Expression of adiponectin, leptin, and their receptors in ovarian endometrioma

Youn Seok Choi, M.D., Ph.D.,[a] Hoon Kyu Oh, M.D., Ph.D.,[b] and Jung-Hye Choi, Ph.D.[c]

[a] Department of Obstetrics and Gynecology and [b] Department of Pathology, School of Medicine, Catholic University of Daegu, Daegu; and [c] Division of Molecular Biology, College of Pharmacy, Kyung Hee University, Seoul, South Korea

Objective: To evaluate the expression of leptin, leptin receptor (ObR), adiponectin, and adiponectin receptor (AdipoR) in ovarian endometriomas compared with normal endometrium, and to analyze relationships among adipokines and their receptors.
Design: Experimental study.
Setting: A clinic for the treatment of endometriosis and basic research laboratories.
Patient(s): Forty-four women with endometriosis and 42 age-matched women with no laparoscopic evidence of endometriosis as control subjects.
Intervention(s): Endometrial tissue samples were obtained during laparoscopic surgery.
Main Outcome Measure(s): Immunohistochemical staining for leptin, ObR, adiponectin, and AdipoR was performed with the use of tissue microarray. Clinical characteristics were reviewed from the patient's medical records. The effect of leptin on the expression of adiponectin was evaluated in endometriotic cell line using real-time reverse-transcription polymerase chain reaction.
Result(s): Positive expression rates of leptin and ObR were significantly higher in ovarian endometrioma compared with normal endometrium, but those of adiponectin and AdipoR were similar (ovarian endometrioma vs. normal endometrium, respectively: leptin 100% vs. 59.5%; ObR 72.7% vs. 33.3%; adiponectin 31.8% vs. 42.9%; AdipoR 88.6% vs. 73.8%). Expression of adipokines and their receptors did not show any correlation with disease stage. A positive correlation was found between expression of ObR and adiponectin (correlation coefficient 0.488; $P=.001$). Leptin treatment in endometriotic cells induced mRNA expression of adiponectin.
Conclusion(s): These data suggest that leptin and its receptor are induced in ovarian endometriomas, and that the leptin/ObR system regulates adiponectin gene expression in endometriotic cells. (Fertil Steril® 2013;100:135–41. ©2013 by American Society for Reproductive Medicine.)
Key Words: Leptin, adiponectin, ObR, AdipoR, ovarian endometrioma

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/choiys-adiponectin-leptin-ovarian-endometrioma/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Endometriosis is a common gynecologic disorder characterized by ectopic implants of endometrium in peritoneal cavity. It is associated with infertility, dysmenorrhea, and chronic pelvic pain. Endometriosis is estimated to affect 6%–10% of the general female population and 15%–50% of reproductive-age women with infertility (1). The pathogenesis of endometriosis is not completely understood. Although no single theory can explain all cases of endometriosis, the most commonly accepted mechanism of disease establishment is retrograde menstrual reflux of endometrial tissue. However, although a majority of women have retrograde menstruation, the much lower prevalence of endometriosis suggests that additional factors determine susceptibility to endometriosis (2). Aberrations in immune function, angiogenesis, and matrix metalloproteinase expression have been suggested as such additional factors.

Leptin, the 16-kd product of the Obese (Ob) gene, is produced predominantly in adipose tissue, and shows a strong positive correlation with total body fat and body mass index (BMI). Leptin acts through its receptor (ObR), which is encoded by the Ob gene. In human tissues, four different ObR variants have been described, but only the long ObR isoform (ObRL) has full signaling potential. The leptin system has been shown to have a role in food intake, basal metabolism, and reproductive function (3). Besides these functions, leptin is now recognized to have immunoregulatory,

Received December 7, 2012; revised and accepted March 7, 2013; published online April 8, 2013.
Y.S.C. has nothing to disclose. H.K.O. has nothing to disclose. J.-H.C. has nothing to disclose.
Supported by a grant of the Korean Health Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (no. A111881).
Reprint requests: Jung-Hye Choi, Ph.D., Division of Molecular Biology, College of Pharmacy, Kyung Hee University, Dongdaemun-Ku, Hoegi-Dong 130-701, Seoul, South Korea (E-mail: jchoi@khu.ac.kr).

Fertility and Sterility® Vol. 100, No. 1, July 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.03.019

proinflammatory, and angiogenic properties, and it is thought to have a role in the pathogenesis of endometriosis (4–9). Several studies showed that peritoneal fluid levels of leptin in women with endometriosis are increased (7, 10–12). Styer et al. (13) showed that leptin signaling is a necessary component in endometriosis-like lesion proliferation, early vascular recruitment, and maintenance of neoangiogenesis in a murine model of endometriosis. Their study found that the disruption of leptin signaling impairs the establishment of endometriosis-like lesions.

