Simvastatin inhibits the proliferation and the contractility of human endometriotic stromal cells: a promising agent for the treatment of endometriosis

Simvastatin significantly inhibited the proliferation of endometriotic stromal cells, attenuated the collagen gel contraction mediated by these cells, and suppressed endometriotic stromal cell attachment to collagen fibers. Simvastatin is considered to be a promising agent for the treatment of endometriosis-associated fibrosis, which is among the major pathologies caused by endometriosis. (Fertil Steril® 2009;92:2097–9. ©2009 by American Society for Reproductive Medicine.)

Histologically, endometriosis is characterized by dense fibrous tissue surrounding the ectopic endometrial glands and stroma (1). During the development and progression of endometriotic lesions, excess fibrosis may lead to scarring, chronic pain, and the alterations of tissue function, all of which are characteristics of this disease (1, 2). Immunohistochemical analysis led Anaf et al. (3) to suggest that endometriotic stromal cells can differentiate into α-smooth muscle actin–positive myofibroblasts. It has been suggested that type I collagen is a major contributor to endometriosis-associated fibrosis (2, 4).

We have established a three-dimensional (3-D) collagen gel culture system with human endometriotic stromal cells as a model of fibrosis formation in endometriosis (5, 6). Endometriotic stromal cells were cultured in floating collagen lattices to induce the reorganization and compaction of collagen fibers, resulting in the contraction of the collagen gels. This culture system provided a model of mechanically relaxed tissue with low tensile strength comparable with that in the early stages of development of endometriotic lesions. Using this model, we demonstrated that endometriotic stromal cells cultured in floating 3-D collagen gel exhibit a more enhanced contractile profile and greater ability to differentiate into a myofibroblast phenotype than do normal endometrial stromal cells (5, 6). Activation of the Ras homology (Rho)–Rho-associated coiled-coil–forming protein kinase (ROCK)–mediated pathway in endometriotic stromal cells may be involved in this phenomenon (5, 6).

Statins are potent inhibitors of cholesterol biosynthesis that are used widely to reduce serum cholesterol levels in patients with hyperlipidemia (7, 8). By competitively inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase to block the conversion of HMG CoA to L-mevalonate, a rate-limiting step in cholesterol synthesis (9), statins reduce the synthesis of isoprenoids such as farnesyl pyrophosphate, a precursor of cholesterol, and geranyl geranyl pyrophosphate, which is synthesized from farnesyl pyrophosphate (10). Both farnesyl pyrophosphate and geranyl geranyl pyrophosphate serve as lipid attachments for a variety of intercellular proteins to the plasma membrane, including small guanosine triphosphate–binding proteins, such as Ras and Ras-like proteins (e.g., Rho, Rac, and Rab), resulting in their activation (11, 12). Rho exists in an inactive guanosine diphosphate–bound cytosolic form, and, on cellular activation, guanosine triphosphate is exchanged and these proteins translocate to the active membrane form (13). The anchoring of Rho to cell membranes requires prenylation (14). In the plasma membrane, Rho is implicated in cytoskeletal responses to extracellular signals and is converted to an active guanosine triphosphate–bound state (15). By inhibiting this isoprenylation, statins lower both membrane levels and the activity of Rho proteins, and they also regulate both the Rho-ROCK and the mevalonate pathways (13, 16).

In the present study, we investigated the effects of simvastatin on the proliferation and the contractility of endometriotic stromal cells. Endometriotic tissues were obtained from premenopausal patients in the mid-to-late proliferative phase who had undergone salpingo-oophorectomy or cystectomy for ovarian endometriotic cysts (N = 8, aged 28–37 years). None of the patients had undergone any hormonal treatments for at least 12 months before the operation. This study was approved by the Institutional Review Board of the Faculty of Medicine at Oita University. Endometriotic stromal cells were isolated from ovarian endometriotic tissues by enzymatic digestion as previously described (17). Isolated endometriotic stromal cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 100 IU/mL of penicillin (GIBCO-BRL, Gaithersburg, MD), 50 mg/mL of streptomycin.
Effects of simvastatin on (A) the proliferation, (B) the contractility, and (C) morphology of endometriotic stromal cells. (A) The cell viability of untreated endometriotic stromal cells was defined as 100%. *P < .0005, **P < .0001 versus untreated controls (Bonferroni-Dunn test). (B) The endometriotic stromal cell–mediated collagen gel contractility was assessed by measuring the gel surface area. The total area of the gel surface after 48 hours under untreated conditions was defined as 100%. The relative gel surface areas were calculated. *P < .0005, **P < .0001 versus untreated controls (Bonferroni-Dunn test). (C) Untreated endometriotic stromal cells cultured in 3-D collagen gels attached to the collagen fibers, and the initially loose network contracted into a dense, tissue-like structure. The cell morphology was dendritic to stellate. In contrast, the contractile force of endometriotic stromal cells treated with 50 μmol/L of simvastatin was weak, and the cell morphology remained round to polygonal; unlike the untreated endometriotic stromal cells, the simvastatin–treated cells did not attach to the collagen fibers.

FIGURE 1

(A) 

(B) 

(C) 

inhibitor of ROCK-I and ROCK-II (19, 20), significantly inhibited endometriotic stromal cell–mediated contractility (5). Interestingly, Y-27632 was found to exert a greater effect on the contractility of endometriotic stromal cells than on the contractility of normal eutopic endometrial stromal cells. Because statins have a long history of clinical use as potent inhibitors of Rho activation (13, 16), it is possible that statins such as simvastatin could be applied to the medical treatment and prevention of endometriosis-associated fibrosis.

Using normal eutopic endometrial tissue explants in a 3-D fibrin gel culture, Esfandiari et al. (21) have demonstrated that lovastatin inhibited angiogenesis and stromal cell proliferation in these tissues. Piotrowski et al. (22) also demonstrated that simvastatin and mevastatin inhibited the proliferation of endometriotic stromal cells, as well as the activation of extracellular signal–regulated kinase in these cells. In these reports, statins are suggested as potentially promising agents for the treatment and prevention of endometriosis. However, none of these previous studies examined endometriotic tissues, which are considered to be functionally different from the normal eutopic endometrium (5, 17, 23).

In the present study, we demonstrated for the first time that simvastatin inhibited the proliferation of endometriotic stromal cells, suppressed the attachment of these cells to collagen fibers, and attenuated the collagen gel contractility mediated by these cells. The inhibition of Rho activation as a result of HMG CoA reductase inhibition is considered to be among the underlying mechanisms responsible for these observations. The present results suggest that statins are promising agents for the treatment of endometriosis-associated fibrosis, one of the major pathogeneses of endometriosis. Although further basic and clinical studies will be necessary, medical treatment that modulates the Rho-ROCK–mediated pathway may provide a novel therapeutic approach to the treatment of endometriosis.

REFERENCES