ORIGINAL ARTICLE: ENDOMETRIOSIS

Expression of microtubule associate protein 2 and synaptophysin in endometrium: high levels in deep infiltrating endometriosis lesions

Martina Gori, B.Sc., Alice Luddi, Ph.D., Giuseppe Belmonte, B.Sc., Paola Piomboni, Ph.D., Claudia Tosti, M.D., Lucia Funghi, Ph.D., Errico Zupi, M.D., Lucia Lazzeri, M.D., Ph.D., and Felice Petraglia, M.D.

Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy

Objective: To assess whether healthy endometrium, eutopic endometrium, and endometriotic lesions express nerve growth factor (NGF), microtubule-associated protein 2 (MAP-2), and synaptophysin (SYP).
Design: Molecular study in tissue extracts.
Setting: University hospital.
Patient(s): A group of women (n = 70), divided as [1] healthy controls (n = 30) and [2] with endometriosis (n = 40), was included.
Intervention(s): From the healthy control group an endometrial specimen was collected by hysteroscopy (proliferative phase, n = 16; secretive phase, n = 14). Endometriotic and endometrial specimens were collected from women undergoing laparoscopic surgery for endometriosis, endometrioma (OMA) (n = 20), or deep infiltrating endometriosis (DIE) (n = 20).
Main Outcome Measure(s): To assess expression of *NGF, MAP-2*, and *SYP* messenger RNA (mRNA) levels in endometrium and in endometriosis by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and protein localization by immunofluorescence. Cultures of human endometrial stromal cells were used to evaluate the effect of tumor necrosis factor (TNF)-α on NGF and SYP.
Result(s): Endometrial tissue from control expressed mRNA for *NGF, MAP-2*, and *SYP*, without any difference between proliferative and secretive phase. The DIE and OMA lesions showed the highest *NGF* mRNA expression, significantly higher than in eutopic endometrium and control. In DIE lesions *SYP* mRNA expression was higher than in OMA or in eutopic endometrium or controls. Immunofluorescence analysis of NGF, MAP-2, and SYP showed a slightly more intense positive signal in endometriotic lesions. Exposure to TNF-α increased NGF and SYP mRNA expression in endometrial culture cells.
Conclusion(s): The present study revealed the presence of two selected neuronal markers, *MAP-2 and SYP* mRNAs and protein expression, in eutopic endometrium and in endometriotic lesions. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, microtubule-associated protein 2, neurogenesis, nerve growth factor, synaptophysin

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/gorim-neuronal-markers-endometriosis/

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.

The pathogenesis of endometriosis involves several different mechanisms, and one of the most intriguing questions is how endometriosis generates pain (1). Endometriosis-related pain results primarily from the interaction among inflammation, angiogenesis, and neurogenesis (2–5). Ovarian endometrioma (OMA) is generally associated with low intensity of pain, whereas deep infiltrating endometriosis (DIE) causes more frequently severe dysmenorrhea, dyspareunia, and chronic pelvic pain (5). Deep infiltrating endometriosis–related pain is also associated with the highest level of stress perception, which may increase the activity of hypothalamic–pituitary–adrenal axis and brain pathways (6, 7). Indeed, women with endometriosis and chronic pain show alterations in central nervous system (8) highlighting that both peripheral

Received April 13, 2015; revised October 19, 2015; accepted October 26, 2015.
M.G. has nothing to disclose. A.L. has nothing to disclose. G.B. has nothing to disclose. P.P. has nothing to disclose. C.T. has nothing to disclose. L.F. has nothing to disclose. E.Z. has nothing to disclose. L.L. has nothing to disclose. F.P. has nothing to disclose.
Reprint requests: Felice Petraglia, M.D., Department of Molecular and Developmental Medicine, University of Siena, Viale Bracci, Siena 53100, Italy (E-mail: felice.petraglia@unisi.it).

Fertility and Sterility® Vol. ■, No. ■, ■ 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.10.024

and central mechanisms play a crucial role in generating endometriosis-related pain and predispose these women to the development of additional chronic comorbidities (9).

Deep infiltrating endometriosis lesions are associated with high concentration of proinflammatory cytokines (10), and inflammation is correlated with neuropathic pain by a stimulation of peripheral nerve sensitization detected in proximity to DIE (11, 12), supporting a spatial relationship between nerve fibers and the lesion. In addition, DIE lesions may infiltrate nerve fibers and cause hyperalgesia, as shown by perineural and interneural invasion in recto-vaginal septum endometriotic lesions, thus assuming that physical compression of nerve fibers may be involved in generating severe pain (13). The presence of nerve fibers in lesions of DIE, OMA, and in peritoneal endometriosis has been shown (14, 15), having sensory and a high density of sympathetic nerves (16). Specific markers (substance P, calcitonin gene-related peptide, acetylcholine, and tyrosine hydroxylase) for sensory, sympathetic, and parasympathetic nerves have been confirmed in peritoneal lesions, with significantly higher expression than in normal peritoneum (14). The pan-neuronal protein marker PGP9.5 was described in cases of OMA and much less in asymptomatic patients (17). Additionally, eutopic endometrium of women with endometriosis showed PGP9.5 expression, but not in healthy women (18, 19); however, other studies were unable to identify PGP9.5 in the eutopic endometrium of women with endometriosis (20). These studies showed no difference in the expression levels of neuronal or neurotrophic markers like PGP9.5 (21).

