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Original Article

Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reduces the size of experimental endometriosis in the rat model

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Abstract

Background: The effect of rosiglitazone, an activator of peroxisome proliferator-activated receptor-gamma, on the growth of ectopic uterine tissue was assessed.

Methods: Endometriosis was surgically induced in 28 rats by transplanting an autologous fragment of endometrial tissue onto the inner surface of the abdominal wall. Four weeks later, rats were randomly grouped and a second laparatomy was performed. The length, width, height and volume of the explants were measured. Rosiglitazone at 0.2 mg/kg/day was orally administered to one group, while vehicle treatment was given to the control group. Four weeks later, rats were sacrificed and ectopic uterine tissues were re-evaluated morphologically and histologically. Scoring system was used to evaluate the preservation of epithelia.

Results: One rat in the study group and two rats in the control group died as a result of complications related to surgery. There was a significant difference in post-treatment length, width, height, and spherical volumes between control and rosiglitazone-treated groups. The epithelia were found to be preserved significantly better in the control group when compared with the rosiglitazone-treated group.

Conclusion: Rosiglitazone was found to cause regression of experimental endometriosis in rats.

Key words: endometriosis, PPAR-γ, rat model, rosiglitazone.

Introduction

A concept of a cell-mediated immunological aetiology for endometriosis has gained wide acceptance. Inflammatory cells and their secretory products have been recognised as important mediators in the pathophysiology of endometriosis.[1,2] An increase in the amount of peritoneal fluid is a characteristic finding in endometriosis, as is the increased presence of various free-floating cells such as macrophages, lymphocytes, eosinophils, natural killer cells, mast cells, mesothelial cells, and endometrial cells, as well as a wide range of soluble substances including autoantibodies, cytokines, growth factors, adhesion molecules, enzymes, hormones, prostaglandins, and reactive oxygen species.[3–11] Although the peritoneal fluid of women with endometriosis contains increased numbers of immune cells, evidence suggests that their actions do more to promote the disease than to prevent it.

Macrophages are a key element in innate immune response, and recent studies suggest that the peritoneal fluid of women with endometriosis contains an increased number of activated macrophages that secrete various local products, such as growth factors and cytokines.[9] The endometriosis-associated inflammatory response, tissue repair, and neovascularisation are dependent on the peritoneal fluid macrophages and their secretory products/cytokines.[12] And it has been shown that endometriotic endometrial cells have the capacity to utilise cytokines to facilitate critical disease processes such as attachment, invasion, angiogenesis, and growth.[2,13–16] From this point, it can be hypothesised that modulation of the secretion of cytokines from macrophages might alter the course of the disease process.

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Recently, we have shown that a peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonist, rosiglitazone, with the ability to modulate macrophages, affected the induction of endometriosis negatively in an experimental rat model;[17] however, the effect of rosiglitazone on the established endometriosis is not known. Based on the above considerations, the purpose of this study was to test if rosiglitazone, an activator of PPAR-γ, could impede the growth of an ectopic uterine tissue in an experimental animal model.

Methods

Twenty-eight female, non-pregnant Wistar albino rats weighing between 170 and 240 g were used as a model for experimental induction of endometriosis. The rats were caged individually in a controlled environment with 12-h light/dark cycles and were fed ad libitum. The animals were sexually mature. 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 the health before the operations.

Endometriosis was surgically induced in rats by the first author by transplanting an autologous fragment of endometrial tissue onto the inner surface of the abdominal wall as proposed by Vernon and Wilson[18] with minor modifications.[19]

The rats were individually caged after the operation and were left for a recovery period of four weeks. After four weeks, rats were randomly assigned (using random number tables) into two groups, each consisting of 14 rats, and daily vaginal smears were performed. A second laparatomy was performed to the rats in oestrous state. All three dimensions (length × width × height in millimetres) of ectopic uterine tissue were measured *in situ* using a caliper by the first author. 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 measurements were recorded.

The first group was assigned as the control group, and a vehicle treatment (3 mL 5% dextrose) was administered daily to prevent any unexpected effect from the gavage methodology of drug administration. Gavage method may be a stressful condition for the rats and it was thought that stress may cause production and release of steroid hormones (glucocorticoids), including the primary stress hormone cortisol, which may affect the immunological milieu of the peritoneal cavity and endometriotic implants. Rosiglitazone maleate at 0.2 mg/kg/day (Avandia®, GlaxoSmithKline, Istanbul, Turkey) was orally (with gavage methodology) administered to the rats in group 2 for 28 days postoperatively. It is stated by the manufacturer that rosiglitazone in this dose does not alter oestrous cyclicity, and the no-effect dose for effects on the placenta, embryo/fetus, and offspring is 0.2 mg/kg/day in rats. During the treatment period, any adverse treatment effects were monitored. During the first four days after the operation, daily vaginal smears were performed and the permanence of the oestrous cycle was confirmed.

