GYNECOLOGY

Surgery accelerates the development of endometriosis in mice

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BACKGROUND: Surgery is currently the mainstay treatment for solid tumors and many benign diseases, including endometriosis, and women tend to receive substantially more surgeries than men mainly because of gynecological and cosmetic surgeries. Despite its cosmetic, therapeutic, or even life-saving benefits, surgery is reported to increase the cancer risk and promotes cancer metastasis. Surgery activates adrenergic signaling, which in turn suppresses cell-mediated immunity and promotes angiogenesis and metastasis. Because immunity, angiogenesis, and invasiveness are all involved in the pathophysiology of endometriosis, it is unclear whether surgery may accelerate the development of endometriosis.

OBJECTIVE: The objective of the study was to test the hypothesis that surgery activates adrenergic signaling, increases angiogenesis, and accelerates the growth of endometriotic lesions.

STUDY DESIGN: This was a prospective, randomized experimentation. The first experiment used 42 female adult Balb/C mice, and the second used 90 female adult Balb/C mice. In experiment 1, 3 days after the induction of endometriosis, mice were randomly divided into 3 groups of approximately equal sizes, control, laparotomy, and mastectomy. In experiment 2, propranolol infusion via Alzet pumps was used to forestall the effect of sympathetic nervous system activation by surgery. In both experiments, mice were evaluated 2 weeks after surgery. Lesion size, hotplate latency, and immunohistochemistry analysis of vascular endothelial growth factor, CD31-positive microvessels, proliferating cell nuclear antigen, phosphorylated cyclic adenosine monophosphate-responsive element-binding protein, β_2-adrenergic receptor (ADRB)-2, ADRB1, ADRB3, ADRA1, and ADRA2 in ectopic implants.

RESULTS: Both mastectomy and laparotomy increased lesion weight and exacerbated hyperalgesia, increased microvessel density and elevated the immunoreactivity against ADRB2, phosphorylated cyclic adenosine monophosphate—responsive element-binding protein, vascular endothelial growth factor, and proliferating cell nuclear antigen but not ADRB1, ADRB3, ADRA1, and ADRA2, suggesting activated adrenergic signaling, increased angiogenesis, and accelerated growth of endometriotic lesions. β-Blockade completely abrogated the facilitory effect of surgery, further underscoring the critical role of β-adrenergic signaling in mediating the effect of surgery.

CONCLUSION: Surgery activates adrenergic signaling, increases angiogenesis, and accelerates the growth of endometriotic lesions in the mouse, but such a facilitory effect of surgery can be completely abrogated by β-blockade. Whether surgery can promote the development of endometriosis in humans warrants further investigation.

Key words: adrenergic receptor, angiogenesis, β-blocker, endometriosis, mouse, surgery

Surgery is now the mainstay treatment for solid tumors and many benign diseases, including endometriosis, defined as the presence of endometrial-like tissues outside the uterine cavity.[1] Remarkably, women tend to receive substantially more surgeries than men mainly because of gynecological surgeries.[2,3]

In the United States, more than 90% of cosmetic and reconstructive surgeries are performed on women.[4] Conceivably, such a predominance of women in plastic surgeries is similar elsewhere in the world.

Despite its cosmetic, therapeutic, or even life-saving benefits, surgery also has its down-side. Extensive animal studies and considerable human studies suggest that surgical stress promotes cancer metastasis.[5-9]

Noncancer-related surgery may also increase the risk of development of cancer. Hysterectomy, for example, is reported to increase the risk of renal cancer,[10,11] and total joint arthroplasty is said to elevate the risk of prostate cancer.[12] Surgical stress is shown to delay prostate involution in mouse.[13] Even 1 single core needle biopsy is reported to promote the distant metastasis of breast cancer in mouse.[14]

Surgery inevitably results in tissue damage, a trauma, or stress to the body. As such, various bioactive molecules are secreted perioperatively, including catecholamines that are known to suppress cell-mediated immunity[15] and promote angiogenesis[16] and metastasis[8] in animal studies.

Currently the pathogenesis of endometriosis, a common disorder among women of reproductive age and a major contributor to pelvic pain and infertility,[17] is poorly understood.[18] Consequently, its effective treatment is still a challenge.[17,19] The quest for novel nonhormonal therapeutics so far has not been successful,[20] and there is no single biomarker that has been unequivocally shown to be clinically useful in diagnosing endometriosis.[21]

Because immunity,[22] angiogenesis,[23] and invasiveness[24] are all involved in the pathophysiology of endometriosis, it is biologically plausible that surgery may also exert its promotional effect on endometriosis. However, to the best of our knowledge, so far there has been no published report on the effect of surgery on the development of endometriosis or on the high recurrence risk of endometriosis.[25] Therefore, we conducted this study and evaluated the effects of surgery, if any, on endometriotic lesion growth and angiogenesis in a mouse model of endometriosis.

Cite this article as: Long Q, Liu X, Guo S-W. Surgery accelerates the development of endometriosis in mice. Am J Obstet Gynecol 2016;215:320.e1-15.

