materials and methods

Twenty-two uteri were obtained upon autopsy of women aged 22–71 years, deceased due to causes not related to disorders of the reproductive system. The study was approved by the Ethics Committee of the Jagiellonian University Medical College. The material was collected 6–22 h after death. Each uterus together with ovaries and cervical portion of the vagina was removed in such a way that relatively long fragments of uterine and ovarian vessels (arteries and veins) were retained.

Immediately after removal, the uteri were perfused via the afferent arteries with prewarmed (37°C), heparinized saline (12.5 IU/ml heparin; Polfa, Poland) containing 3% dextrane (70 kDa) and 0.025% lidocaine (Lignocaine; Polfa), until the fluid outflowing via the veins was completely transparent (~5 min). Next, perfusion was continued using a solution of 0.66% paraformaldehyde/0.08% glutaraldehyde.
(Sigma) in 0.1 mol/l cacodylate buffer, pH 7.4, supplemented with 0.2% lidocaine. Finally, the vascular system was injected with 60–80 ml of Mercox CL-2R resin (Vilene Comp. Ltd, Japan) containing 0.0625 mg/ml methyl acrylate polymerization initiator (Vilene Comp. Ltd) and the uteri were left in a warm water bath (56°C) for several hours to allow polymerization and tempering of the resin.

When the polymerization was completed, the uterine tissues were macerated for 5–6 days by repeated baths in 10% potassium hydroxide at 37°C followed by washing with warm (50–55°C) running tap water. The obtained vascular casts were washed for the next 4–5 days in multiple changes of distilled water under mild vacuum conditions, cleaned in 5% trichloroacetic acid for 1–2 days, washed again in distilled water for 2–3 days and freeze-dried in a lyophilizer (Liavog G2; Aqua Fina, Germany).

The freeze-dried casts were examined macroscopically, gently dissected to expose the vasculature of myomata and stored in an exiccator containing phosphorus pentoxide until the microscopic examination. They were then mounted onto copper plates using colloidal silver and ‘conductive bridges’ (Lametschwandtner et al., 1980) and coated with gold. The casts were examined using a JEOL SEM 35-CF scanning electron microscope at 20–25 kV.

Results

Among 22 uteri prepared for corrosion casting, only five yielded casts of acceptable quality. The examined uteri contained multiple leiomyomata of different size (Figure 1). The present observations are limited to intramural fibroids, since the vascular casts of the exophytic, subserosal tumours were always considerably damaged.

The smallest fibroids (1–3 mm) were usually almost avascular, being surrounded by relatively dense myometrial vascular network composed mostly of capillaries and containing a few larger vessels, both arteries and veins (Figure 2). This network did not substantially differ from the vasculature of areas occupied by normal myometrium. In larger myomata (up to 1 cm), the density of the surrounding vessels increased, and a few small vessels (capillaries, arterioles and small arteries) invading the lesion could also be observed (Figure 3). Occasionally, such myomata contained one or two larger, tortuous vessels (usually artery, sometimes accompanied by vein) traversing the tumour, but they seldom gave any branches on the territory of the lesion (Figure 4).

Large myomata (>1 cm) contained a chaotic network of blood vessels—mostly capillaries, arterioles and venules. Two patterns of arterial supply were observed: either two or three larger arteries penetrated towards the central areas of the tumour, giving relatively few side branches, or multiple smaller and shorter arteries supplied the tumour from the periphery branching relatively early into arterioles and capillaries. In routine histological sections, such larger vessels are usually observed in the connective tissue septa separating the myomatous foci. The vascular density of large myomata was variable, but generally it seemed to be lower than or similar to that of the areas of unchanged myometrium. Inside such tumours, several small (1–2 mm), roundish avascular areas occasionally penetrated by a few capillaries were observed (Figure 5).

A characteristic feature of larger fibroids was ‘vascular capsule’—an extremely dense vascular network at the border between the tumour and the surrounding myometrium, often separated from the unchanged myometrial tissue by a narrow avascular cleft (Figures 5 and 6). Capillaries, arterioles and venules, tending to form parallel arrays, were again the predominant components of this network, although larger vessels were encountered more frequently than inside the tumour (Figure 6a–c). The veins were often flattened, showing signs of compression by the tumour (Figure 3).

