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Statins Inhibit Monocyte Chemotactic Protein 1 Expression in Endometriosis

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Abstract

Statins are potent inhibitors of the endogenous mevalonate pathway. Besides inhibiting cholesterol biosynthesis, statins may also demonstrate anti-inflammatory properties. Inflammation is implicated in the attachment and invasion of endometrial cells to the peritoneal surface and growth of ectopic endometrium by inducing proliferation and angiogenesis. In this study, the effect of statins on monocyte chemotactic protein 1 (MCP-1) expression in endometriotic implants in nude mouse model and in cultured endometriotic cells was evaluated. In mouse model, simvastatin decreased MCP-1 expression in a dose-dependent manner in endometriotic implants ($P < .05$). Similarly, both simvastatin and mevastatin revealed a dose-dependent inhibition of MCP-1 production in cultured endometriotic cells ($P < .01$). This inhibitory effect of the statins on MCP-1 production was reversed by the downstream substrates of the mevalonate pathway. Moreover, statins decreased MCP-1 messenger RNA expression in cultured endometriotic cells ($P < .05$). In conclusion, statins exert anti-inflammatory effect in endometriotic cells and could provide a potential treatment of endometriosis in the future.

Keywords

endometriosis, statin, MCP-1, inflammation

Introduction

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, more commonly known as statins, are potent inhibitors of the endogenous mevalonate pathway, which directs the biosynthesis of cholesterol and isoprenoids.[1] Statins therefore inhibit not only the biosynthesis of cholesterol but also of isoprenoid intermediates such as geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP), which serve as lipid attachments for a variety of intracellular proteins to the plasma membrane, including small guanosine triphosphate-binding proteins, such as Ras and Ras-like proteins (eg, Rho, Rac, and Rab), resulting in their activation (Figure 1).[2,3] Isoprenylation of these proteins, in turn, regulates the downstream signaling pathways involved in cytoskeletal organization, motility, membrane trafficking, transcriptional activation, and cell proliferation and differentiation.[4] Therefore, statins may interfere with these processes by depleting intracellular GGPP and FPP, resulting in inhibition of protein isoprenylation.

Statins have also been shown to affect the expression, secretion, and function of a variety of immune mediators, resulting in the modulation of both adaptive and innate immune functions.[5] Therefore, statins have been considered as a treatment of various inflammatory diseases, including multiple sclerosis, rheumatoid arthritis, and endometriosis.[6–10]

Endometriosis is a proinflammatory disease defined by the presence of viable endometrial tissue outside the uterine cavity. The peritoneal fluid of women with endometriosis contains increased numbers of immune cells that would be expected to clear endometrial cells from the peritoneal cavity seem to allow endometriosis implants to persist and progress by secreting growth factors and cytokines.[11] Moreover, cytokines secreted by ectopic endometrial cells also promote survival and growth of endometriotic implants.[11] Monocyte chemotactic protein 1 (MCP-1), a chemokine that attracts and activates

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monocytes/macrophages, is elevated in the peritoneal environment of women with endometriosis and plays an important role in the pathogenesis of endometriosis.[12] Although MCP-1 is mainly secreted by immune cells, other cell types including ectopic endometriosis implants express this chemokine.[13] Monocyte chemotactic protein 1 stimulates endometrial cell attachment to extracellular matrix and angiogenesis.[14–16] It also plays an important role in the growth and maintenance of ectopic endometrial tissue by not only stimulating macrophages to secrete growth factors and cytokines but also by stimulating endometrial cell proliferation directly.[12]

Given the importance of MCP-1 in the pathogenesis of endometriosis, taken together with immunomodulatory effect of statins, our hypothesis is that MCP-1 expression in endometriotic cells can be inhibited by statins. Therefore, we evaluated the effect of statins on MCP-1 expression in endometriotic implants in a mouse model and in endometriotic cells in cell culture.

Materials and Methods

Tissue Collection

For nude mouse experiments, normal endometrial tissues (n = 6) were acquired by Pipelle (Unimar, Inc, Wilton, Connecticut) biopsy during the proliferative phase of the menstrual cycle from 6 healthy volunteer donors (aged 18-45 years, 29.3 ± 5.4 years [mean ± standard deviation]) exhibiting normal menstrual cycles and no history of endometriosis. For endometrial stromal cell cultures, eutopic and ectopic endometrial samples (n = 7) were obtained from women with endometriosis (aged 24-41 years, 30.6 ± 3.1 years) undergoing laparoscopy for infertility or pelvic pain. Individuals with a recent (<3 months) history of hormone therapy were excluded. Biopsies were washed in prewarmed, phenol red-free Dulbecco modified Eagle medium (DMEM)/Ham F-12 Medium (DMEM/F-12; Sigma, St Louis, Missouri) to remove residual blood and mucous before culturing. Informed consent was obtained before biopsy, and the use of human tissues was approved by the Human Investigation Committee of Yale University and Vanderbilt University's Institutional Review Board and Committee for the Protection of Human Subjects.

