Effect of Peroxisome Proliferator–Activated Receptor-γ Agonist Rosiglitazone on the Induction of Endometriosis in an Experimental Rat Model

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OBJECTIVE: *To assess the effect of the peroxisome proliferator–activated receptor (PPAR)-γ agonist rosiglitazone on the induction of endometriosis in a rat model.*

METHODS: *Endometriosis was surgically induced in 28 rats by transplanting an autologous fragment of endometrial tissue onto the inner surface of the abdominal wall. Group I was assigned as control and no medication was administered. Starting 3 days before the operation and continuing for 4 weeks, 0.2 mg/kg/d rosiglitazone was administered to the study group orally. Four weeks later rats were killed and ectopic uterine tissues were evaluated morphologically and histologically. Scoring systems were used to evaluate preservation of epithelia.*

RESULTS: *Four rats in the study group and one rat in the control group died of complications related to surgery. There was a significant difference in post-treatment spherical volumes (64.00 mm^3 [interquartile range (IQR): 354.42] vs 41.60 mm^3 [IQR: 37.87], P = .018) and explant weights (77.97 mg [IQR: 431.27] vs 47.24 mg [IQR: 43.01], P = .005) between control and rosiglitazone-treated groups. The epithelia were found to be preserved significantly better in the control group when compared with the roziglitazone-treated group (2.00 [IQR:2.00] vs 0.00 [IQR:2.25], P = .014).*

CONCLUSIONS: *Rosiglitazone was found to affect the induction of endometriosis negatively in this experimental rat model and seemed to interfere with the growth and maintenance of the uterine explant. (J Soc Gynecol Investig 2006;13:58–62)*

KEY WORDS: Rosiglitazone, experimental endometriosis model, rat, peroxisome proliferator–activated receptor.

Endometriosis is characterized by the presence of tissue resembling endometrial gland and stroma outside the uterus. It affects up to 10% of women of reproductive age and as many as 30% to 50% of all infertile women.[1,2] The pathogenesis of endometriosis remains unclear, although Sampson's implantation theory is widely accepted.[3] This theory is based on the assumption that viable endometrial cells from retrograde menstruation reach the abdominal cavity, implant, and grow to some extent on the peritoneum.

Almost all women, about 90%, with patent fallopian tubes have reflux menstruation into the peritoneal cavity[4]; however, endometriosis is found in only about 10% of women of reproductive age. This suggests that there may be genetic and immunologic factors that determine a woman's susceptibility to endometriosis.[5,6]

It has been stated that the monocyte/macrophage system in the peritoneal cavity is basically a "disposal system" that removes all debris including endometrial cells from the abdominal tubal ostii.[7] This system may play a role in the development of endometriosis. An increased number of macrophages and greater concentration of its secretory products such as interleukin-1, tumor necrosis factor, and growth factors has been described in the peritoneal fluid of women with endometriosis.[8–10] It has been proposed that the cytokines elaborated from activated peritoneal macrophages mediate many of the symptoms associated with the endometriosis syndrome.[11] Therefore, it can be speculated that modulation of these cytokines might alter the course of the disease.

A new class of macrophage-modulating factors includes the peroxisome proliferator–activated receptors (PPARs). PPARs are ligand-dependent transcription factors of the nuclear hormone receptor super family. The role of these receptors in macrophage biology has not been well defined, but recent insights into the role of PPAR-γ in the monocyte lineage indicate that it regulates macrophage activation and inflammatory response.[12] Thiazolidinediones activate PPAR-γ and are

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among the compounds with the highest affinity and efficacy on PPAR-γ.

It is possible to utilize animal models to study events involved in the pathogenesis and pathophysiologies of endometriosis that cannot be accessible in humans. Particularly the successful development and setting up of surgically transplanted endometrial tissue in the rat, which shows histologic transformations similar to those seen in human endometriotic lesions, has offered a research tool to study this enigmatic disease.[13] Based on the above considerations, the purpose of this study was to evaluate the effect of a thiazolidinedione, rosiglitazone, on the development of an experimental endometriosis model.