No arteriovenous anastomoses were observed in the investigated material.

Discussion

In this study, fresh uterine specimens from hysterectomized patients could not be used because whole organs are required for resin injection and at least fragments of surgically removed uterine tissue must be collected for histopathological examination. The application of corrosion casting and SEM to autopsy material bears a risk of poor tissue preservation and only single papers report successful casting of such material (Banya et al., 1989; Murakami et al., 1994). This obviously limits corrosion casting studies of human tissues and organs. However, we were able to obtain acceptable casts from ~20% of uteri collected upon autopsy 6–12 h after death of the patient. This demonstrates that—considering all the limitations—the technique can be successfully applied to human organs obtained upon autopsy.

In his classic paper, Faulkner (1944) described the vascular system of myoma as ‘a mass of proliferating arteries’. The present observations have not confirmed such a view, showing that arteries are not the predominant vessel type in the myomata (the technique used by Faulkner did not visualize capillaries) and that the vascular density of myomata demonstrated by SEM seems to be lower than or similar to that of unchanged myometrium. However, it seems possible that Faulker’s description refers to the ‘vascular capsule’, a zone of very high vascular density revealed by corrosion casting around the tumour. Such structure has not been explicitly described in the previous publications, although ‘venous plexus’ (Farrer-Brown et al., 1970) or ‘vascular plexus’ (Awataguchi, 1982) were mentioned to occur around the periphery of myomata. In a recent immunocytochemical/morphometric study, Casey et al. (2000) reported significantly higher microvascular density in the adjacent myometrium than in small and large myomata. In our material, ‘vascular capsule’ was a constant feature of all myomata except for the smallest ones and it reached the highest density of blood vessels in large tumours.

The corrosion casting technique did not reveal extremely dilated veins in the myometrium and inner layer of myometrium described by Farrer-Brown et al. (1970) in their dye-injection study, although flattening of venous vessels surrounding the tumours, also reported by these authors, was observed in our material and seemed to result from the compression exerted by the tumour. Since these authors injected the veins, the reported dilatation might have been artificially produced by the injection pressure, whereas in the present study the resin was injected via arteries and filled the veins under low pressure after passing through the capillary bed.
Although some differences in blood flow revealed by colour Doppler sonography have been demonstrated between outer myometrial and submucosal leiomyomata (Tsuda et al., 1998), no differences in the vascular pattern of tumours originating from those two locations have been observed in this study.

Figure 1. Uterine specimen prepared for inspection in SEM. Note multiple myomata of different sizes (encircled). Asterisks: contact bridges. Bar = 1 cm.

Figure 2. Four small avascular myomata, ~2 mm in diameter (asterisks), surrounded by myometrial vasculature. Bar = 1 mm.

Figure 3. A larger myoma (~4 mm in diameter) with a few vessels invading the tumour from the periphery. Note the ‘vascular capsule’ surrounding the myoma and flattened, compressed veins (asterisks). Bar = 1 mm.

Figure 4. Highly tortuous artery and vein traversing a nearly avascular myoma without giving off branches on the territory of the tumour. Bar = 1 mm.

Figure 5. Fragment of a large (3 cm) myoma showing a chaotic network of blood vessels (mainly capillaries), several small avascular spaces (asterisks, probably necrotic foci) and the surrounding vascular capsule (VC). Bar = 1 mm.
Figure 6. The ‘vascular capsule’ surrounding large (2 cm) myoma. (A) The inner aspect of the capsule (the vasculature of myoma was removed) showing larger vessels entering the capsule from periphery (arrowheads). (B) Higher magnification of the outer aspect of the capsule demonstrating the high density of the capillaries. (C) The capsule and adjacent myometrium; note parallel arrays of extremely dense capillaries and of larger vessels forming the capsule (C), separated from the myometrial vasculature (M) by a narrow avascular cleft (asterisk). Bars = (A) 1 mm, (B) 500 μm, (C) 500 μm.
The capillaries present in myomata had typical appearance. We did not encounter thick capillary vessels with irregular profiles, observed in corrosion casts of some malignant tumours investigated earlier in our laboratory (Bugajski et al., 1989; Miodoński et al., 1998). The relatively low vascular density of myomata points to lower intensity of angiogenesis in this benign tumour, as compared with that of rapidly growing malignant neoplasms in which the accelerated angiogenesis may lead to formation of morphologically abnormal capillary vessels. As recently reported (Hong et al., 2001), angiogenic growth factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor show significantly stronger expression in leiomyosarcoma that in leiomyoma. In our study on urinary bladder cancer (Miodoński et al., 1998), we suggested that VEGF may promote formation of wide, irregular capillaries observed in the exophytic part of that tumour.