Starting 28 days after the operation and lasting for five days, daily vaginal smears were performed and all rats that were in oestrous were sacrificed and laparotomy was performed while the rats were fixed in the supine position. The sizes of the implants were measured again with the same method, with the same caliber, by the same investigator who was blinded to the groups. Tissues were photographed using a digital camera and then excised. Then tissue samples were fixed in 10% buffered formalin solution for 24 h. 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 haematoxylin and eosin.

The histological diagnosis of endometriosis was based on the morphological identification of endometrial glandular tissue and stroma; glands and stroma of the endometrial type, with epithelial lining and luminal formation. In microscopic examination, the preservation of endometrial tissue was semiquantitatively evaluated according to a scoring system[17] by the fifth author in a blinded fashion.

Statistical analysis was accomplished on a personal computer using SPSS demo version 12.0 (SPSS Inc. Chicago, IL, USA). It was found that variables were not normally distributed. The data were expressed as the median (minimum–maximum). For the analyses of the pre- and post-treatment height, width, length and spherical volume in the treatment group and first and second measurements of these parameters in the control group, Wilcoxon signed-rank test was used. For the comparison of epithelial preservation scores between groups, Mann–Whitney *U*-test was used. A *P*-value of < 0.05 was assumed to be significant.

Results

One rat in the study group and two rats in the control group died just a couple of days after the second operation as a result of complications related to surgery. The standardised 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. Side-effects on the oestrous cycle were determined by vaginal smears. Effects of the treatment on the endometrium were evaluated histologically. Determination of the occurrence of any other side-effects was done by observation. Weight of the rats was measured and general appearance of rats, presence of anorexia (loss of appetite), and colour of urine were observed.

Morphologically, the implants were cystic (Fig. 1). The pre- and post-treatment measurements of height, length, width and volume of the implants in the rosiglitazone-treated group were significantly different; however, the differences of these parameters were not statistically significant in the control group (Table 1).

The histopathological finding of endometrial glands and stroma in the surgical site of implantation of endometrial squares allowed the diagnosis of experimental endometriosis.

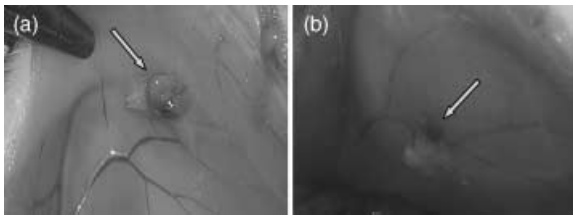

Figure 1 Gross morphological appearances of implanted uterine autografts. (a) In the control group (white arrow). (b) In the rosiglitazone treated group (white arrow).

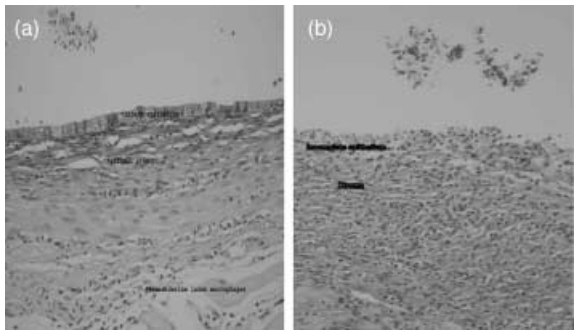

Figure 2 Histology of the ectopic endometrium. (a) In the control, non-treated group (haematoxylin and eosin (H&E) stain, ×20 magnification) (Note the preserved epithelial linings). (b) In the rosiglitazone-treated group (H&E stain, ×4 magnification) (Note the destroyed epithelium).

Histologically, epithelia of the cystic implants in the control group were found to be more persistent when compared with the rosiglitazone-treated group (Fig. 2). The median score of the preservation of epithelium in the rosiglitazone-treated group was 1 (0–2), whereas it was 2.5 (0–4) in the control group ($P = 0.039$). The inner lining of the cysts contained simple columnar epithelium. The uterine autograft from rosiglitazone-treated rats showed marked epithelial changes (Fig. 2).