MATERIALS AND METHODS

Twenty-eight female, non-pregnant Wistar albino rats weighing between 190 and 230 g were used as a model for experimental induction of endometriosis. The rats were caged individually in a controlled environment with 12-hour light/dark cycles and were fed ad libitum. The animals were sexually mature and demonstrated normal estrous cycle changes in uterine histology (data not shown). The guidelines for the care and use of the animals approved by the local institution were followed. All rats were observed for several days to ascertain health before the operation.

Before the operation, rats were randomly assigned into two groups each consisting of 14 rats. The first group was assigned as the control group and no medication was administered. Rats in group 2 received 0.2 mg/kg/d oral rosiglitazone maleate (Avandia, GlaxoSmithKline, Istanbul, Turkey) starting 3 days before the operation and continuing for 28 days postoperatively. It is stated by the manufacturer that rosiglitazone in this dose does not alter estrous cyclicity and the no-effect dose for the placenta, embryo/fetus, and offspring is 0.2 mg/kg/d in rats.

Endometriosis was surgically induced in rats by transplanting an autologous fragment of endometrial tissue onto the inner surface of the abdominal wall as proposed by Vernon and Wilson[13] with modifications by Lebovic et al.[14] Briefly, each rat was anesthetized with an intramuscular injection of ketamine (70 mg/kg Ketalar, Eczacibasi, Istanbul, Turkey). Before surgery, the abdominal skin was shaved and antisepsis was obtained by 10% povidone iodine solution. Using sterile techniques, a 5-cm vertical midline incision was made and both uterine horns were exposed. A distal segment, 1 cm in length, was resected from the right uterine horn. The segment was placed in phosphate-buffered saline at 37C and split longitudinally, and a 5- × 5-mm piece was sectioned. This piece of uterine tissue was transplanted without removing the myometrium onto the inner surface of the right abdominal wall with the serosal surface apposed and secured with single nonabsorbable 5-0 polypropylene suture at the middle to the abdominal wall. Before closure of the abdominal wall, 2 mL of saline was administered into the abdominal cavity to prevent drying and minimize adhesion formation. The abdominal incision was closed in two layers with the use of a simple interrupted 4-0 polyglactin 910 suture for the peritoneum-fascia and for the skin. The operation was limited to 25 minutes for each rat to control the effect of room air tissue drying. All surgeries were performed by the second author.

The rats were individually caged after the operation and were left for a recovery period of 4 weeks. During this period, 0.2 mg/kg/d rosiglitazone was administered to the rats in the study group. During the treatment period, any adverse treatment effects were monitored. Vaginal smears were performed on all rats after drug treatments to ensure that their reproductive cycles were not disrupted at the beginning and at the end of the study. At the day of operation, four rats in the control group and four rats in the treatment group were in proestrus stage and three rats in the control group and five rats in the treatment group were in estrus stage. During the first 5 days after the operation, daily vaginal smears were performed and the permanence of the estrous cycle was confirmed.

Starting 28 days after the operation and lasting 4 days, daily vaginal smears were performed and all rats that were in estrus were killed and a second-look laparotomy performed while the rats were fixed in the supine. All three dimensions (length × width × height in millimeters) of ectopic uterine tissue were measured in situ using a caliper by the first author, who had no prior knowledge of which group was being evaluated. The spherical volume of each ectopic uterine tissue was calculated using the prolate ellipsoid formula: $V\ (mm^3) = 0.52 \times A \times B \times C$, where A, B, and C denote width, length, and height, respectively. Tissues were photographed using a digital camera and then excised and weighed (in milligrams). Then tissue samples were fixed in 10% buffered formalin solution for 24 hours. After fixation, routine tissue processing procedure was performed, and then sampled tissues were embedded in paraffin. Paraffin wax blocks were cut in 4 μm thickness. Prepared sections were stained with hematoxylin-eosin.

