Combined inhibition of vascular endothelial growth factor (VEGF), fibroblast growth factor and platelet-derived growth factor, but not inhibition of VEGF alone, effectively suppresses angiogenesis and vessel maturation in endometriotic lesions

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BACKGROUND: Angiogenesis represents the crucial step in the pathogenesis of endometriosis, because endometriotic lesions require neovascularization to establish, proliferate and invade inside the peritoneal cavity. To elucidate the role of angiogenic factors, we investigated in vivo whether blockade of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) affects angiogenesis of ectopic endometrium. METHODS: Mechanically isolated endometrial fragments were transplanted into the dorsal skinfold chamber of hormonally synchronized hamsters. Subsequently, we analysed the effect of the VEGF inhibitor SU5416 and the combined VEGF, FGF and PDGF inhibitor SU6668 on angiogenesis of the ectopic endometrium over a time-period of 14 days using intravital fluorescence microscopy. RESULTS: Selective blockade of VEGF resulted in a slight reduction of microvessel density when compared to control animals. In contrast, combined inhibition of all three growth factors significantly suppressed angiogenesis of endometrial grafts, as indicated by a reduced size of the microvascular network and a decreased microvessel density. This was caused by an inhibition of blood vessel maturation. CONCLUSIONS: Vascularization of endometriotic lesions is not solely driven by VEGF, but depends on the cross-talk between VEGF, FGF and PDGF. Thus, the combined inhibition of these growth factors may represent a novel therapeutic strategy in the treatment of endometriosis.

Key words: angiogenesis/dorsal skinfold chamber/endometriosis/growth factors/intravital fluorescence microscopy

Introduction

Endometriosis is characterized by the presence of endometrial tissue at ectopic sites. Today, the implantation theory of Sampson (1927) is the most widely accepted aetiology of this common gynaecological disease, which means that during menstruation endometrial fragments pass backward along the Fallopian tubes and implant inside the peritoneal cavity. Recently, several studies have proposed that angiogenesis represents an important step during this process, because similar to tumour metastases, endometriotic implants require neovascularization to establish, proliferate and invade (Fujishita et al., 1999; Taylor et al., 2002; Gescher et al., 2003). In fact, a characteristic clinical feature of endometriotic lesions is their dense vascularization (Nisolle et al., 1993). This led to the idea that the suppression of blood vessel development by inhibition of specific angiogenic factors may be a novel therapeutic opportunity in the treatment of endometriosis (Hull et al., 2003; Nap et al., 2004; Olive et al., 2004).

During the last years, several angiogenic factors could be identified, which are involved in the advancement of endometriosis, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF).

VEGF is probably the most studied angiogenic factor. The biological effects of VEGF are mediated by two high-affinity receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1/KDR), which are expressed on the surface of microvascular endothelial cells (de Vries et al., 1992; Millauer et al., 1993). These two receptors serve distinct functions such as endothelial cell proliferation and chemotaxis (Waltenberger et al., 1994). Interestingly, peritoneal fluid concentrations of VEGF from patients with endometriosis correlate significantly with the stage of the disease (Shifren et al., 1996). Moreover, it has been shown that endometriotic lesions themselves produce VEGF, predominantly in the epithelial cell layer (Goteri et al., 2004).

FGF is a potent pleiotropic heparin-binding mitogen for vascular endothelial cells which binds to specific FGF receptors
with intrinsic tyrosine kinase activity and synergistically acts with VEGF in stimulating new vessel formation (Seghezzi et al., 1998; Klint and Claesson-Welsh, 1999). FGF and its mRNA have also been detected in endometriotic lesions and correlate with the lesion diameters (Di Blasio et al., 1995).

PDGF signals through two cell-surface tyrosine kinase receptors, PDGFR-α and PDGFR-β, and induces angiogenesis by up-regulating VEGF production and modulating the proliferation of pericytes and fibroblast-like cells surrounding the endothelium (Sato et al., 1993; Lindahl et al., 1997). Immunohistochemical studies indicate that PDGF is localized in human endometrium and that PDGFR-β staining intensity is increased during the proliferative phase in luminal and epithelial cells (Chegini et al., 1992). Furthermore, in vitro studies could demonstrate that PDGF plays an important role in stromal cell proliferation during endometriosis (Surrey and Halme, 1991).