Discussion

A unifying hypothesis to explain endometriosis has not been elucidated as yet, but numerous investigations have implicated disturbances in the immune response as fundamental to its aetiology and pathogenesis.[20] The monocyte/macrophage system seems to play an important role in the development of endometriosis. In endometriotic peritoneal fluid, the concentration and numbers of macrophages are significantly increased; however, instead of acting as scavengers to eliminate ectopic endometrial cells, activated macrophages and circulating monocytes can promote the disease by secreting growth factors and cytokines that stimulate proliferation of ectopic endometrium and inhibit their scavenger functions.[21,22] These macrophages under basal and stimulated conditions produce higher levels of tumour necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-8, IL-10, and smooth muscle contracting prostaglandins such as PGE2 and PGF2α than the macrophages of healthy women.[23,24] Resident or inflammatory peritoneal leucocytes, endothelial cells, and misplaced endometrial cells also synthesise various chemokines, such as CCL2 (also known as monocyte chemotactic protein or MCP1), and CCL5 (also known as RANTES (regulated on activation, normal T-cell expressed and secreted)), which increase leucocyte-trafficking and activation.[7,8] In addition, monocytes from women with endometriosis and or their secretory products seem to enhance autologous endometrial cell proliferation and decrease endometrial cell apoptosis, whereas monocytes from fertile women without endometriosis suppress endometrial cell proliferation.[25] Therefore, it can be hypothesised that by blocking early monocyte infiltration, the subsequent activation of other cellular inflammatory mediators might be prevented.

Recently, we have shown that a PPAR-γ agonist rosiglitazone, with macrophage modulation properties, interfered with the development of an endometriosis model in rats.[17] Briefly, endometriosis was surgically induced in 28 rats by the same method used in present study. Group 1 was assigned as control and no medication was administered. Starting three days before the operation and continuing for four weeks, 0.2 mg/kg/day rosiglitazone was administered to the study group orally. Four weeks later, rats were sacrificed and ectopic uterine tissues were evaluated with respect to successful establishment of endometriosis. There was a

Table 1 Comparison of the first and second measurements of length, width, height and volume in the control and rosiglitazone-treated groups

	Control group $n = 12$			Rosiglitazone-treated group $n = 13$		
	First measurement Median (min–max)	Second measurement Median (min–max)	P	First measurement Median (min–max)	Second measurement Median (min–max)	P
Length	8 (5–10)	8.5 (4–10)	0.196	5 (3–11)	5 (1–10)	0.004
Width	7 (4–9)	8 (4–9)	0.334	5 (2–9)	4 (1–9)	0.021
Height	5 (3–8)	6 (3–9)	0.068	4 (1–9)	3 (1–7)	0.01
Volume	165.1 (41.6–576)	191.9 (24.9–576)	0.333	52 (3.1–463.3)	23.4 (0.5–327.6)	0.008

Length, width and height are in mm, volume is in mm^3.

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significant difference in the post-treatment spherical volume between control (median: 64.00 mm^3, interquartile range (IQR): 354.42) and rosiglitazone (median: 41.60 mm^3, IQR: 37.87) treatment groups. The present study showed that rosiglitazone treatment was not only effective on growth of endometrial explants as reported before[17] but also causes regression of established lesions.

A novel approach to treating endometriosis is to target the inflammatory response associated with the disease. Pentoxifylline treatment, a phosphodiesterase inhibitor, appears to decrease inflammation and has proven efficacious in the treatment of endometriosis in animal models.[26,27] Danazol, progestogens, and GnRH-agonists like hormonal treatments for endometriosis also work as immune modulators and seem to be relatively non-specific inhibitors of various inflammatory pathways.[28–30] In cells of the immune system, PPAR-γ activators were shown to inhibit the activation of cytokines and other inflammatory response genes such as IL-2, IL-6, IL-8, and TNF-α.[31] Rosiglitazone seems to reduce the size of the endometrial explants significantly in the rat model possibly by inhibiting the secretion of various cytokines from 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]

In addition, it has been shown that polymorphisms of the PPAR-γ2 gene may be associated with endometriosis.[35,36] Dogan *et al.* suggested that the 12-Pro allele of PPAR-γ2 may have protective effects avoiding the development and progression of endometriosis.[35] Although Kiyomizu *et al.* in their study did not confirm this and did not find a significant difference with respect to 12Ala frequency between patients with endometriosis and the control women, they found that the PPAR-γ 161C genotype and allele frequencies were significantly increased in patients with endometriosis. They suggested that the PPAR-γ 161CC genotype could be a genetic risk factor for endometriosis.[36] From this point, endometriosis seems to be associated with PPAR-γ and it would not be imprudent to think agents with PPAR-γ-modulating properties may affect the course of the disease.

Our observation that PPAR agonists induce regression in endometriosis is consistent with recent reports. The effect of another thiazolidinedione, ciglitazone, on endometriosis was shown by Lebovic *et al.*[19] They showed that ciglitazone reduced the size of experimentally established endometriosis in the rat model after four 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. Secondly, there is disadvantage of extrapolating data across species and immunological 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 regression of endometriosis in an animal model was assessed in the present study. Rosiglitazone was found to reduce the size of the endometrial explants significantly in this rat model. Endometriosis is associated with an abnormal inflammatory response and rosiglitazone as a thiazolidinedione may be a helpful anti-inflammatory agent in the treatment of the disease. More experiments on animal models and clinical trials would be helpful in determining the utility of this novel class of compounds for women with endometriosis.

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