The histologic diagnosis of endometriosis was based on the morphologic identification of endometrial glandular tissue and stroma: glands and stroma of the endometrial type, with epithelial lining and luminal formation. By microscopic examination, the preservation of endometrial tissue was semiquantitatively evaluated according to a scoring system developed by the fifth author

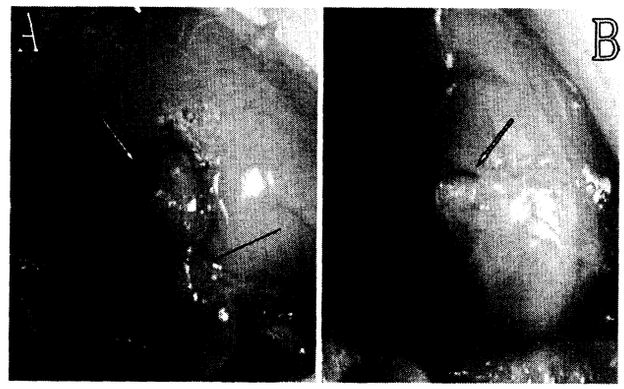

Figure 1. Gross morphologic appearances of implanted uterine autografts. (A) In the control group (*white arrow*); note the adhesion between the graft and the intestine (*black arrow*). (B) In the rosiglitazone-treated group (*white arrow*).

Table 1. Comparison of Volume, Weight, and Preservation of Endometrium Between Control and Treatment Groups

Variable	Control median (IQR*)	Roziglitasone-treated group median (IQR)	*U*	*P*
Volume	64.00 (354.42)	41.60 (37.87)	27.00	.018
Weight	77.97 (431.27)	47.24 (43.01)	20.00	.005
Preservation of endometrium	2.00 (2.00)	0.00 (2.25)	27.00	.014

as follows: a score of 4 indicates glandular structures lined with well-preserved, complete endometrial epithelium and presence of stroma in the whole endometriosis focus; a score of 3, only glandular structures lined with the complete endometrial epithelium without stromal areas in the endometriosis focus; a score of 2, glandular structures lined with discontinuous destroyed epithelium in incomplete stromal areas in the endometriosis focus; a score of 1, only glandular structures lined with incomplete destroyed epithelium, without stroma in the endometriosis focus; and a score of 0, no endometrial epithelium or stroma. The fifth author evaluated sections from each explant in a blinded fashion.

Statistical analysis was accomplished on a personal computer by using statistical program for social sciences version 12.0 (SPSS 12.0, demo, SPSS Inc, Chicago, IL). Lilliefors adjusted Kolmogorov-Smirnov test was used to test whether the variables used in the study were normally distributed. It was found that none of the variables tested were normally distributed (in volume and weight variables, there was a normal distribution in the treated group [*P* close to 1]; however, these variables were not normally distributed in the control group [*P* close to 0] and normal distribution could not be provided by transformation), and thus statistical analyses were performed by a nonparametric test (Mann-Whitney *U* test) and the data expressed as the median and interquartile range (IQR). A *P* value of less than .05 was considered significant.

RESULTS

Four rats in the study group and one rat in the control group died the day after the operation due to complications related to surgery. The standardized surgical procedures and the administration of the protocols were well tolerated by the remaining animals. All laparotomy sites were intact and none of the animals had an incisional hernia. No side effects related to medication were observed in the treatment group.

Morphologically the implants were cystic (Figure 1). There were adhesions between the implants and other abdominal viscera, particularly in the control group (Figure 1). There was a significant difference ($P = .018$) in spherical volume between control (median: 64.00 mm^3, IQR: 354.42) and the study group treated with rosiglitazone (median: 41.60 mm^3, IQR: 37.87) (Table 1). Similarly, the two groups were significantly different with respect to weight; median: 77.97 mg (IQR: 431.27) for the control group and 47.24 mg (IQR: 43.01) for rosiglitazone-treated group ($P = .005$) (Table 1).