Human Tissue Preparation for Endometriosis Model

Endometrial biopsies were dissected into small cubes (1 × 1 mm^3) and 8 to 10 pieces of tissue per treatment group were suspended in tissue culture inserts (Millipore, Bedford, Massachusetts). Cultures were maintained under serum-free conditions in DMEM/F-12 supplemented with 1% insulin–transferrin–selenium (ITS+; Collaborative Biomedical, Bedford, Massachusetts), 0.1% Excyte (Miles Scientific, Kankakee, Illinois), and 1× antibiotic/antimycotic solution and incubated at 37°C in a humidified chamber with 5% CO_2. All tissues were treated with 1 nmol/L 17β-estradiol (Sigma) and maintained in culture for 24 hours before injection into mice.

Nude Mice Endometriosis Model

Previous studies using the nude mouse model of experimental endometriosis were conducted using subperitoneally placed human tissues to assess the impact of different compounds.[10,17] Five-week-old ovariectomized female nude mice (NCr strain, n = 15) were purchased from Harlan Sprague Dawley (Indianapolis, Indiana). After a 1-week acclimation period, mice were implanted with a slow-release silastic capsule containing 8 mg 17β-estradiol (in cholesterol) 24 hours before injection of human tissues. Endometrial tissues, obtained and cultured as described above, were washed in phosphate-buffered saline (PBS) and injected into mice subperitoneally along the ventral midline just below the umbilicus. Twenty-four hours after tissue injection, mice were randomly assigned to one of the following treatment regimens: vehicle only, 5 mg/kg simvastatin, or 25 mg/kg simvastatin. Simvastatin (Sigma) was suspended in a 50:50 mix of water and PEG300 (Sigma) and provided to mice by gavage daily for 10 days. Twenty-four hours after the last treatment, mice were sacrificed, and endometriosis implants were removed and fixed.

MCP-1 Immunohistochemistry

Formalin-fixed paraffin-embedded samples were cut into 5-µm sections. After deparaffinization, slides were boiled in 10 mmol/L citrate buffer (pH 6.0) for 15 minutes for antigen retrieval. Then, sections were immersed in 3% hydrogen peroxide (in 50% methanol/50% distilled water) for 15 minutes to block endogenous peroxidase activity. After washing with Tris-buffered saline (TBS), 3 times for 5 minutes, slides were then incubated in a humidified chamber with 5% blocking horse serum (LabVision, Fremont, California) in TBS for 30 minutes at room temperature. Afterward, excess serum was drained and sections were incubated with horse monoclonal anti-mouse MCP-1 antibody (10 µg/mL) in 1% blocking horse serum in TBS (R&D Systems, Inc, Minneapolis, Minnesota) overnight at 4°C in a humidified chamber. For negative controls, normal horse IgG isotype was used at the same concentrations. The sections were washed 3 times for 5 minutes with TBS and then biotinylated goat anti-horse antibody (1.5 mg/mL; Vector Laboratories, Burlingame, California) was added at 1:400 dilution for 30 minutes at room temperature. The antigen–antibody complex was detected using a strep–avidin–biotin–peroxidase kit (Vector Laboratories). 3,3-Diaminobenzidine tetrahydrochloride dihydrate (DAB; Vector Laboratories) was used as the chromogen, and sections were counterstained with hematoxylin.

The intensity for MCP-1 immunoreactivity was semiquantitatively evaluated using the following intensity categories: 0, no staining; 1+, weak but detectable staining; 2+, moderate or distinct staining; and 3+, intense staining. For each tissue, a histological score (HSCORE) value was derived by summing the percentages of cells that stained at each intensity category and multiplying that value by the weighted intensity of the staining, using the formula HSCORE = Σ Pi (i + 1), where

i represents the intensity scores, and Pi is the corresponding percentage of the cells. In each slide, 5 randomly selected areas were evaluated under a light microscope (×40 magnification), and the percentage of the cells for each intensity within these areas was determined at different times by 2 investigators blinded to the type and source of the tissues. The intraindividual and interindividual coefficients of variation were 10% and 12%, respectively, for the HSCORE evaluation. The average score of 2 was used.