In light of the fact that not only VEGF but also FGF and PDGF are expressed in endometriotic lesions, it is reasonable to speculate that the process of vascularization is not solely driven by VEGF, but may depend on the interaction of all three growth factors, including redundant angiogenic actions. In consequence, this would indicate that combined inhibition of these three growth factors might be more effective in the treatment of endometriotic lesions than antagonizing VEGF signal transduction alone. In order to confirm this hypothesis, we transplanted mechanically isolated endometrial fragments into the dorsal skinfold chamber of hormonally synchronized Syrian golden hamsters. With the use of the small molecule angiogenesis inhibitor SU5416, which solely inhibits the activity of VEGF, and SU6668, which is a multipotent inhibitor of the tyrosine kinase activity of VEGF, FGF and PDGF receptors (Mendel et al., 2000; Hoekman, 2001), we then analysed the role of the three growth factors on the process of angiogenesis and vascularization of endometriotic lesions.

Materials and methods

Animals

The experiments were conducted in accordance with the German legislation on protection of animals and the NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, Washington, USA), and were approved by the local governmental animal care committee. Eight to 10 week old female Syrian golden hamsters with a body weight of 60–80 g were used for the study. The animals were housed one per cage and had free access to tap water and standard pellet food (Altromin, Lage, Germany) throughout the experiment.

Preparation of the dorsal skinfold chamber

The dorsal skinfold chamber preparation contains one layer of striated muscle, subcutaneous tissue and skin and allows for intravital microscopic observation of the microcirculation in the awake animal over a time period of 2–3 weeks. The chamber technique and its implantation procedure have been described previously in detail (Menger et al., 2002). Briefly, under sodium pentobarbital anaesthesia (50 mg/kg body weight i.p.), two symmetrical titanium frames were implanted on the extended dorsal skinfold of the hamsters, so that they sandwiched the double layer of skin. One layer of skin was then completely removed in a circular area of ~15 mm in diameter, and the remaining layers (consisting of striated skin muscle, subcutaneous tissue and skin) were covered with a removable coverslip incorporated into one of the titanium frames. After the preparation, the animals were allowed to recover from anaesthesia and surgery for ≥48 h.

Isolation and transplantation of endometrial fragments

For isolation of endometrial fragments, each hamster equipped with a dorsal skinfold chamber was anaesthesitized with pentobarbital sodium (50 mg/kg body weight intraperitoneally). After laparotomy, one uterine horn was aseptically removed and placed in a 30 mm diameter Falcon plastic Petri dish filled with 37°C warm Dulbecco’s modified Eagle’s medium (10% fetal calf serum, 0.1 mg/ml gentamicin) and the fluorescent dye bisbenzimide H33342 for staining of endometrial tissue (200 μg/ml; Sigma, Deisenhofen, Germany). The uterine horn was opened longitudinally and the endometrium was dissected from the uterine muscle under a stereo microscope. Then, the endometrium was transferred into 37°C warm bisbenzimide H33342-free Dulbecco’s modified Eagle’s medium and microdissected into endometrial fragments of comparable size. For autologous transplantation of endometrial fragments, the cover glass of the dorsal skinfold chamber was removed and three or four endometrial fragments were placed on the striated muscle within the chamber. After transplantation into the dorsal skinfold chamber, the specific fluorescence/background fluorescence ratio is high enough to precisely delineate the stained endometrial tissue from the non-stained surrounding host tissue (Vollmar et al., 2001).

All animals were hormonally synchronized, i.e. in the same stage of the 4 day estrus cycle in the hamster, and endometrial fragments were transplanted on estrus. Synchronization was performed according to the method of Gross by administration of two subcutaneous injections of 7.5 μg/135 g body weight estradiol (Sigma), given 24 h apart, followed by one injection of 1.0 mg/135g body weight of progesterone (Sigma), given 20 h after the last estradiol injection (Gross, 1977).