The histopathologic finding of endometrial glands and stroma in the surgical site of implantation of endometrial squares allowed the diagnosis of experimental endometriosis. Histologically, epithelia of the cystic implants in the control group were found to be more persistent when compared with the rosiglitazone-treated group (Figure 2). The median scores were 2.00 (IQR: 2.00) and 0.00 (IQR: 2.25), respectively, with a *P* value of .014. Cystic implants in the control group were more similar to the eutopic endometrium because they both contained endometrial epithelium and stromal cells (Figures 2 and 3). The inner lining of the cysts contained simple columnar epithelium. The uterine autograft from rosiglitazone-treated rats showed marked epithelial changes (Figure 2). The eutopic endometria of the control and treatment groups were histologically similar (Figure 3).

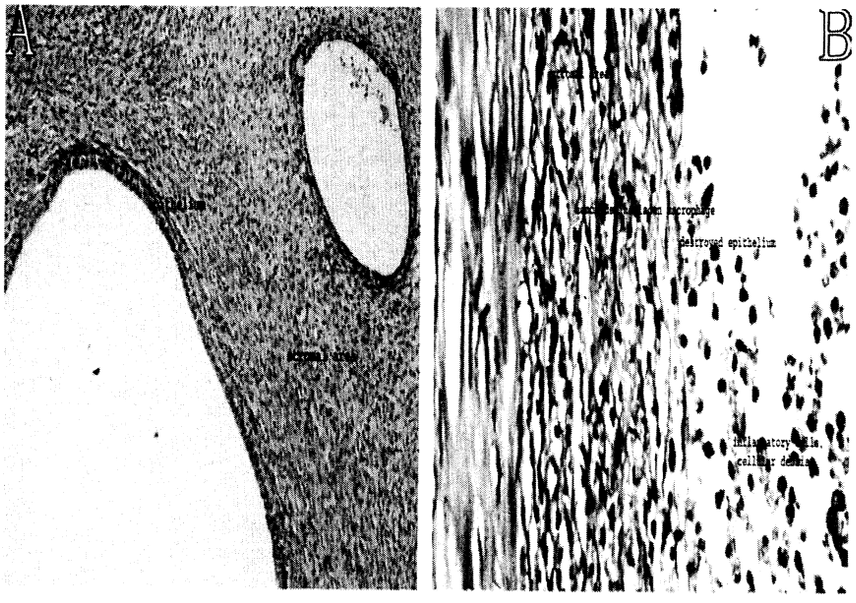

Figure 2. Histology of the ectopic endometrum. (A) In the control, nontreated group (note the preserved epithelial linings). (B) In the rosiglitazone-treated group (note the destroyed epithelium).