As can be concluded from the vascular architecture of the myomatous uteri, the most intense angiogenesis occurs around the periphery of the myoma. The fibroids have been shown to produce a variety of angiogenic growth factors, including epidermal growth factor, VEGF, platelet-derived growth factor, transforming growth factor-α and -β, basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF) and adrenomedullin (ADM) (Harrison-Woolrych et al., 1994, 1995; Mangrulkar et al., 1995; Vollenhoven et al., 1995; Arici and Sozen, 2000; Dixon et al., 2000; Hague et al., 2000; Hong et al., 2001). These factors, released from the tumour, influence angiogenesis not only inside the myoma, where the process may be obstructed by the compact character of tumour tissue, but also in the surrounding myometrium. Among the angiogenic factors, bFGF and ADM are the likely candidates to promote angiogenesis around myomata, since they were found to occur in larger quantities in myomata than in the unchanged myometrial areas (Mangrulkar et al., 1995; Hague et al., 2000).

ADM is produced upon hypoxia—a natural consequence of the avascularity of small myomata—and its expression correlates with the vascular density of leiomyoma and the myometrium of leiomyoma-bearing uteri. Its release from the tumour was suggested to stimulate angiogenesis in the surrounding myometrium (Hague et al., 2000). The possible concentration gradient of the centrifugally released factor (the highest concentration at the tumour periphery) might be responsible for the formation of the ‘vascular capsule’ around the tumour. Large quantities of bFGF are stored in the extracellular matrix of leiomyomata (Mangrulkar et al., 1995), hence its action in larger tumours, containing connective tissue septa and surrounded by connective tissue capsule, could also significantly contribute, respectively, to the development of new vessels inside the tumour and at its periphery.

The vascular patterns observed in leiomyomata of various size allow us to propose a pathway of vascular system development in these tumours. The small myomatous foci compress the pre-existing blood vessels, induce their regression, leading to formation of transiently avascular regions inside such tumours. Subsequently, the density of blood vessels increases in the direct surroundings of the myoma, and, as it grows in size, new blood vessels penetrate the tumour from its periphery where ‘the vascular capsule’ is being formed, and provide origin to vascular network observed inside the larger leiomyomata. Small avascular areas observed in the latter tumours probably represent either necrotic or newly developing myomatous foci.

This concept does not quite agree with the earlier suggestion (Farrer-Brown et al., 1970) that the arterial pattern of a myoma represents an expansion of the pre-existing supply to that area of myometrium. It does correspond, however, with the recently proposed model of angiogenesis in growing tumours (Holash et al., 1999a,b): the pre-existing vasculature is first co-opted by the tumour, then undergoes regression and the angiogenesis starts at the periphery of the tumour leading to invasion of new vessels into the tumour which promote its further growth. Such sequence of events was also considered in the case of myomata (Casey et al., 2000).

In our material, the earliest stage of vessel co-option by newly developing myomatous foci cannot be visualized, because such areas would not differ in the vascular architecture from the surrounding normal myometrial tissue. However, large, branchless arteries and veins occasionally found inside small, almost avascular leiomyomata probably represent co-opted vessels which, due to their size, are more resistant to tumour-induced regression than smaller, thin-walled vessels.

The role of vascular abnormalities in the development of clinical symptoms associated with leiomyomata, such as abnormal uterine bleeding, was first proposed nearly a century ago (Sampson, 1912). Contemporary studies demonstrated dysregulation of various growth factors and their receptors in the myomatous uteri (Stewart and Nowak, 1996). Further research aimed at disclosing the successive angiogenic events in the course of leiomyoma growth and also at explaining the involvement of specific growth factors in that process will help not only to elucidate the pathogenesis of this common tumour, but also to design new therapeutic strategies.

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