Adiponectin is another adipocytokine, a ~30-kd polypeptide that is highly expressed and secreted by adipocytes and circulated at high levels in the bloodstream. Adiponectin levels both in adipose tissue and in circulation are negatively correlated with obesity (14, 15). Two types of adiponectin receptors (AdipoR1 and -R2) are described, and adiponectin acts via those receptors. Adiponectin plays an important role in regulating energy metabolism and insulin sensitivity (15). In addition to its role in energy metabolism, adiponectin has been shown to have antiinflammatory, antiangiogenic, and antiatherosclerotic effects (16–18). In contrast to leptin, adiponectin concentrations in serum and peritoneal fluid of women with endometriosis are lower than those of women without endometriosis (14, 17). These findings suggest that adiponectin may be an antiendometriotic factor (14, 17, 18), but the role of adiponetin in pathogenesis of endometriosis is still uncertain.

As suggested by these studies, the leptin system has a role in the pathogenesis of endometriosis via inflammatory and angiogenic effects, whereas the adiponectin system may have a possible role as a counter-regulatory function to the leptin system via antiinflammatory and antiangiogenic effects. However, the relationship among leptin, ObR, adiponectin, AdipoR, and endometriosis has not been investigated in human tissue. In the present study, we evaluated the expression of leptin, ObR, adiponectin, and AdipoR in ovarian endometriomas with the use of immunohistochemistry and analyzed the relationship among leptin, ObR, adiponectin, AdipoR, and endometriosis.

MATERIALS AND METHODS

Patients

A total of 44 patients with ovarian endometriomas who underwent laparoscopic surgery from January 1, 2009, to December 31, 2009, at the Department of Obstetrics and Gynecology were enrolled as the ovarian endometrioma group. The study was approved by the Institutional Review Board of Daegu Catholic University Medical Center. We selected age-matched 42 patients who underwent hysterectomy because of myoma or carcinoma in situ of uterine cervix as the control group. The sample size (N) was calculated for the chi-square test with the following formula:

$$N = \frac{\left\{Z\,\alpha\sqrt{[2p(1-p)]} + Z\,\beta\sqrt{[p1(1-p1)+p2(1-p2)]}\right\}^2}{d^2}$$

The sample size was 41.9 to satisfy the following conditions: effect size (d) 0.3, predictive positive proportion in normal endometrium (p1) 0.3, predictive positive proportion in ovarian endometriomas (p2) 0.6, statistical power $(1-\beta)$ 80%, and significance level (α) 0.05 (two-sided test). A pathologist reviewed the endometrial specimens to confirm absence of any other pathologic disease. All resected specimens were stained with hematoxylin and eosin (H&E) and histologically examined for diagnosis. The histologic slides of the excised tissues were evaluated by one pathologist (H.K.O.), who was blinded to the clinical variables of the patient. Clinical characteristics, such as age, stage, height, weight, and BMI, were evaluated based on medical records. Endometriosis was classified according to the American Society for Reproductive Medicine. The size of the endometrioma was measured by ultrasonogram before surgery, and the longest diameter was used for analysis. The endometrial phase was decided by menstrual cycle date in patients with regular menstrual cycles and by pathologic finding of endometrial dating in patients with irregular menstrual cycles.

Construction of Tissue Microarrays

Representative paraffin tumor blocks were selected according to the primary evaluation of H&E-stained slides before they were prepared for tissue microarrays (TMAs). Two tissue cores (1 mm in diameter) were taken from ovarian endometriomas or normal endometrial tissue blocks with a manual punch arrayer (Quick-Ray; Uni-Tech Science). The cores were placed in a new recipient paraffin block that ultimately contained 72–96 tissue cores. Each array block contained control tissue samples. Multiple sections (5 μm in thickness) were cut from the TMA blocks and then mounted onto microscope slides. The TMA H&E-stained sections were reviewed under light microscopy to confirm the presence of representative ovarian endometriomas or normal endometrium.

Immunohistochemical Staining

Immunohistochemistry was conducted on 5-μm-thick TMA tissue sections with the use of the Bond Polymer Intense Detection System (Leica Microsystems) according to the manufacturer's instruction with minor modifications. The 5-μm-thick sections of formalin-fixed and paraffin-embedded TMA tissues were deparaffinized with Bond Dewax Solution (Leica Microsystems), and an antigen retrieval procedure was performed with the use of Bond ER Solution (Leica Microsystems) for 30 minutes at 100°C. The endogenous peroxidase was quenched by 5-minute incubation with hydrogen peroxide. Sections were incubated for 15 minutes at ambient temperature with a rabbit polyclonal antileptin antibody (ab16227, 1:150; Abcam), a rabbit polyclonal anti–ObR antibody (ab60042, 1:150; Abcam), a mouse monoclonal antiadiponectin antibody (ab22554, 1:150; Abcam), and a goat polyclonal anti-AdipoR antibody (ab77611, 1:200; Abcam) with the use of a biotin-free polymeric horseradish peroxidase–linker antibody conjugate system in a Bond-Max automatic slide stainer (Leica Microsystems). Human adipocytes (leptin and adiponectin), hepatocellular carcinoma (ObR), and normal liver (AdipoR) tissues were used as positive control tissues. The positive