Isolation and Culture of Endometriotic Stromal Cells

Endometriotic tissues were minced with a sterile surgical blade and digested in Hank balanced salt solution (Sigma-Aldrich) containing collagenase B (1 mg/mL, 15 U/mg; Roche, Indianapolis, Indiana), deoxyribonuclease I (0.1 mg/mL, 1500 U/mg; Roche), penicillin (200 U/mL), and streptomycin (200 mg/mL) for 60 minutes at 37°C with agitation. The dispersed endometrial cells were separated by filtration through a wire sieve (73-μm-diameter pore; Sigma-Aldrich) and were cultured in DMEM Ham F-12 (1:1 vol/vol; Sigma-Aldrich) containing fetal bovine serum (10% vol/vol; Invitrogen, Carlsbad, California). The cultures were maintained in a standard 95% air/5% CO_2 incubator at 37°C.

After one passage, endometriotic stromal cells (ESCs) were plated and grown to pre- or full confluence. Endometriotic stromal cells after first passage were assayed immunocytochemically using specific cell-surface markers and were found previously to contain 0% to 7% epithelial cells, no detectable endothelial cells, and 0.2% macrophages.[18,19] The ESCs were treated with serum-free, phenol red-free media (Sigma-Aldrich) for 24 hours before treatments. Each experiment with ESCs was repeated at least 3 times using cells prepared from endometrial tissue specimens obtained from at least 3 different patients.

Enzyme-Linked Immunosorbent Assay

Confluent ESC cultures were treated with vehicle (control), a concentration gradient of mevastatin (1-10-30 μmol/L) or simvastatin (1-10-30 μmol/L) with or without the addition of the isoprenoid intermediates, FPP (3-10-30 μmol/L), or GGPP (3-10-30 μmol/L) for 24 hours. After the experiments, supernatants were collected and the amount of MCP-1 was quantified using specific enzyme-linked immunosorbent assay (ELISA) according to instructions provided by the manufacturer (R&D Systems Inc). The sensitivity of MCP-1 ELISA was 5 pg/mL. The intra- and interassay coefficients of variation of MCP-1 ELISA were 4.2% and 5.9%, respectively. According to the manufacturer, there is no significant cross-reactivity or interference with other known cytokines in these assays.

Level of MCP-1 was normalized to the total cell culture protein content as measured by Bradford protein assay (Bio-Rad Laboratories, Hercules, California). Briefly, after collecting culture supernatants and washing the monolayers with Hank balanced salt solution, the cells were harvested using a cell scraper in cold PBS. After the centrifugation, the cell extraction buffer (20 mmol/L Tris-HCl buffer with 150 mmol/L NaCl, 1% Triton X-100, 1 mmol/L phenylmethylsulfonylfluoride, and complete protease inhibitor cocktail; Roche) was added to the cell pellets and sonicated for 5 seconds. After the final centrifugation, the supernatant was collected, and protein content was measured at 650 nm with a multi-well plate reader.

Semiquantitative Reverse Transcriptase (RT)-Polymerase Chain Reaction Analysis

Confluent human ESC cultures were treated with vehicle (control), a concentration gradient of mevastatin (1-10 μmol/L) or simvastatin (1-10 μmol/L) for 6 hours. At the end of experimental cell incubations, total RNA was extracted by Trizol reagent (Gibco BRL, Rockville, Maryland) according to the manufacturer's instructions. RNA was treated for genomic DNA contamination using DNase I (Ambion Austin, Texas). The quality and concentration of RNA were determined by measuring the absorbance at 260 and 280 nm. Amplifications were carried out by 30 cycles of polymerase chain reaction (PCR) in which the initial 5-minute denaturation at 94°C was followed by a "touchdown" program for 10 cycles of 92°C for 30 seconds, 65°C for 20 seconds (−1°C per cycle), and 72°C for 1 minute per kilobase and then 20 cycles of 92°C for 30 seconds, 55°C for 20 seconds, and 72°C for 1 minute per kilobase in a volume of 20 μL containing ×1 PCR buffer (Roche), 0.125 mmol/L of each dNTP, 0.5 μmol/L of each primer, and 2 units of SuperTaq polymerase. Semiquantitative RT-PCR was performed using primers specific for MCP-1 and β-actin. The primers used for amplification: MCP-1: sense, 5′-ATGCAATCAATGCCCCAGTC-3′, antisense, 5′-TGCAGATTCTTGGGTTGTGG-3′; and β-actin: sense, 5′- TTGCTGATCCACATCTGCTG-3′, antisense, 5′-GACAGGATGCAGAAGGAGAT-3′. The PCR products and molecular weight markers were fractionated in 2% agarose gels containing ethidium bromide (10 mg/mL) and visualized by ultraviolet light. The intensity of each band was normalized to its corresponding β-actin band to compare values semiquantitatively between samples.

