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COMMENTARY

Antiangiogenic therapies in endometriosis

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Oral contraceptives, androgenic agents, progestins and gonadotropin-releasing hormone analogues have all been successfully used in the treatment of endometriosis. However, none of these drugs can eradicate the disease. It is widely accepted that the growth of newly formed blood vessels is essential for the establishment and growth of endometriotic lesions; therefore, inhibition of angiogenesis may offer a new option for treatment of this disorder. In this paper, we reviewed anti-vascular endothelial growth factor agents and other angiostatic drugs (i.e., TNP470, endostatin, anginex, rapamycin) that have been studied in laboratory and animal models of endometriosis. Although preliminary results are interesting, further investigations are required before clinical trials can be planned in humans.
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Abbreviations: CAM, chicken chorioallantoic membrane; VEGF, vascular endothelial growth factor

Endometriosis is a benign, oestrogen-dependent, gynaecological disorder characterized by the presence of endometrial tissue (glands and stroma) outside the uterine cavity (mainly on the pelvic peritoneum, the ovaries and in the rectovaginal septum). It is estimated to occur in up to 10% of women of reproductive age (Eskenazi and Warner, 1997); it is typically associated with infertility and pain symptoms (including dysmenorrhoea, dyspareunia and chronic non-menstrual pain). It is well known that endometriotic lesions can be removed at surgery determining a significant improvement in pain symptoms (Ford *et al*., 2004); however, the disease may recur after surgical excision and patients may not accept a second operation. In addition, some women may desire to avoid or delay surgery preferring medical therapy.

Currently available medical therapies are designed to suppress oestrogen synthesis, inducing atrophy of ectopic endometriotic implants or interrupting the cycle of stimulation and bleeding. Oral contraceptive, androgenic agents, progestins and gonadotropin-releasing hormone analogues have all been successfully used in the treatment of endometriosis. However, none of these drugs can eradicate the disease and, in some cases, the substantial side effects limit the long-term use of these therapies. For these reasons, new drugs are under development (Ferrero *et al*., 2005).

Angiogenesis is a prerequisite for the development of endometriosis. According to the transplantation theory (Sampson, 1927), shed endometrial fragments lodged in the peritoneal cavity require the establishment of a new blood supply for the survival of implants and the development of the disease. In line with this hypothesis, early endometriotic lesions are characterized by dense vascularization (Nisolle *et al*., 1993), and several angiogenic factors (such as vascular endothelial growth factor (VEGF), interleukin-8, placental growth factor) have been shown to contribute to the establishment and progression of the disease. Assuming that the growth of newly formed blood vessels is of pivotal importance in the development of endometriosis, inhibition of angiogenesis seems to offer a new option for treatment. Both anti-VEGF agents and other angiostatic drugs have been evaluated in different laboratory and animal models of endometriosis.

The effect of angiogenesis inhibition was initially studied by using a model in which human endometrium was implanted into nude mice (Hull *et al*., 2003). Immediately after implantation of cultured human endometrium fragments, two VEGF-A inhibitors were administered: a truncated soluble inhibitory receptor (sflt-1) and an affinity-purified antibody to human VEGF-A. Both the angiostatic agents were effective in preventing blood vessel growth and development of endometriotic explants in this animal model. It was subsequently demonstrated that the transplantation of human endometrium onto the chicken chorioallantoic membrane (CAM) leads to a strong angiogenic response and to the formation of endometriosis-like lesions (Nap *et al*., 2005). Administration of angiostatic agents (TNP470, endostatin, anginex and anti-human VEGF antibody) significantly inhibited this angiogenic response and reduced the formation of endometriosis-like lesions. Furthermore, the lesions in the CAMs treated with

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angiostatic agents showed significantly more necrosis. These findings suggest that the endometrium induces a strong angiogenic response while implanting in the ectopic site and this response seems to be crucial for the survival of the lesions.

