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The effect of captopril on endometriotic implants in a rat model

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ABSTRACT

Objective: To determine the effects of captopril on experimentally induced endometriosis in a rat model.
Study design: Twenty-four adult, mature female Wistar-Albino rats in which endometriotic implants were induced by transplanting autologous uterine tissue to ectopic sites on the peritoneum. After the endometriotic implants were formed surgically, the 24 rats were randomly divided into three groups. Group 1 (captopril group, eight rats) were given 50 mg kg^{-1} d^{-1} of oral captopril for 21 d. Group 2 (leuprolide acetate group, eight rats) were given a single 1 mg kg^{-1} subcutaneous injection of leuprolide acetate. Group 3 (control) were given no medication and served as controls (eight rats). The surface area of the endometriotic implants and the score of histologic analysis. Also, VEGF and MCP-1 levels in peritoneal fluids and bloods were analyzed.
Results: At the beginning of the medical treatment, the mean surface areas of the endometriotic implants were comparable in all three groups. At the end of the treatment the mean implant surface area in the captopril group and leuprolide acetate group was less than that in the control group. Mean histopathological examination score for the implants post treatment was lower in the captopril and leuprolide acetate groups. Peritoneal fluids VEGF level in the captopril and leuprolide acetate groups was lower than that in the control group. The post-treatment MCP-1 level was also lower in the captopril and leuprolide acetate groups than in the control group. The serum VEGF and MCP-1 levels post treatment were significantly lower in the captopril and leuprolide acetate groups than in the control group.
Conclusion: Administration of captopril reduced the size and progression of endometriotic lesions in a rat model.

Introduction

Endometriosis is the presence of both endometrial gland and stromal tissue outside the uterus, such as the ovaries, fallopian tubes, and pelvis [1]. Patients with endometriosis may have dysmenorrhoea, dyspareunia, pelvic pain, and infertility. Endometriosis is present in ≥22% of asymptomatic women, ≥45% of women with pelvic pain, and 38% of all infertile women [2,3]. Several theories have been developed in an attempt to explain the etiology of endometriosis, including retrograde menstruation, implantation theory, metaplasia of the peritoneum, and blood or lymphatic spread [4]; however, endometriosis is thought to have a polygenic-multifactorial pattern of inheritance [5].

Angiogenesis is required for endometriotic implants to spread to the peritoneal cavity. Several studies have reported an increase in angiogenesis around peritoneal endometriotic implants [6–9]; therefore, the growth of endometriotic implants might be limited via inhibition of angiogenesis, as previously reported [10,11]. According to the implantation theory of the etiology of endometriosis, angiogenesis is among the major factors associated with the initiation and progression of the disease. As such, during the last decade many studies have focused on the mechanisms of endometriosis in an effort to determine the importance of angiogenesis in the treatment of endometriotic lesions [11]. Furthermore, enzyme-linked immunosorbent assay of peritoneal fluids, histological, immunohistochemical, and gene expression analysis of endometriotic tissue, cell culture systems, and sophisticated in vivo animal models have been employed in the analysis of blood vessel development in ectopic endometrium and the efficacy of angiogenesis inhibitors. Such research has confirmed that angiogenesis plays a crucial role in the

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development of endometriosis, and may have a role in the treatment of the disease and its symptoms [11].

Several experimental studies have examined the role of anti-angiogenic agents, including vascular endothelial growth factor (VEGF) inhibitors, endogenous angiogenesis inhibitors, statins, COX-2 inhibitors, immunomodulators, dopamine agonists, PPAR agonists, and other agents [11]. Moreover, captopril (D-3-mercapto-2-methylpropanoyl-L-proline) – an inhibitor of angiotensin-converting enzyme (ACE) – is also an effective inhibitor of angiogenesis [12,13]. Today, captopril is commonly used to manage hypertensive disorders; however, it has been also been shown to limit the progression of several angiogenesis-dependent diseases, including arthritis, diabetic retinopathy, atherosclerosis, and cancer, due to its inhibition of new blood vessel growth [14]. As such, the present study aimed to investigate the effect of captopril on experimentally induced endometriosis in a rat model.

Materials and methods

Animals

The study included 24 Wistar-Albino rats that were provided by Gazi University, Animal Reproduction Center. The animals were housed in the university's animal laboratory under a controlled environment of 22 °C and a 12-h light/dark cycle. Standard rat feed and reverse-osmosis-purified water were provided ad libitum. All rats were given 1 week of acclimation to this environment before experimentation. The Gazi University Committee on the Use and Care of Animals approved the study protocol, and all procedures complied with the 1996 National Academy of Science's Guide for Care and Use of Laboratory Animals.