Statistical Analysis

Because the data from immunohistochemistry, ELISA, and semiquantitative RT-PCR analysis were normally distributed (as determined by Kolmogorov-Smirnov test), comparisons of samples were analyzed with 1-way analysis of variance followed by post hoc Holm-Sidak test. Statistical calculations were performed using SigmaStat for Windows, version 3.0 (Jandel Scientific Corp, San Rafael, California). Statistical significance was defined as $P < .05$.

Results

Statins Inhibit MCP-1 Protein Expression in Nude Mouse Endometriosis Model

To determine the effect of statins on MCP-1 expression in vivo, we initially established experimental endometriosis on the

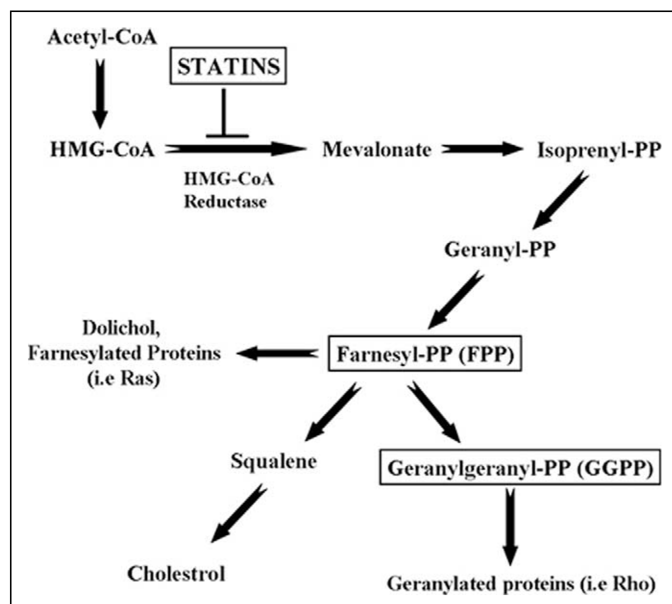

Figure 1. Effects of statin on mevalonate pathway. The beneficial effects of statins may extend beyond their effects on serum cholesterol levels. The inhibition of HMG-CoA reductase blocks the production of mevalonic acid, a precursor for both cholesterol and several isoprenoid intermediates. Some of these intermediates are substrates for the posttranslational isoprenylation of various proteins involved in cell signaling. HMG-CoA indicates 3-Hydroxy-3-methylglutaryl coenzyme A.

surface of the peritoneum in estradiol-treated nude mice. Twenty-four hours after creation of endometriosis model, mice were treated either with vehicle only, 5 mg/kg simvastatin, or 25 mg/kg simvastatin by gavage daily for 10 days. Twenty-four hours after the last treatment, mice were sacrificed.

Both epithelial and stromal cells in ectopic endometriosis implants showed cytoplasmic MCP-1 immunoreactivity. However, epithelial staining was more intense compared to the stromal staining in all samples ($P < .05$; Figure 2). Simvastatin decreased the MCP-1 expression in a dose-dependent manner with the greatest inhibition achieved at 25 mg/kg in both epithelial and stromal endometriosis cells ($P < .05$; Figure 2).

Statins Inhibit MCP-1 Protein Secretion in Cultured ESCs

Endometriotic stromal cells were treated with vehicle (control), a concentration gradient of mevastatin (1-10-30 μmol/L) and simvastatin (1-10-30 μmol/L) for 24 hours and supernatants were collected for the quantification of MCP-1 using ELISA. Both statins caused an inhibition of MCP-1 production by ESCs. Mevastatin revealed a dose-dependent inhibition of MCP-1 production with the greatest inhibition achieved at 30 μmol/L ($P < .01$), resulting in 61% $\pm$ 5% (mean $\pm$ standard error of the mean [SEM]) inhibition compared to control (Figure 3). Similarly, simvastatin also reduced MCP-1 levels in a dose-dependent manner with the greatest inhibition achieved at 30 μmol/L ($P < .01$), resulting in 69% $\pm$ 7% decrease in MCP-1 production when compared to control levels (Figure 3).