Intravital fluorescence microscopy

For in vivo microscopic observation, the animals were immobilized in a Plexiglas tube and the dorsal skinfold preparation was attached to the microscopic stage. After i.v. injection of 0.15 ml 5% fluorescein isothiocyanate (FITC)-labelled dextran 150 000 (contrast enhancement by intravascular staining of plasma), intravital fluorescence microscopy was performed using a modified Leitz Orthoplan microscope with a 100 W mercury lamp attached to a Ploemo-Pak illuminator with blue, green and UV filter blocks (Leitz, Wetzlar, Germany) for epi-illumination. The microscopic images were recorded by a charge-coupled device video camera (CF8/1 FMC; Kappa GmbH, Gleichen, Germany) and transferred to a video system for off-line evaluation. With the use of ×5, ×6.3, ×10 and ×20 long-distance objectives, magnifications of ×108, ×136, ×216 and ×432 were achieved on a 14-inch video screen (PVM 1444; Sony, Tokyo, Japan).

Microcirculatory analysis

Quantitative off-line analysis of the videotapes was performed by means of the computer-assisted image analysis system CapImage (Zeintl, Heidelberg, Germany) and included the determination of the size of the transplanted endometrial fragments (mm²), the size of the blood-perfused microvascular networks (given in percentage of the size of the grafts), the microvessel density, i.e. the length of red blood cell (RBC)-perfused microvessels per observation area (cm²/mm²), the diameters of the microvessels (μm) and the centreline RBC velocity VRBC (μm/s). Volumetric blood flow (VQ) of individual microvessels was calculated from VRBC and diameter (d) for each microvessel as:

\[ VQ = \pi \times (d/2)^2 \times VRBC/K (pl/s) \]
where $K (= 1.3)$ represents the Baker–Wayland factor (Baker and Wayland, 1974), considering the parabolic velocity profile of blood in microvessels.

**Experimental protocol**

A first group of eight female hamsters were treated with SU5416 (25 mg/kg body weight/day i.p. in 150 μl DMSO), whereas a second group ($n = 4$) received SU6668 (75 mg/kg body weight/day i.p. in 150 μl DMSO). Animals of the control group ($n = 5$) were treated with the vehicle DMSO (150 μl i.p.). In all, 25 and 15 endometrial fragments were transplanted into the dorsal skinfold chambers of the SU5416 and the SU6668 group respectively. Control hamsters received a total of 18 endometrial fragments.

The macroscopic appearance of the skinfold chamber preparations and the implanted grafts were documented daily. Intravital fluorescence microscopic analysis of the microcirculation was performed on days 0 (day of transplantation), 2, 4, 7, 10 and 14 after transplantation of endometrial fragments. Measurements on vascular density and microhaemodynamics included only newly formed microvessels, which could be clearly distinguished by their glomerular-like arrangement from the autochthonous host striated muscle microvessels, which display the typical parallel arrangement of muscle capillaries (Menger and Lehr, 1993). Microvessel density was measured within three regions of interest per graft and observation time-point. Microvascular diameters and microhaemodynamic parameters were determined by analysing 10 microvessels per region of interest. Microvessels were selected randomly inasmuch as those microvessels were chosen for analysis of diameter, which crossed a vertical line drawn over the centre of the video screen. In all microvessels selected, both vessel diameter and $V_{RBC}$ were determined for subsequent calculation of $VQ$. At the end of the in vivo experiments, i.e. day 14 after transplantation of endometrial fragments, the animals were killed with an overdose of pentobarbital, and the dorsal skinfold chamber preparations were processed for haematoxylin–eosin staining and immunohistochemistry.

**Histology and immunohistochemistry**

For light microscopy, formalin-fixed specimens of the dorsal skinfold chamber were embedded in paraffin. Sections 4 μm thick were cut and stained with haematoxylin and eosin according to standard procedures. For immunohistochemical detection of pericytes lining the wall of newly formed blood vessels within the endometrial grafts, desmin staining was performed by a mouse monoclonal anti-desmin antibody as primary antibody (1:50; Dako Cytomation, Hamburg, Germany). A goat anti-mouse antibody (1:200; Amersham, Freiburg, Germany) served as secondary antibody. 3,3′-Diaminobenzidine was used as chromogen. The sections were counterstained with haemalum and examined by light microscopy (BX60; Olympus, Hamburg, Germany).

**Statistics**

Data were first analysed for normal distribution and equal variance. Differences between groups were then calculated by analysis of variance (ANOVA) followed by the appropriate post hoc test compensating for multiple comparisons. To test for time-effects within each experimental group, ANOVA for repeated measures was applied. This was followed by a post hoc paired comparison, including correction of the $α$-error according to Bonferroni probabilities for repeated measurements (SigmaStat; Jandel Corporation, San Rafael, CA, USA). All data are given as means ± SEM. Statistical significance was accepted for a value of $P < 0.05$.

