Effect of a statin on an in vitro model of endometriosis

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Objective: To determine the inhibitory effect of a statin on angiogenesis in a three-dimensional (3-D) culture of human endometrial fragments in vitro. Angiogenesis has been proposed as an important mechanism in the pathogenesis of endometriosis, and statins (3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors) have been shown to have anti-inflammatory and anti-angiogenic activity.
Design: Experimental in vitro study of human endometrial biopsies and 3-D culture in fibrin matrix.
Setting: Research laboratory at a university-affiliated infertility center.
Patient(s): Forty-six normal ovulating women undergoing infertility treatment.
Intervention(s): Endometrial samples obtained from the fundus of the uterine cavity were minced, and the fragments were placed in a three-dimensional fibrin matrix culture system.
Main Outcome Measure(s): Presence or absence of proliferation of stromal cells and invasion of the fibrin matrix, presence or absence of vessel sprouting, and immunohistochemical characterization of cellular components.
Result(s): During the 1st week of culture, invasion of stromal cells into the fibrin matrix occurred in the control group and in some wells outgrowths were observed. After 2 weeks, endometrial glands were observed in the outgrowths at a distance from the main tissue and were growing in conjunction with new vessel formation until the end of culture period. A concentration-dependent effect of lovostatin was seen on cell growth and angiogenesis in the experimental groups. In the presence of 5 and 10 μM of statin, angiogenesis was abolished, and cell proliferation was inhibited. In the presence of 1 μM of lovastatin, angiogenesis was reduced, but cell proliferation was not affected.
Conclusion(s): The statins were shown to be effective in inhibiting the mechanisms of cell proliferation and angiogenesis in an experimental model for the development of endometriosis-like tissue. (Fertil Steril® 2007;87: 257–62. ©2007 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, in vitro, fibrin matrix, invasion, angiogenesis, statin

Endometriosis is the presence of endometrial glandular and stromal cells outside of the uterine cavity. This disease is found in about 10% of women of reproductive age and in up to 50% of women with infertility (1). In these women, quality of life often is negatively affected by chronic pelvic pain, severe dysmenorrhea, and dyspareunia. No medical therapy has been demonstrated to be effective in eradicating the disease or in preventing it without unacceptable side effects. Surgery continues as the first-line treatment to eradicate endometriotic lesions, but recurrence of the condition occurs in ≤47% of women (2, 3).

Several theories of the etiology of endometriosis have been proposed, including metaplastic alteration of peritoneal surface epithelial cells, immune-system abnormalities, and vascular dissemination of endometrial cells (4). The most widely accepted hypothesis for the development of endometriosis is retrograde menstruation through the fallopian tubes into the peritoneal cavity. It has been shown that endometrial fragments in peritoneal cavity, similarly to tumor metastases, follow two basic steps to generate endometriosis: implantation and acquisition of a new blood supply through angiogenesis. Angiogenesis, the development of new capillaries from preexisting blood vessels (5), has been proposed as an important mechanism in the pathogenesis of endometriosis.

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The 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors, referred to as statins, are used widely for lowering cholesterol synthesis by blocking conversion of HMG-CoA to mevalonate. Several clinical trials have demonstrated that statins are effective for both the primary and secondary prevention of coronary artery diseases. In addition, statins have been shown to have anti-inflammatory and anti-angiogenic activity. Statins have biphasic potential either to promote or inhibit angiogenesis (6). Low statin doses induce a proangiogenic effect through the serine–threonine protein kinase Akt activation and increase nitric oxide production, whereas high statin doses may decrease protein prenylation and inhibit cell growth.

The aim of the present study was to determine the potential inhibitory effect of a statin (lovastatin; Sigma, St. Louis, MO) on angiogenesis in a three-dimensional fibrin matrix culture of human endometrial fragments in vitro that we have described elsewhere as a possible endometriosis model (7).