We further investigated whether the inhibitory effects of these statins could be reversed by adding the downstream substrates FPP and GGPP to the treatments. Thus, the isoprenoid intermediates, FPP (3-10-30 μmol/L) and GGPP (3-10-30 μmol/L) were added to the cultures together with the most effective concentration of simvastatin (30 μmol/L) for 24 hours, and MCP-1 was quantified by ELISA. All doses of GGPP and FPP at 30 μmol/L significantly increased MCP-1 expression compared to control group ($P < .05$; Figure 4). Both GGPP (all doses) and FPP (at 30 μmol/L) reversed the effect of simvastatin on MCP-1 expression and increased the MCP-1 levels to the control levels. Monocyte chemotactic protein 1 levels were significantly higher in endometriotic cells treated with simvastatin and GGPP (all doses) or FPP (at 30 μmol/L) compared to those only treated with simvastatin ($P < .05$; Figure 4).

Statins Inhibits MCP-1 Messenger RNA Expression in Cultured ESCs

Endometriotic stromal cells were treated with vehicle (control), a concentration gradient of mevastatin (1-10 μmol/L) and simvastatin (1-10 μmol/L) for 6 h. Both mevastatin and simvastatin treatments decreased MCP-1 messenger RNA expression in cultured ESC compared to control at all doses ($P < .05$, Figure 5).

Discussion

In this study, we demonstrated the inhibitory effect of statins on MCP-1 expression in endometriotic implants in a mouse model and in ESCs in culture. This inhibitory effect of the statins on MCP-1 production was reversed by the downstream substrates of the mevalonate pathway (ie, GGPP and FPP). These findings suggest that isoprenoid generation through the mevalonate pathway may be one of the mechanisms for MCP-1 expression in endometriotic cells.

Numerous theories of the pathogenesis of endometriosis have been proposed. The most widely accepted theory is that the disorder originates from retrograde menstruation of endometrial tissue sloughed through patent fallopian tubes into the peritoneal cavity.[20] The development of the disease in the pelvis has been attributed to attachment and invasion of endometrial fragments to the peritoneum, establishment of blood supply, and presence of a suboptimum immune response that does not adequately clear the implants, resulting in their continued survival and growth.[11] However, retrograde menstruation is a common phenomenon affecting 76% to 90% of women of reproductive age and does not correlate with the prevalence of endometriosis. Some factors including proinflammatory peritoneal environment and immunologic alterations were proposed to render certain women susceptible to attachment and survival of ectopic endometrial tissues in the peritoneal cavity.[21] Monocyte chemotactic protein 1 is one of the key chemokines in the creation of inflammatory environment in endometriosis.[12] Moreover, MCP-1 may directly stimulate endometrial cell attachment to extracellular matrix,