**Results**

After isolation and transplantation, the initial size of the endometrial grafts was comparable in SU5416-treated (0.56 ± 0.05 mm²) and SU6668-treated animals (0.55 ± 0.10 mm²), and did not significantly differ from that of DMSO-treated controls (0.48 ± 0.01 mm²). In all three experimental groups, typical signs of angiogenesis, such as sinusoidal sacculations and sprout formation, were observed as soon as day 2 after transplantation. During the following days, the capillary sprouts interconnected with each other and finally developed new glomerulus-like microvascular networks. However, whereas in controls and SU5416-treated animals the whole area of the endometrial grafts was almost completely vascularized by day 4 after transplantation (Figure 1A and C), SU6668-treated animals showed a significant restriction of angiogenesis with a reduced vascularized area of only ~55–70% of the grafts’ size from day 4 to the end of the 14 day observation period (Figure 1B and C).

![Figure 1](image-url)  
**Figure 1.** (A, B) Intravital fluorescence microscopy of the newly formed microvasculature of endometrial grafts at day 7 after autologous transplantation into the dorsal skinfold chamber of a control (A) and a SU6668-treated (B) Syrian golden hamster. While the endometrial graft of the control animal already exhibits a complete glomerular-like microvascular network, substantial parts of the endometrial graft in the SU6668-treated hamster still lack vascularization (asterisks). Also, note the still increased and heterogeneously distributed capillary diameters in the endometrial tissue of the SU6668-treated animal (B), indicating lack of maturation of the newly formed microvessels. Blue-light epi-illumination with contrast enhancement by 5% FITC-labelled dextran 150 000 i.v. Scale bars = 150 μm. (C) Vascularized area (%) of endometrial grafts' size from day 4 to the end of the 14 day observation period.
The newly formed microvascular networks in control hamsters presented with a microvessel density of ∼300 cm/cm² throughout the observation period (Figure 2A and C). In contrast, endometrial grafts of SU5416-treated animals exhibited a significantly lower density of microvessels of ∼220–250 cm/cm² at day 10 and day 14 (Figure 2C). In SU6668-treated hamsters the anti-angiogenic effect was markedly more pronounced, in that endometrial grafts finally exhibited a microvessel density of only 50% (∼150 cm/cm²) of that measured in DMSO-treated controls (Figure 2B and C).

In control animals and SU5416-treated hamsters, the size of the endometrial grafts showed only a minor reduction to ∼80% when compared to the size initially measured after transplantation. In contrast, SU6668 treatment resulted in a marked decrease of the graft size at day 14 to only ∼55% of that measured at day 0 (Figure 3).

In endometrial grafts of both control and SU5416-treated animals, sequential analysis of the diameters of the newly formed microvessels revealed a significant decrease from ∼16–17 μm at day 2 to ∼11 μm at day 14 after transplantation (Figure 4A and C). In contrast, capillaries of SU6668-treated hamsters presented with constantly elevated diameters of ∼16–17 μm throughout the 14 day observation period (Figure 4B and C). Interestingly, these new blood vessels were characterized by a pronounced heterogeneity of vessel calibre distribution, which was due to multiple vessel wall irregularities (Figure 4B).

Centreline red blood cell velocity progressively increased in endometrial grafts from ∼20–45 μm/s at day 2 to ∼130–200 μm/s until the end of the experiment and did not show significant differences between the three experimental groups (Table I). Correspondingly, volumetric blood flow of endometrial grafts ranged from 4–9 pl/s at day 2 to 10–15 pl/s at day 14 after transplantation (Table I).

In all groups, histological examination of the dorsal skinfold preparations at day 14 after transplantation revealed typical endometriotic lesions, which consisted of cyst-like dilated endometrial glands (Figure 5A). In control animals and SU5416-treated hamsters, endometriotic lesions presented with a vascularized endometrial stroma containing mature blood vessels as shown by immunohistochemical detection of pericytes lining the vessel walls (Figure 5B). In contrast, pericytes were absent within endometrial grafts of SU6668-treated hamsters indicating disturbed vessel maturation (Figure 5C).