Large interest is devoted to factors and mechanisms through which nerve fibers grow in endometrium and in lesions of women with endometriosis. The first question is whether healthy endometrial cells may be the source of nerve growth factor (NGF) and neuronal markers. Nerve growth factor and other neurotrophins, such as neurotrophin 3 (NT-3), neurotrophin 4 (NT-4), neurotrophin 5 (NT-5), and brain-derived neurotrophic factor, are expressed in healthy endometrium, eutopic endometrium, and endometriotic lesions (22, 23). Tumor necrosis factor (TNF)-α increases NGF expression in cultured human endometrial stromal cells (HESCs), and this mechanism may stimulate nerve growth also in lesions (5), supporting the endometrial source of NGF. What is not yet defined is the mechanism of action of NGF in stimulating local neurogenesis.

Macrophages and their products can directly stimulate the synthesis of NGF, which plays a crucial role for the survival, development, and function of neurons in both the central nervous system and peripheral nervous system (24). Interestingly, the presence of inflammatory cells near the new peri-endometriotic nerves seems to be more pronounced in patients with more severe symptoms (25). These new nerve fibers seem to be surrounded by mast cells that have also been associated with other pathologic conditions in which pain is a predominant symptom and that can share a high comorbidity rate with endometriosis, such as interstitial cystitis/painful bladder syndrome and irritable bowel syndrome (26, 27).

Microtubule-associated protein 2 (MAP-2) and synaptophysin (SYP) are selective neuronal markers that reflect the degree of neuronal differentiation. Microtubule-associated protein 2 is an early neuronal marker, a neuron-specific protein that stabilizes microtubules in the dendrites of postmitotic neurons (28); SYP, a membrane glycoprotein of presynaptic vesicles, may be regarded as the most important differentiated postmitotic neuronal cell marker (29, 30).

The aim of the present study was to assess the expression of NGF, MAP-2, and SYP by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and immunofluorescence in healthy endometrium, in eutopic endometrium, and in lesions of OMA and DIE. Furthermore, NGF and SYP messenger RNA (mRNA) expression in HESCs after TNF-α exposure was investigated.

MATERIALS AND METHODS

The present study included a group of 70 women including healthy women (n = 30) and endometriotic patients (n = 40). The study was approved by the local human investigation board, and informed consent was obtained from all patients before inclusion. Current infections, endocrine disorders, or the use of hormonal treatment within the past 3 months were common exclusion criteria. A complete medical history, physical examination, and transvaginal ultrasound evaluation were performed for each patient. All sonographic examinations were performed by the same examiner (L.L.), with experience in transvaginal ultrasound (TVS) for endometriosis.

From the healthy control group an endometrial specimen (n = 30) was collected by hysteroscopy. These women underwent laparoscopy for tubal sterilization and were operated during the proliferative (n = 16) or secretory (n = 14) phase of the menstrual cycle. The specimens were collected by hysteroscopy with biopsy and grasping forceps, semirigid, double-action jaws, 5 Fr. The diagnosis of previous or concurrent endometriosis in these women was clinically excluded.

In endometriotic patients laparoscopic surgery was performed in the week following the end of menstrual bleeding in a group with OMA (n = 20) and in a group with DIE (n = 20) (patients with both OMA and DIE were excluded), classifying the stage of the disease according to the American Society for Reproductive Medicine classification (31). Specimens of endometriotic tissue were collected during the laparoscopic procedure by using scissors, without bipolar energy. In the case of OMA, endometriotic tissue was carefully stripped from the lining cyst wall, avoiding taking any of the normal ovarian cortex. Endometrioma and DIE were subsequently histologically confirmed by pathologists. Endometrial specimens from some patients with OMA (n = 12) or DIE (n = 12) were also collected by hysteroscopy (resulting in the proliferative phase).

A fragment of endometrial or endometriotic tissue was immediately submerged in liquid nitrogen to allow subsequent RNA extraction and RT-PCR.

RNA Extraction and Complementary DNA Preparation

For the total RNA extraction, frozen tissue samples were disrupted, homogenized, and processed for RNA extraction using the RNeasy Protect Mini kit according to the manufacturer's