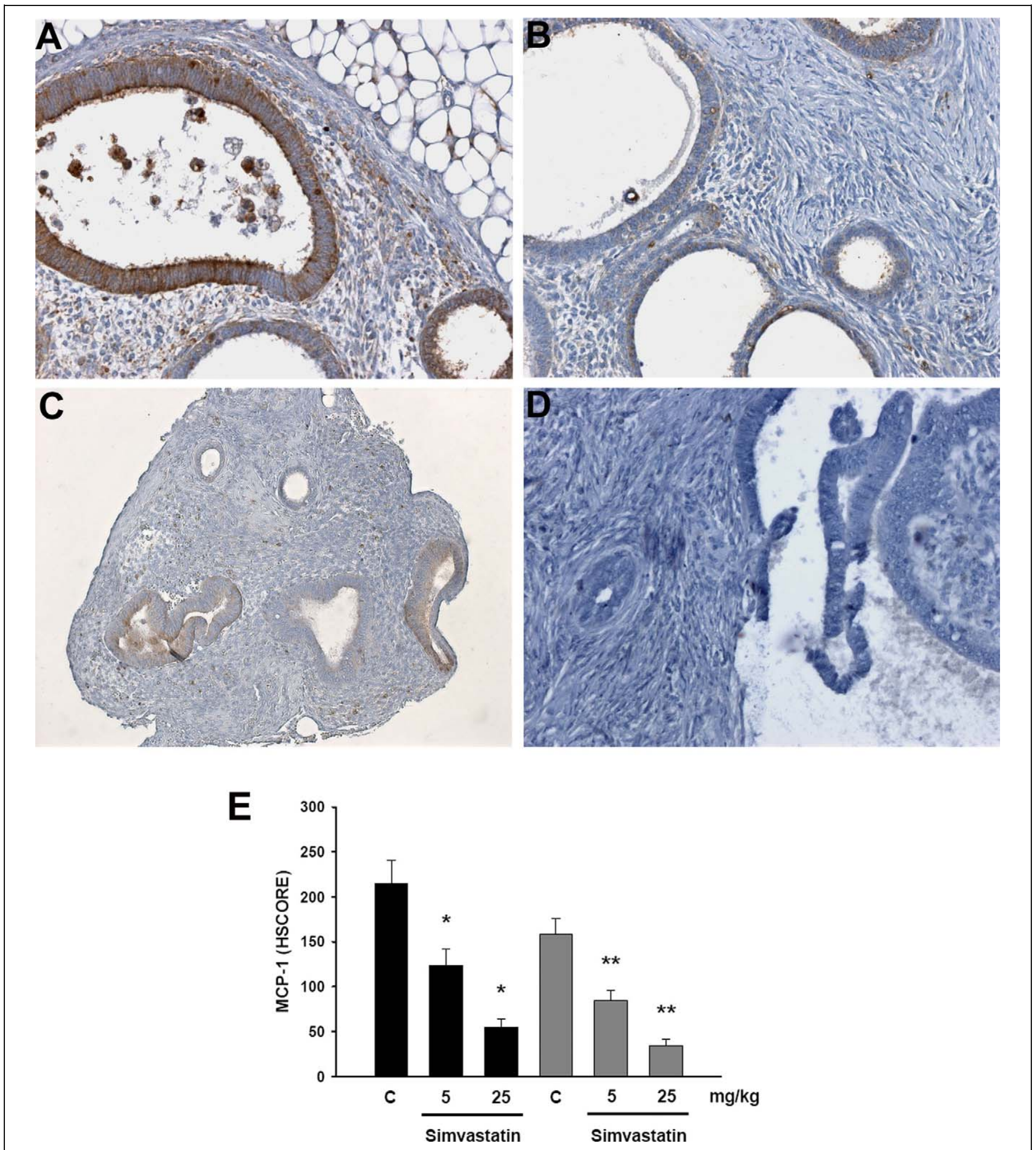

Figure 2. Monocyte chemotactic protein 1 (MCP-1) immunoreactivity in nude mouse endometriosis model. Twenty-four hours after creation of endometriosis model, mice were treated either with (A) vehicle only, (B) 5 mg/kg simvastatin, or (C) 25 mg/kg simvastatin by gavage daily for 10 days. (D) Negative control. (E) Simvastatin decreased the MCP-1 expression in a dose-dependent manner with the greatest inhibition achieved at 25 mg/kg simvastatin in both epithelial (black bars) and stromal (gray bars) endometriosis cells (mean $\pm$ standard error of the mean). *$P < .05$ versus control (epithelial cell), **$P < .05$ versus control (stromal cell).

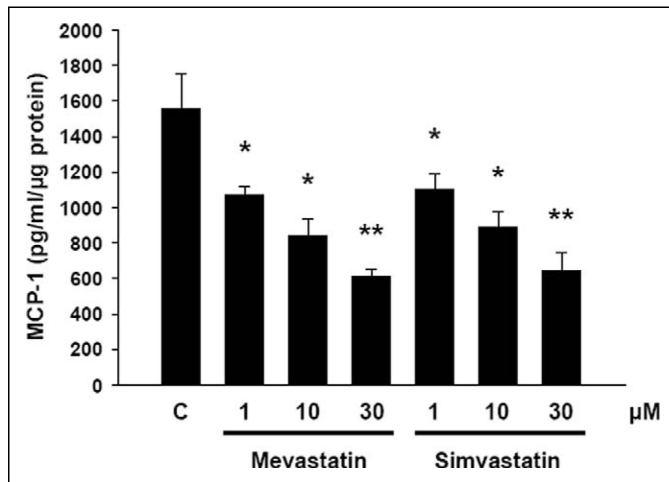

Figure 3. Effects of statins on monocyte chemotactic protein 1 (MCP-1) secretion in cultured endometriotic cells. Endometriotic stromal cells were treated with vehicle (C), a concentration gradient of mevastatin (1-10-30 μmol/L) or simvastatin (1-10-30 μmol/L) for 24 hours. Both statins caused dose-dependent inhibition of MCP-1 production with the greatest inhibition achieved at 30 μmol/L (mean ± standard error of the mean; n = 3). **P* < .05 versus control, ** *P* < .01 versus control.