**Discussion**

In the present study we could demonstrate in vivo that angiogenesis and vascularization of endometriotic lesions is significantly down-regulated by combined inhibition of VEGF, FGF and PDGF, but not by antagonizing VEGF alone. For our study, we used the two small molecule tyrosine kinase inhibitors SU5416 and SU6668, which previously have been shown to inhibit angiogenesis and growth of tumours in a variety of cancer models (Vajkoczy et al., 1999; Laird et al., 2000; Ning et al., 2002; Bergers et al., 2003). The major advantages of
these anti-angiogenic compounds are their lipophilic nature and their low molecular weights, guaranteeing a rapid endothelial uptake during the presence within the vascular compartment, and thus, a rapid binding to the intracellular domain of tyrosine kinase receptors (Fong et al., 1999). Moreover, the doses of SU5416 (25 mg/kg body weight/day) and SU6668 (75 mg/kg body weight/day) chosen in the present study have been proven to be efficacious without significant toxicity in mice (Fong et al., 1999; Ning et al., 2002).

Combined inhibition of VEGF, FGF and PDGF by SU6668 effectively suppressed the process of vascularization of the endometrial fragments, as indicated by a significantly reduced size and density of the newly developing microvascular networks. This was accompanied by the regression of the endometrial transplants, resulting in a decreased size of the transplants when compared with that of controls and SU5416-treated animals at the end of the observation period, i.e. day 14 after transplantation. Because SU6668 is an angiogenesis inhibitor, which targets not only the VEGF receptor VEGFR-2 on endothelial cells (like SU5416), but also the receptors FGFR-1 and PDGFR-β on stromal cells and pericytes, our findings suggest that the targeting of the interaction of the endothelium with the perivascular cells represents an effective strategy to counteract angiogenesis and vascularization of ectopic endometrial tissue.

As described previously in detail, angiogenesis is a complex dynamic process which is characterized by a coordinated sequence of cellular interactions (Carmeliet, 2000). Upon angiogenic stimulation, vascular endothelial cells are activated and migrate into the interstitium, resulting in the formation of capillary sprouts. Endothelial cells behind the migrating endothelium of the sprouts proliferate so that the newly developing blood vessel elongates. Subsequently, the maturation of the new blood vessel proceeds with the recruitment of mesenchymal cells differentiating into pericytes, which themselves stabilize the vessel wall, resulting in a regular shape of the vessel and a reduction in the vessel calibre. Especially this last step, indicating maturation of the newly formed microvessels, is mediated by PDGF, which is presumably secreted by endothelial cells in response to VEGF (Hellström et al., 2001). As recently reported, lack of pericyte stabilization results in an extensive variability of the diameters of the newly formed capillaries (Hellström et al., 2001), and immature blood vessels without pericytes finally undergo regression (Papetti and Herman, 2002). Correspondingly, we found that blood vessels in endometrial grafts of SU6668-treated hamsters exhibited vessel wall irregularities and constantly elevated diameters throughout the observation period. Moreover, pericytes lining the vessel walls were absent within these grafts. In contrast,

### Table 1. Centreline red blood cell velocity (μm/s) and volumetric blood flow (pl/s) of endometrial grafts after autologous transplantation into the dorsal skinfold chambers of hormonally synchronized Syrian golden hamsters, which were treated daily with SU5416, SU6668 or vehicle (Control)

<table>
<thead>
<tr>
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<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
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<tr>
<td>Centreline red blood cell velocity (μm/s)</td>
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<tr>
<td>SU5416</td>
<td>33.8 ± 6.4</td>
<td>118.8 ± 25.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>168.9 ± 30.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141.7 ± 23.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>141.1 ± 11.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>SU6668</td>
<td>45.8 ± 12.3</td>
<td>70.0 ± 26.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.4 ± 21.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.2 ± 39.8</td>
<td>132.3 ± 56.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>24.0 ± 8.2</td>
<td>138.7 ± 38.1</td>
<td>192.0 ± 20.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>168.4 ± 12.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>197.7 ± 38.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Volumetric blood flow (pl/s)</td>
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<tr>
<td>SU5416</td>
<td>5.9 ± 1.6</td>
<td>12.1 ± 2.2</td>
<td>15.1 ± 2.4</td>
<td>12.6 ± 1.8</td>
<td>9.9 ± 1.0</td>
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<tr>
<td>SU6668</td>
<td>8.6 ± 0.8</td>
<td>10.1 ± 3.5</td>
<td>12.8 ± 1.7</td>
<td>14.4 ± 3.6</td>
<td>14.5 ± 4.0</td>
</tr>
<tr>
<td>Control</td>
<td>3.7 ± 1.3</td>
<td>16.0 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.4 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9 ± 1.5</td>
<td>15.0 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
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Means ± SEM.

