Resolvins RvD1 and 17(R)-RvD1 alleviate signs of inflammation in a rat model of endometriosis

Natalia Dmitrieva, Ph.D., Gregory Suess, B.S., and Russell Shirley, B.S.

Program in Neuroscience, Florida State University, Tallahassee, Florida

Objective: To study the effects of two resolvins of D series, RvD1 and 17(R)-RvD1, on inflammatory signs associated with endometriosis (ENDO).
Design: In vivo research study.
Setting: Research laboratory.
Animal(s): Female Sprague-Dawley rats.
Intervention(s): Intravenous or intraperitoneal injections of RvD1 (300 ng/kg) or 17(R)-RvD1 (300 and 900 ng/kg) in rats with surgically induced ENDO.
Main Outcome Measure(s): Vascular permeability of ectopic endometrial growths was assessed by Evans Blue extravasation; vaginal hyperalgesia was assessed with telemetered visceromotor response.
Result(s): Both resolvins, but not vehicle, significantly decreased vascular permeability in ectopic endometrial tissue. 17(R)-RvD1 also significantly alleviated severity of vaginal hyperalgesia.
Conclusion(s): Our results suggest that RvD1 and 17(R)-RvD1 can be considered for further investigation of their therapeutic potential for treating ENDO. (Fertil Steril® 2014;102: 1191–6. ©2014 by American Society for Reproductive Medicine.)
Key Words: Proresolving lipid mediator, docosahexaenoic acid, omega-3 polyunsaturated fatty acid, cyclo-oxygenase 2, hyperalgesia, endometrium

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/dmitrieva-rvd1-17r-rvd1-inflammation-rat-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.

Endometriosis (ENDO) is a common chronic, estrogen-dependent, painful condition (1–3). The incidence of ENDO is estimated to be approximately 7% but can be as high as 10%–15% among women of reproductive age (1, 4–6). Women with ENDO often experience dysmenorrhea (excessive menstrual pain), dyspareunia (vaginal hyperalgesia), dyschezia (pain with defecation), and chronic pelvic pain, which can co-occur with a range of other painful conditions (7). Symptoms can range from moderate to severe. A rat model of ENDO has been developed (8). Similar to women, rats with ENDO exhibit abnormal sensitivity in the pelvic area (i.e., vaginal hyperalgesia [9] that can be assessed with the visceromotor response [VMR]) (10, 11).

Evidence from human and animal studies suggests that abnormal immune, vascular, and neural activities in ectopic endometrium contribute to inflammatory signs and symptoms associated with ENDO. Ectopic endometrial growths in women and rats become highly vascularized, infiltrated with polymorphonuclear cells and activated macrophages (12–14), and densely innervated with sensory and sympathetic fibers (15–17). Similar to other inflammatory conditions, vessel permeability to blood proteins in endometrial growths in women (14, 18) and rats (17, 19) is significantly increased. Ectopic endometrial tissue produces inflammatory prostaglandins, cytokines, and growth factors (17, 20–24). Most of these inflammatory mediators facilitate plasma protein extravasation into surrounding tissue (25–31) that can activate visceral nociceptors (26, 32–34). The resultant neural activity (35, 36) further exacerbates blood vessel leakage. In rats with ENDO, this neurogenic component of plasma protein extravasation was found to become significant in the early stages of cyst development (19). Activated nociceptors are known to contribute to increased sensory sensitivity, including pain (37, 38).

Received March 31, 2014; revised and accepted June 30, 2014; published online August 11, 2014.
N.D. has nothing to disclose. G.S. has nothing to disclose. R.S. has nothing to disclose.
This research was partially funded by Bayer HealthCare Pharmaceuticals (Grant4Tagerts).
Reprint requests: Natalia Dmitrieva, Ph.D., Program in Neuroscience/Psychology, Florida State University, 1107 West Call St., Tallahassee, Florida 32306-4301 (E-mail: dmitrieva@psy.fsu.edu).

Fertility and Sterility® Vol. 102, No. 4, October 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.06.046

Resolvins (an acronym of "resolution phase interaction products") are proresolving lipid mediators that received their name for their distinctive role in resolution of acute immune inflammatory response (down-regulation of leucocyte infiltration, facilitation of phagocytotic activity, and apoptotic cell removal) (39). Resolvins are biosynthesized from omega-3 polyunsaturated fatty acids by 5- and 15-lipoxygenases and cyclo-oxygenase 2 (COX2), enzymes that are well known for their roles in production of proinflammatory lipids. Resolvin RvD1 [17(S)-resolvin D1] is produced from docosahexaenoic acid in the site of inflammation by leukocytes and endothelial cells (40, 41). Biosynthesis of RvD1 requires 5- and 15-lipoxygenases, whereas the final step in production of its stable analog 17(R)-RvD1 requires aspirin-acetylated COX2 (41). Recent studies in animal models demonstrated the effectiveness of low doses of RvD1 and 17(R)-RvD1 in reducing inflammatory signs, including pain and edema (42–45). To produce its peripheral effects, RvD1 activates two high-affinity G protein–coupled receptors expressed in peripheral leukocytes, macrophages, and endothelial cells, namely ALX/FPP2 and CPR32 (46, 47). The existence of spinal sites of RvD1 actions has been also suggested (45).