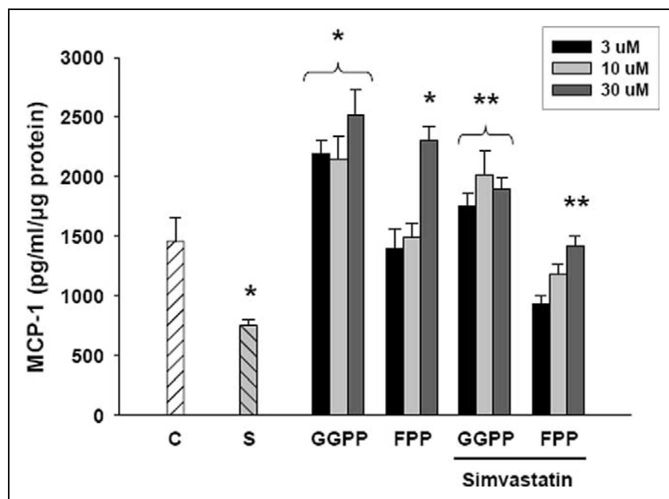

Figure 4. Effects of isoprenoid intermediates on MCP-1 secretion in cultured endometritic cells. The isoprenoid intermediates, FPP (3-10-30 μmol/L) and GGPP (3-10-30 μmol/L) were added to the cultures together with the most effective concentration of simvastatin (S, 30 μmol/L) for 24 hours. All doses of GGPP and FPP at 30 μmol/L significantly increased MCP-1 expression compared to control group. Both GGPP (all doses) and FPP (at 30 μmol/L) reversed the effect of simvastatin on MCP-1 expression and increased the MCP-1 levels to the control levels. Monocyte chemotactic protein 1 (MCP-1) levels were significantly higher in endometriotic cells treated with simvastatin and GGPP (all doses) or FPP (at 30 μmol/L) compared to those only treated with simvastatin (mean ± standard error of the mean; n = 3). **P* < .05 versus control, ***P* < .05 versus simvastatin only. GGPP indicates geranylgeranyl pyrophosphate; FPP, farnesyl pyrophosphate.

endometrial cell proliferation, and angiogenesis.[12,14,16] Therefore, we can speculate that statins may alleviate the inflammatory peritoneal environment and may slow down the

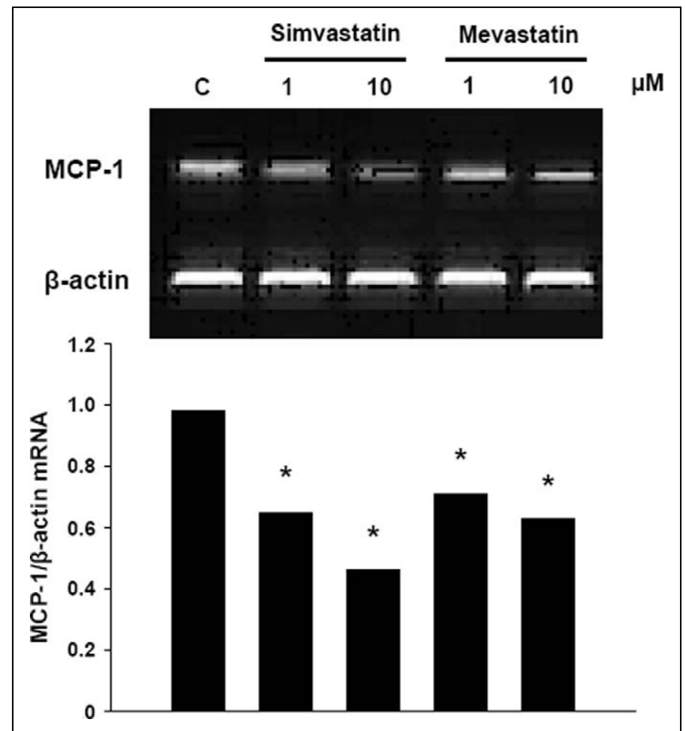

Figure 5. Effects of statins on MCP-1 mRNA expression in cultured endometriotic cells. Endometriotic stromal cells were treated with vehicle (C), a concentration gradient of mevastatin (1-10 μmol/L) or simvastatin (1-10 μmol/L) for 6 hours. Both mevastatin and simvastatin treatments decreased MCP-1 mRNA expression in cultured endometriotic stromal cell compared to control at all doses. The intensity of each band was normalized to its corresponding β-actin band. Data are representative of 3 independent experiments. **P* < .05 versus control. MCP-1 indicates monocyte chemotactic protein 1; mRNA, messenger RNA.

establishment and progression of endometriosis by inhibiting MCP-1 production.