<sup>a</sup>P < 0.05 versus day 2 within each individual group.
Anti-angiogenic treatment of endometriosis

Capillary diameters in SU5416 and control animals continuously decreased over the 14 day vascularization period, finally reaching a size of \(\sim 11\ \mu m\) after maturation. However, the blood perfusion of endometrial grafts in SU6668-treated hamsters was not affected by the suppression of vessel maturation as indicated by comparable values of red blood cell velocity and volumetric blood flow in all three experimental groups.

Although SU5416 treatment resulted in a slight but significant reduction of microvessel density when compared to the control group, it was surprising that the selective inhibition of VEGF did not show more inhibitory effects on the process of vascularization of the endometrial grafts. In fact, in the endometriosis model used in the present study, hypoxia-induced VEGF release (Shweiki et al., 1992), has to be considered as an important driving force for the development of new blood vessels, because the endometrial grafts lack an initial vascular supply after transplantation into the dorsal skinfold chamber. Thus, they are solely dependent on oxygen diffusion similar to endometrial fragments which reach the peritoneal cavity by retrograde menstruation. Therefore, we suggest that the anti-angiogenic effect of selective VEGFR-2 inhibition by SU5416 might be, at least in part, overwhelmed by an increased (redundant) activity of other angiogenic factors, which have been shown to be involved in the pathogenesis of endometriosis, such as FGF and PDGF, but also epidermal growth factor (EGF) (Huang and Yeh, 1994), platelet-derived endothelial cell growth factor (PD-ECGF) (Fujimoto et al., 1999), endometriosis protein-I (ENDO-I, endometriotic haptoglobin) (Piva and Sharpe-Timms, 1999) or interleukin-8 (IL-8) (Ryan et al., 1995).

Moreover, previous studies in nude mice demonstrated that inhibiting VEGF by application of selective VEGF antibodies could completely suppress the formation of endometriotic lesions (Hull et al., 2003). This was not the case in our study. However, the previous studies further reported that VEGF antibodies were less effective in reducing lesion formation when endometrial tissue explants had been exposed to progesterone (Hull et al., 2003). Thus, anti-VEGF therapy may be more effective in inhibiting overall lesion formation if given first-time in the proliferative, rather than in the secretory, phase of the menstrual cycle. In the present study, we transplanted endometrial fragments on estrus, i.e. at the beginning of the secretory phase. This might explain why the establishment of endometriotic lesions was not completely inhibited by the application of SU5416 directly after transplantation.

In summary, our findings demonstrate that the combined inhibition of the angiogenic factors VEGF, FGF and PDGF effectively suppresses the process of angiogenesis and vascularization of ectopic endometrial tissue. We therefore conclude that the application of anti-angiogenic compounds, which target simultaneously different mechanisms of blood vessel development, may represent a future therapeutic strategy in the treatment of endometriosis.

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Figure 5. Haematoxylin–eosin-stained cross-section of an endometrial graft at day 14 after transplantation into the dorsal skinfold chamber of a Syrian golden hamster of the control group (A). The graft reveals typical signs of an endometriotic lesion such as cyst-like dilated endometrial glands (g) with an intact glandular epithelium surrounded by a richly vascularized endometrial stroma (asterisks). (B) Immunohistochemical detection of desmin reveals the presence of mature blood vessels (asterisks) as indicated by pericytes lining the vessel walls (arrows) within the stroma of an endometrial graft of the control group as well as within the subcutaneous host tissue located below the striated muscle (m). In contrast, newly developed blood vessels (asterisks) within an endometrial graft of a SU6668-treated hamster lack pericytes, indicating disturbed vessel maturation (C). Scale bars: (A) 120 \(\mu m\); (B, C) 80 \(\mu m\).
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