MATERIALS AND METHODS

Patients

Endometrial biopsies were performed as part of a diagnostic workup in some premenopausal women who were referred to the Toronto Centre for Advanced Reproductive Technology for infertility treatment. Exclusion criteria included any endometrial abnormality (polyps, hyperplasia, or cancer) and administration of any hormones, GnRH agonist therapy, or intrauterine device within the last 3 months. The Research Ethics Committee of Mount Sinai Hospital in Toronto approved the performance of endometrial biopsy and the use of fragments of human endometrium as described in Three-Dimensional In Vitro Culture of Endometrial Fragments. A written informed consent describing the procedures and aims of the study was obtained from each donor in compliance with regulations concerning the use of human tissues. Endometrial samples were collected from a total of 46 normal ovulating women (mean age, 37.3 y [range, 31–44 y]) on cycle days 19–24. The biopsies were obtained from the fundal region of the uterine cavity with an endometrial sampling device (Endocell; Wallach Surgical Devices Inc., Orange, CT). The presumptive diagnoses of infertility included unexplained infertility (n = 21), male-factor infertility (n = 12), female age of >40 y (n = 8), tubal disease (n = 2), oocyte donor (n = 1), endometriosis (n = 1), and combined male factor and endometriosis (n = 1). In all patients, menstrual dating was performed according to the last menstrual period.

Materials

Cell-culture medium, supplements, and all other chemicals not listed in this section were obtained from Sigma Chemical Co. (Oakville, ON, Canada). Plastics for cell culture were supplied by Falcon (Becton Dickinson Labware, Franklin Lakes, NJ). Target Retrieval Solution and monoclonal antibodies against CD31 were purchased from DAKO Diagnostic (Mississauga, ON, Canada). We obtained 4′,6-diamidino-2-phenylindole from Sigma Chemical Co. (St. Louis, MO).

Three-Dimensional In Vitro Culture of Endometrial Fragments

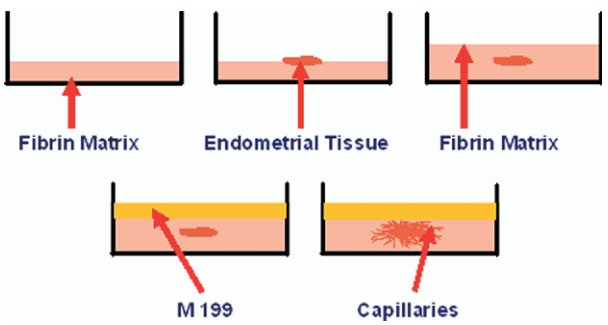
Tissue cultures were divided into four groups on the basis of the concentrations of lovastatin in the culture media: [1] 1 μM lovastatin (group 1, n = 10), [2] 5 μM lovastatin (group 2, n = 21), [3] 10 μM lovastatin (group 3, n = 15), and [4] the control group (n = 46; for each endometrial biopsy, half of the samples were cultured in wells receiving no lovastatin). The total number of culture wells (both control and treatment with statin) was 112, 346, and 154 for groups 1, 2, and 3, respectively.

Each endometrial biopsy collected was placed in cold sterile phosphate-buffered saline (PBS) solution containing 2.5 mg/mL of amphotericin B plus 50 mg/mL of gentamycin and immediately was cut into approximately 1-mm fragments with fine dissecting forceps and a scalpel. These explants were cleared of residual clots and placed in PBS before their use. Cultures were performed in 24-well culture plates; 0.5 mL of a solution of fibrinogen (3 mg/mL in Medium 199) was added to each well and mixed with 15 mL of thrombin (50 National Institutes of Health U/mL in 0.15 M NaCl). Endometrial fragments quickly were placed in the center of the wells after clot formation and were covered by an additional 0.5 mL per well of the fibrinogen–thrombin solution, to hold them at the same level between the two clots.

After gel formation, 1 mL per well of Medium 199, supplemented with 5% of heat-inactivated fetal bovine serum, 0.1% e-aminocaproic acid, L-glutamine (2 mM), and antibiotics (streptomycin, 50 mg/mL; penicillin, 50 IU/mL; and amphotericin B, 2.5 mg/mL) added (Fig. 1). Explants

FIGURE 1

Schematic presentation of three-dimensional culture of endometrial fragments. M199 represents 1 mL of medium 199 added on top of the fibrin matrix.



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