The effects of statins on the development and growth of endometriosis were studied in animal models. In nude mouse endometriosis model, simvastatin (5 and 25 mg/kg) induced a dose-dependent reduction in the number and size of endometriotic lesions.[10] Similarly, in homologous model of endometriosis, whereby rats underwent surgical establishment of autologous uterine tissues to ectopic sites, atorvastatin (2.5 mg/kg) therapy led to a significant reduction of the mean area of endometriotic implants.[22] The proposed mechanisms of actions of statins on endometriosis implants are stimulation of apoptosis, inhibition of endometrial cell proliferation, blockage of angiogenesis, disruption of adherence and invasion of endometrial cells to peritoneum, and decrease in oxidative stress and inflammation.[10,22–26]

Decreased sensitivity of endometrial tissue to spontaneous apoptosis contributes to the implantation and growth of endometrium at ectopic sites.[27] Ectopic endometriosis implants demonstrate high ERK1/2 signaling pathway activity resulting in enhanced cell survival.[28] Statins may decrease the proliferation of endometrial stromal cells by blocking the activation of

this pathway.[24,25,28] Statins may also induce apoptotic cell death by stimulating caspase 3/7 in endometrial stromal cells.[26]

In order to implant and grow, refluxed endometrial cells need to establish cell-to-cell or cell-to-extracellular matrix interactions with the peritoneal lining and invade extracellular matrix after initial attachment. Since MCP-1 may regulate endometrial cell attachment to extracellular matrix, statins may disrupt this initial attachment. The matrix metalloproteinases (MMPs), zinc-dependent endopeptidases, which degrade all components of the extracellular matrix, are upregulated in ectopic endometriosis cells in response to inflammatory peritoneal environment and are essential for the invasive nature of the endometriotic implant.[29] Statins inhibit several MMPs in different cell types, including MMP-3 and MMP-7, both of which are particularly upregulated in endometriosis.[30–32] Moreover, simvastatin decreases MMP-3 expression in cultured endometrial stromal cells.[10]

Statins may also protect from development of endometriotic implants by reducing angiogenesis, as demonstrated in animal endometriosis models and in cell culture.[10,22,23] Statins may block angiogenesis by decreasing MCP-1-induced vascular endothelial growth factor by endometrial stromal cells.[16] These observations are consistent with other reports documenting statin-induced inhibition of angiogenesis in models such as a xenograft model of human colon cancer in mice and myocardial ischemia in swine.[33,34]

Statins may also prevent the development of endometriosis with their anti-inflammatory and antioxidant properties. To our knowledge, our study demonstrates the anti-inflammatory effect of statins on endometriosis implants for the first time. The expression of MCP-1 is strongly enhanced after stimulation with tumor necrosis factor α and interleukin 1β, both are found in high concentrations in peritoneal fluid of endometriosis patients.[35,36] The MCP-1 gene expression is regulated primarily at the level of transcription by transcription factors and signaling pathways including mitogen-activated protein kinase and nuclear factor-kappa β. Statins may block the activation of these regulatory molecules resulting in downregulation of MCP-1 production.[37,38] The growth of endometrial stromal cells is stimulated by moderate oxidative stress and inhibited by antioxidants.[39] In vitro, statins have direct antioxidant activity: simvastatin is the most effective as an antihydroxyl radical antioxidant, whereas fluvastatin was the most effective as an antiperoxyl radical antioxidant.[40]

Statins are the principal therapy for hypercholesterolemia due to their ability to inhibit the synthesis of cholesterol with relatively mild side effects. However, mounting evidence suggests that some of the clinical benefits of statins may not be attributed to the lipid-lowering properties of these drugs. Our study suggests that statins also have anti-inflammatory properties in endometriosis and could provide a potential treatment of endometriosis. Before using statins in clinical practice, further studies are needed to better delineate the effect of statins on endometriosis using primate models or with clinical trials in women with endometriosis.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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