Obviously, preventing the development of new endometriotic lesions is not sufficient as a therapy for endometriosis. In the clinical setting, treatment is initiated in women after endometriosis has been diagnosed, at which point the lesions have already been present for a period of time. Therefore, the treatment should aim to inhibit the maintenance and growth of established lesions. In line with these observations, in another study, endometriotic lesions were allowed to form in nude mice for over 3 weeks after transplantation of human endometrial tissue before evaluating the effect of angiostatic agents (Nap *et al*., 2004). TNP470, endostatin, anginex and anti-human VEGF antibody significantly decreased microvessel density and the number of established endometriotic lesions; in particular, endostatin was the most effective inhibitor of microvessel density. The effectiveness of endostatin in suppressing the growth of endometriotic lesions was subsequently evaluated in the mouse autograft model (Becker *et al*., 2005). When endostatin was initiated immediately after surgical transplantation, it inhibited the growth of surgically induced endometriosis by approximately 50%; however, this therapy did not determine the regression of established lesions (Becker *et al*., 2005). More recently, it has been shown that two synthetic fragments of the endostatin molecule show inhibitory activity on the growth of endometriotic lesions (Becker *et al*., 2006). The efficacy of anti-VEGF agents in the treatment of endometriosis was confirmed in the Rhesus monkeys autograft model (Park *et al*., 2004); in fact, the administration of an immunopurified antibody blocking VEGF receptor (anti-Flk1 antibody) determined a significant inhibition of endometriosis explants formation.

It has recently been proved that female Syrian golden hamsters equipped with dorsal skinfold chambers represent a novel endometriosis model for repetitive *in vivo* analyses of angiogenesis in ectopic endometrial tissue (Laschke *et al*., 2005). By using this model to autotransplant autologous endometrium, it has been demonstrated that angiogenesis and vascularization of endometriotic lesions are significantly downregulated by simultaneous inhibition of VEGF, fibroblast growth factor and platelet-derived growth factor, but they are not reduced by antagonizing VEGF alone (Laschke *et al*., 2006b).

In the current issue of the *British Journal of Pharmacology*, Laschke *et al*. (2006a) demonstrate that the administration of immunosuppressive doses of rapamycin significantly reduce the size of endometriotic lesions induced in the dorsal skinfold chambers of Syrian golden hamsters. This effect was associated with the inhibition of VEGF-mediated angiogenesis as indicated by a suppression of endothelial cell sprouting *in vitro* and a reduction of microvessel density in endometriotic lesions *in vivo*. Moreover, rapamycin directly inhibited cell proliferation within the endometrial tissue, whereas manifestation of apoptotic cell death remained unaffected. These observations are of particular interest as rapamycin has been widely used in humans. This compound is currently used for the prevention of allograft rejection following renal and other solid organ transplantation (McAlister *et al*., 2001; Chueh and Kahan, 2005; Lee and Chapman, 2005; Mota, 2005) and for incorporating into drug-eluting stents to prevent re-stenosis following coronary angioplasty (Kastrati *et al*., 2005). Experience in the transplanted patients suggests that long-term use of rapamycin is safe and well tolerated.

In conclusion, the administration of antiangiogenic drugs has been proved to reduce the establishment, maintenance and progression of endometriotic lesions in different laboratory and animal models; however, further investigations are required before clinical trials can be planned in humans. In particular, it would be advisable to study the safety and efficiency of the antiangiogenic substances in non-human primate model of endometriosis, such as baboons. The role of antiangiogenic compounds in the treatment of endometriosis remains to be defined. It appears unlikely that antiangiogenic drugs may cure the symptoms caused by large endometriotic nodules that are mainly composed by fibromuscular tissue (Itoga *et al*., 2003); on the contrary, these agents may have a role in the postoperative treatment of endometriosis to increase the pain-free interval and decrease the recurrence of the disease.

References

Becker CM, Sampson DA, Rupnick MA, Rohan RM, Efstathiou JA, Short SM *et al.* (2005). Endostatin inhibits the growth of endometriotic lesions but does not affect fertility. *Fertil Steril* **84** (Suppl 2), 1144–1155.