Surgical procedure

All the rats were anesthetized via intramuscular administration of 50 mg kg^{-1} of ketamine hydrochloric acid (Ketalar, Eczacıbası Warner-Lambert Ilaç Sanayı, Levent, Istanbul, Turkey) and 7 mg kg^{-1} of xylazine hydrochloric acid (Rompun, Bayer ŞiŞli, Istanbul, Turkey). They rates were immobilized on a standard rat surgery board. Under aseptic conditions a ventral midline incision was made to expose the reproductive organs. All rats underwent three surgeries (laparotomies), as explained below.

Laparotomy 1

Ectopic endometrium was induced surgically, as described in ref. [15]. The left uterine horn was ligated at both the uterotubal junction and cervical end, and then removed. A 7 mm segment of the excised horn was cut and placed in sterile isotonic saline. A trimmed 5 mm × 5 mm section of the endometrium was then transplanted into the peritoneal cavity (Fig. 1a and b), with the epithelial lining of the segment in apposition to the ventrolateral body wall adjacent to a large vessel using sterile 4–0 silk. The midline abdominal incision was closed with chromic catgut sutures. The skin incision was closed with a horizontal mattress. After the laparotomy 1, all rats were observed for 21 d in their cages without any medication.

Laparotomy 2

Two rats died during the period of discovered endometrial implants. The remaining rats underwent laparotomy 2 (exploratory laparotomy) to look for endometrial implants and collect peritoneal fluid for measurement of vascular endothelial growth factor (VEGF) and monocyte chemotactic protein-1 (MCP-1) levels. To assess the level of VEGF and MCP-1 in peritoneal fluid, peritoneal lavage with 3 mL of saline was performed and samples immediately sent to the laboratory at the beginning of the laparotomy. The length and width (mm) of the implants were measured microscopically, and the surface area of each implant was calculated as length × width and recorded. Then, the laparotomy was closed. Following the laparotomy 2, all rats were allowed a resting period of 3 d and then they were randomly divided into three groups. Rats in the captopril group ($n = 7$) were given 50 mg kg^{-1} d^{-1} of oral captopril for 21 d (Bristol Meyers/Squibb, Princeton, NJ) [16]. Rats in the leuprolide acetate group ($n = 8$) were given a single 1 mg kg^{-1} subcutaneous injection of leuprolide acetate depot formulation (Lucrin, Abbott, Cedex, Istanbul, Turkey). The leuprolide acetate dose was based on a previous study in which 1 mg kg^{-1} was observed to be optimal for female rats [17]. Rats in the control group ($n = 7$) were given no medication. Oral medications were administered by laboratory personnel via an orogastric tube. All the rats were observed for 21 d.

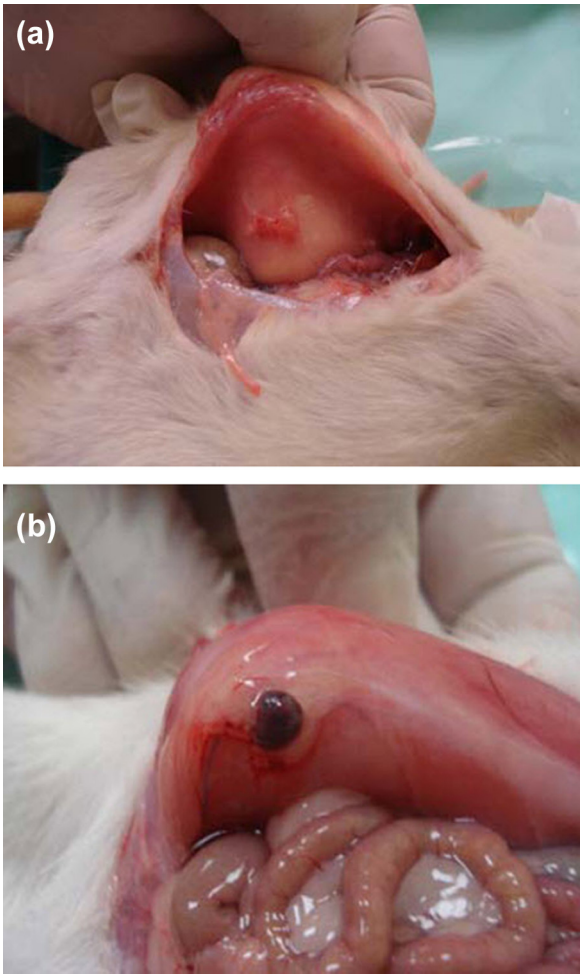

Fig. 1. (a and b) Discovered endometrial implants.

Laparotomy 3

Laparotomy 3 was performed after 21 d of medical treatment. During the surgery the length and width of the implants were measured microscopically, and the surface area of each implant was again calculated. The implants were then excised and fixed in 10% formalin for histopathological examination. Peritoneal lavage with 3 mL of saline was performed again to assess the VEGF and MCP-1 levels in peritoneal fluid. In addition, after treatment we