0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2016.02.055

We evaluated the effects of 2 modes of surgery, one mimicking laparotomy and the other, mastectomy. Conceivably, mastectomy is more localized and less invasive or traumatic than laparotomy and may have a less direct effect on lesion growth. Representing different stress levels, these 2 modes should help us to assess the effect of surgery, if any, on the development of endometriosis more reliably.

In addition, given the finding that the β_2 adrenergic receptor appears to be involved in surgery-facilitated endometriosis development, we evaluated whether β-blockade could forestall the promotional effect induced by surgery.

Materials and Methods

Animals

A total of 132 7 week old virgin female Balb/C mice were purchased from the SLAC Experimental Animal Company (Shanghai, China) and used for this study. All mice were maintained under controlled conditions with a 12 hours light/12 hours dark cycle and had access to food and water ad libitum.

All experiments were performed under the guidelines of the National Research Council's *Guide for the Care and Use of Laboratory Animals*[26] and were approved by the Institutional Experimental Animals Review Board of Shanghai Obstetrics and Gynecology Hospital, Fudan University.

Induction of endometriosis

We used an established mouse model of endometriosis by intraperitoneal injection of endometrial fragments as described[27-29] and also used in our previous studies.[30] Briefly, 7 week old donor mice were initially injected with 100 μg/kg estradiol benzoate (Animal Medicine Factory, Hangzhou, China). One week later they were killed, and their uteri were removed and harvested. The uterine tissues were seeded in a Petri dish containing warm sterile saline and split longitudinally with a pair of scissors.

Two uterine horns from each mouse were first minced with scissors, ensuring that the maximal diameter of the fragment was consistently smaller than 1 mm. Then the fragments were injected intraperitoneally to recipient mice. Two recipient mice received the fragment preparation derived from 1 donor mouse. To eliminate any potential bias, endometrial fragments from 3 donor mice were mixed together and injected intraperitoneally to 6 mice, 2 each from 1 of the 3 groups for experiment 1 or 1 each from the 6 groups for experiment 2.

Models of surgical stress

Mice were anaesthetized with 300 mg/kg chloral hydrate and exposed to an experimental mastectomy or laparotomy as described previously.[8] Mastectomy was performed by the midline chest wall skin incision and removal of 1 of the right mammary tissues from the chest wall, followed by the closure of the skin of chest wall with 3−4 surgical clips.

The laparotomy was done by a 3 cm midline abdominal incision followed by the externalization of intestines for a period of 4 minutes[31]; at the same time, the small intestine was rubbed with 2 saline-soaked cotton swabs in 4 different locations to simulate a surgical procedure. The intestine was then returned to the abdominal cavity and irrigated with saline, and the abdominal wall was closed with surgical clips. After surgery, all mice were administrated with penicillin of 40,000 U intramuscularly to prevent infection.

Mouse experiment 1: surgery accelerates the growth of endometriotic lesions

After 1 week of acclimation, 28 mice were randomly divided into 3 groups: the control group (n = 9), the mastectomy group (n = 10), and the laparotomy group (n = 9). Before induction of endometriosis, the baseline body weight and hotplate latency were measured and recorded. Three days after the induction, simulated mastectomy and laparotomy were performed (see *Models of surgical stress* discussed in the text previously).

To rule out any possible effect of anesthesia on endometriosis development, mice in the control group did not receive any surgery but were anaesthetized in the same manner as the other 2 groups. Fourteen days after surgery, body weight and hotplate latency were measured before mice were killed by cervical dislocation. The abdominal cavities were immediately opened up and all lesions were excised and processed for quantification and immunohistochemistry analysis. The extent of the induced endometriosis was evaluated by assessing the weight of all lesions from each mouse.

The hotplate test was performed with a commercially available Hot Plate Analgesia Meter (model BME-480; Institute of Biomedical Engineering, Chinese Academy of Medical Sciences, Tianjin, China) as reported previously[32] and is described in more detail in the Supplementary Appendix.

Mouse experiment 2: intervention by β-blockade

To further confirm the role of β-adrenergic signaling in surgery-accelerated lesion growth and to evaluate the effect of β-blockade, we used propranolol hydrochloride (5 mg/kg per day; Sigma, St Louis, MO), a nonspecific β-adrenergic receptor (ADRB) antagonist, given via Alzet miniosmotic pumps (model 1002; DURECT Corp, Cupertino, CA), which ensured consistent and controlled release of contents for 14 days. For controls, Alzet pumps containing only phosphate-buffered saline (PBS) of the same amount as propranolol was used. The minipumps containing PBS or propranolol were inserted on the nape of the neck 7 days before surgery (ie, 3 days before the induction of endometriosis).

After 3 days of acclimation, 60 mice were randomly divided into 6 groups of equal sizes, which received the following treatments: control (no surgery) plus PBS, mastectomy plus PBS, laparotomy plus PBS, control plus propranolol (no surgery), mastectomy plus propranolol, and laparotomy plus propranolol.

Before the insertion of the pumps and the induction of endometriosis, body weight and hotplate latency were measured and recorded. Surgery was performed 3 days after the induction of endometriosis (see *Models of surgical stress* discussed in previous text). Mice in the control group were only anesthetized similarly to those receiving simulated surgery.