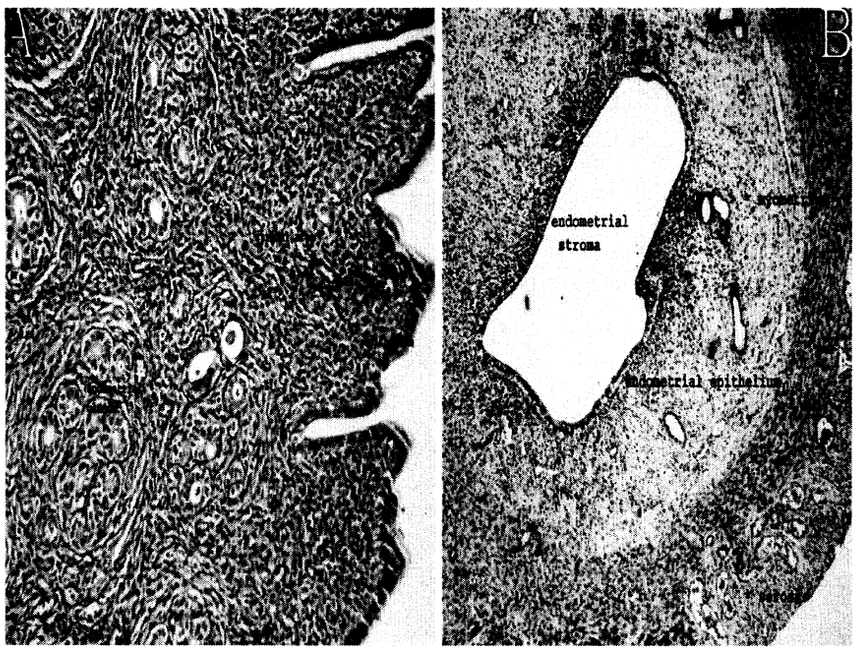

Figure 3. Histology of the eutopic endometria of (A) control group (hematoxylin-eosin stain, 20× magnification) and (B) rosiglitazone-treated group (hematoxylin-eosin stain, 5× magnification).

DISCUSSION

Despite many years of investigation, the pathogenesis of endometriosis is still poorly understood. Recently inflammatory cells and their secretory products have been recognized as important mediators of the pathophysiology of endometriosis.[15,16] It has been proposed that the cytokines elaborated from activated peritoneal macrophages mediate many of the symptoms associated with the endometriosis syndrome.[11] Several macrophage-derived cytokines, such as interleukin (IL)-1, platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), and interleukin-6, have been found to be increased in pelvic fluid from endometriosis subjects.[17–20] In addition, recruitment of some other chemokines has been discovered in peritoneal fluid of women with endometriosis.[21–23] It appears that multiple types of inflammatory cells are recruited from the circulation and accumulate in the peritoneal cavity and within endometriotic implants themselves.[24]

Recently, ligands for PPAR-γ have been shown to inhibit the expression of various cytokines in monocytes and macrophages, principally by preventing the activation of NF-B/Rel by an unknown mechanism.[25,26] On the basis of these observations, we postulated that a PPAR-γ agonist thiazolidinedione, rosiglitazone, could affect the induction of endometriosis negatively in an experimental rat model. It was found that when compared with the control group, rosiglitazone suppressed the growth of ectopic uterine tissue significantly with regard to volume and weight of the explants.

It has been reported that TNF-α and interleukin-8 are able to stimulate the growth of ectopic endometrial stromal cells and that cytokines act as messengers between stromal and epithelial cells.[27] IL-8, with its pro-inflammatory, growth-promoting, and angiogenic properties, may be an important factor responsible for neovascularization and along with TNF-α for the inflammation in and around the ectopic endometrial lesions.[28,29] Interleukin-1β is thought to serve as a mediator between peritoneal fluid macrophages and the promotion of endometriotic implant growth and angiogenesis.[16,30] In addition, monocyte chemotactic protein-1 (MCP-1) plays a role in the growth and maintenance of ectopic endometrial tissue[31] and macrophages by producing cytokines such as TNFα, and may stimulate the production of MCP-1.[31] Rosiglitazone seems to interfere with the growth and maintenance of the uterine explant possibly by inhibiting the expression of various cytokines in macrophages. There is also considerable evidence suggesting that PPAR-γ ligands, such as thiazolidinediones, are potent cell growth inhibitors[32,33] and inducers of apoptosis.[32,34]

The effect of another thiazolidinedione, ciglitazone, on endometriosis was shown by Lebovic et al.[14] They found that ciglitazone reduced the size of experimentally established endometriosis in the rat model after 4 weeks of administration.

There are some limitations in the current study that must be acknowledged. The semiquantitative evaluation of the preservation of endometrial tissue with a scoring system is subjective and there may be interobserver variability. Second, although it has been noted that rat endometriotic tissues and cells perform in a similar manner to human endometriotic cells in organ explant culture and isolated cell culture,[35–37] there is a disadvantage in extrapolating data across species and immunologic properties of species are different.

In conclusion, considering the macrophage modulation properties of PPARs, the effect of a PPAR ligand agonist, rosiglitazone, on the induction of endometriosis in an animal model was assessed in the present study. Rosiglitazone was found to affect the induction of endometriosis negatively in this experimental rat model and seemed to interfere with the growth and maintenance of the uterine explant.

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