Becker CM, Sampson DA, Short SM, Javaherian K, Folkman J, D'Amato RJ (2006). Short synthetic endostatin peptides inhibit endothelial migration *in vitro* and endometriosis in a mouse model. *Fertil Steril* **85:** 71–77.

Chueh SC, Kahan BD (2005). Clinical application of sirolimus in renal transplantation: an update. *Transpl Int* **18:** 261–277.

Eskenazi B, Warner ML (1997). Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* **24:** 235–258.

Ferrero S, Abbamonte LH, Anserini P, Remorgida V, Ragni N (2005). Future perspectives in the medical treatment of endometriosis. *Obstet Gynecol Surv* **60:** 817–826.

Ford J, English J, Miles WA, Giannopoulos T (2004). Pain, quality of life and complications following the radical resection of rectovaginal endometriosis. *BJOG* **111:** 353–356.

Hull ML, Charnock-Jones DS, Chan CL, Bruner-Tran KL, Osteen KG, Tom BD *et al.* (2003). Antiangiogenic agents are effective inhibitors of endometriosis. *J Clin Endocrinol Metab* **88:** 2889–2899.

Itoga T, Matsumoto T, Takeuchi H, Yamasaki S, Sasahara N, Hoshi T *et al.* (2003). Fibrosis and smooth muscle metaplasia in rectovaginal endometriosis. *Pathol Int* **53:** 371–375.

Kastrati A, Dibra A, Eberle S, Mehilli J, Suarez de Lezo J, Goy JJ *et al.* (2005). Sirolimus-eluting stents vs paclitaxel-eluting stents in patients with coronary artery disease: meta-analysis of randomized trials. *JAMA* **294:** 819–825.

Laschke MW, Elitzsch A, Scheuer C, Holstein JH, Vollmar B, Menger MD (2006a). Rapamycin induces regression of endometriotic lesions by inhibiting neovascularization and cell proliferation. *Br J Pharmacol* **149:** 137–144.

Laschke MW, Elitzsch A, Vollmar B, Menger MD (2005). *In vivo* analysis of angiogenesis in endometriosis-like lesions by intravital fluorescence microscopy. *Fertil Steril* **84** (Suppl 2), 1199–1209.

Laschke MW, Elitzsch A, Vollmar B, Vajkoczy P, Menger MD (2006b). 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. *Hum Reprod* **21:** 262–268.

Lee VW, Chapman JR (2005). Sirolimus: its role in nephrology. *Nephrology (Carlton)* **10:** 606–614.

McAlister VC, Peltekian KM, Malatjalian DA, Colohan S, MacDonald S, Bitter-Suermann H *et al*. (2001). Orthotopic liver transplantation using low-dose tacrolimus and sirolimus. *Liver Transpl* **7:** 701–708.

Mota A (2005). Sirolimus: a new option in transplantation. *Expert Opin Pharmacother* **6:** 479–487.

Nap AW, Dunselman GA, Griffioen AW, Mayo KH, Evers JL, Groothuis PG (2005). Angiostatic agents prevent the development of endometriosis-like lesions in the chicken chorioallantoic membrane. *Fertil Steril* **83:** 793–795.

Nap AW, Griffioen AW, Dunselman GA, Bouma-Ter Steege JC, Thijssen VL, Evers JL *et al*. (2004). Antiangiogenesis therapy for endometriosis. *J Clin Endocrinol Metab* **89:** 1089–1095.

Nisolle M, Casanas-Roux F, Anaf V, Mine JM, Donnez J (1993). Morphometric study of the stromal vascularization in peritoneal endometriosis. *Fertil Steril* **59:** 681–684.

Park A, Chang P, Ferin M, Xiao E, Zeitoun K (2004). Inhibition of endometriosis development in Rhesus monkeys by blocking VEGF receptor: a novel treatment for endometriosis. *Fertil Steril* **82:** S71.

Sampson JA (1927). Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the pelvic cavity. *Am J Obstet Gynecol* **14:** 422–469.