Resolvins can produce anti-inflammatory actions at doses at least 10–100 times lower than opioids and COX2 inhibitors without adverse effects (42, 43, 45, 48, 49). Experimental findings suggest that resolvins can effectively alleviate some chronic inflammatory signs. Thus, 17(R)-RvD1 and its precursor have alleviated chronic inflammatory signs and abnormal sensory sensitivity in a mouse model of arthritis (43). Together this information suggests that RvD1 and 17(R)-RvD1 have the potential to alleviate inflammatory signs associated with ENDO. If so, these resolvins should reduce plasma protein extravasation in ectopic growths and alleviate ENDO-induced vaginal hyperalgesia.

MATERIALS AND METHODS

This study was approved by the Florida State University Animal Care and Use Committee (protocol #1239).

Subjects

Female Sprague-Dawley rats were used. Estrous cycle was determined daily by cytologic examination of vaginal lavage collected approximately 2 hours after lights on (50). All experiments were done 10–12 weeks after ENDO surgery.

Endometriosis Surgeries

Endometriosis surgeries were performed under ketamine/xylazine anesthesia (73/8.8 mg/kg intraperitoneal [IP]) as previously described (51). Briefly, the rat was kept warm on a heating pad. A small incision was made in the middle of the abdomen. An approximately 1-cm segment of the middle part of one uterine horn was clamped and excised. Four 2 mm × 2 mm pieces of the excised uterine tissue were sutured on alternate mesenteric cascade arteries. Muscle and skin were sutured separately. Bupivacaine was given locally and butorphanol SC immediately after surgery to alleviate postoperative pain.

RvD1 and 17(R)-RvD1 Treatments for Evans Blue Assessment

All rats were in proestrus on the day of the experiment. Rats were first anesthetized with urethane (1.2 g/kg) and placed on a warm heating pad. The jugular vein was exposed through an incision and catheterized; the skin was sutured.

Rats in one group received an injection through the jugular catheter of a single dose of RvD1 (300 ng/kg in 0.15 mL of phosphate-buffered saline [PBS]), which was flushed through with 0.1 mL heparinized saline. Rats in another group were injected via the jugular catheter with a similar volume of PBS. Three hours later, Evans Blue (EB) was injected.

Rats in three other groups were injected (IP) with one of two doses of a stable analog 17(R)-RvD1 (300 or 900 ng/kg in 0.15 mL of PBS) or PBS. Evans Blue was injected via the jugular vein 3 hours later.

Evans Blue dye extravasation in ectopic growths, which measures vascular permeability, was used as an indirect assessment of neurogenic, mainly C-fiber activity (35). Evans Blue (50 mg/kg in saline) was delivered through the catheter. Thirty minutes later, excess dye was rinsed out of blood vessels by delivering approximately 200 mL of saline through the catheter. Cysts were harvested, weighed, and incubated in formamide at 60°C for 48 hours. Optical density of the sample and standard solutions was measured spectrophotometrically ($\lambda = 620$ nm). The amount of EB extracted from each sample was calculated as milligrams of EB per gram of tissue.

The differences between the RvD1- and vehicle-treated groups were analyzed with Student's t test, and between 17(R)-RvD1 and vehicle with analysis of variance (ANOVA) followed by post hoc Dunnett tests, with significance set at $P \leq .05$.

Telemetric Probe Implantation

Seven weeks after ENDO surgery, a telemetric probe (TA11CTAF40; DSI) was implanted under aseptic conditions and ketamine/xylazine anesthesia under the skin of the right abdominal flank, as described by Dmitrieva et al. (51). Electrodes were tunneled under the skin and implanted in the left inguinal muscle. Experiments began approximately 7 days after implantation.

17(R)-RvD1 Treatment and Assessment of Vaginal Nociception

17(R)-RvD1 treatment and assessment of vaginal nociception in conscious ENDO rats (n = 5) were based on telemetered VMR to vaginal distention. The rat was loosely enclosed in a transparent Plexiglas box. The box was placed on the receiver. A small balloon (approximately 10-mm diameter when fully inflated) connected to a pressure transducer was inserted into the middle of the vaginal canal. After a 10-minute resting period, the balloon was inflated by an infusion pump (0.3 mL/min, 1 mL maximum). Electrical activity from the inguinal muscle was radio-relayed to the receiver, synchronized with the signal from the pressure transducer, and processed by a Ponemah Telemetry System (DSI).