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Effect of imatinib on growth of experimental endometriosis in rats

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ABSTRACT

Objective: Currently, medical and surgical treatment options for endometriosis are limited due to suboptimal efficacy, and also safety and tolerance issues. Long-term use of gonadotrophin-releasing hormone analogs, androgenes, and the danazol, which are widely used drugs for endometriosis, is usually not possible due to their suboptimal safety and tolerance profile. The lack of an effective, tolerable and safe treatment option for endometriosis makes animal models of experimental endometriosis necessary to study candidate drugs. The aim of this study was to investigate the efficacy of imatinib on the experimental endometriosis in a rat model.

Study design: Endometriosis was induced by autotransplantation of uterine tissue into the peritoneal cavity. Twenty-four rats, which had visually confirmed endometriotic implants on subsequent laparotomy, were randomized into three groups to receive imatinib (25 mg/kg/day, p.o.), anastrozole (0.004 mg/day, p.o.), or normal saline (0.1 mL, i.p.) for 14 days. After removal of endometriotic tissue and H & E staining, endometriosis score was determined according to a semiquantitative histological classification. Also, immunostaining with primary antibodies including VEGF, CD117, and Bax were used for immunohistochemical (IHC) examination.

Results: Both anastrozole and imatinib suppressed the growth of endometriotic tissue and reduced the number of ovarian follicles. Although the difference was not statistically significant, imatinib was less effective than anastrozole for treatment of endometriosis.

Conclusion: Imatinib effectively treats experimental endometriosis by its inhibitor effects on angiogenesis and cell proliferation.

Introduction

Endometriosis is a common, chronic, estrogen-dependent inflammatory disease and affects 5–10 of reproductive-age women [1]. The exact prevalence of endometriosis is unknown but estimated to range from 2 to 10% in women of childbearing age. Its prevalence rises up to 50% in women with infertility [1]. Endometriosis causes chronic pelvic pain and it is another important problem associated with time lost from work and significant physical and social debility [2]. Currently, medical and surgical treatment modalities are not successful as expected in endometriosis. Because of recurrence after surgery, long-term medical management is an important option.

Current medical management of endometriosis includes gonadotrophin-releasing hormone analogs, androgenes, and the danazol. Suboptimal safety and tolerance problems limits their long-term use. Long-term suppression can be produced by oral contraceptives and progestings that have adverse effects including weight gain and acne and hirsutism related to their androgenic effects [3–8]. Therefore, the testing of candidate drugs for effective treatment of endometriosis are currently performed on animal models of experimental endometriosis. Numerous drugs have been tested for the treatment of endometriosis, including dinitrosyl iron complexes [9], steroid compounds developed as inhibitors of key enzymes of steroid synthesis (17α-hydroxylase/17,20-lyase, steroid sulfatase, 5α-reductases, aromatase and 17β-hydroxysteroid dehydrogenases) [10], kisspeptin antagonists [11], statins [12], protease-activated receptor 2 antagonist [13], and selective progesterone receptor modulator [14]. Novel drugs for suppressing endometriotic tissue target hormonal pathways via GnRH,

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estrogen and/or progesterone receptors, or modulate nonhormonal pathways including endometriosis-associated inflammation, cell proliferation, angiogenesis, adhesion and/or tissue invasion [8].

Receptor tyrosine kinase (RTK) inhibitors are among the novel adjuvant therapeutic option for several tumors including endometrial cancer for their important roles in cellular signaling, determining key roles in diverse biological processes like growth, differentiation, metabolism, and apoptosis in response to external and internal stimuli. [15,16]. Anti-endometriotic effects of RTK inhibitors in experimental conditions are under investigation: sorafenib [17,18], a multi-RTK inhibitor; cediranib [19], a RTK inhibitor potently inhibits VEGF receptor-2; and bevacizumab [18,20,21], and sunitinib and pazopanib [22].

Most tyrosine kinase receptor inhibitors are designed to target the epidermal growth factor receptor (EGFR), platelet derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGFR) tyrosine kinase families. Imatinib is a RTK inhibitor used as an oral targeted therapy, which can inhibit BCR/Abl, PDGF-R, c-KIT, c-fms, TCR/Abl, Lck, FLT-3 and MAPKs activities on various cell types [15,23]. Regarding their pharmacokinetic properties and clinical success in the management of several cancer types, it is difficult to predict which type of RTK inhibitor will be successful in the suppression of development of endometriosis. To the best of our knowledge, there was one study used imatinib on the endometriotic tissue in a murine model to assess the role of being homozygous for a colony-stimulating factor 1 mutation on the rate of lesion formation [24]. The aim of this study was to investigate the effect of imatinib on the experimental endometriosis in a rat model. The effect of imatinib on angiogenesis, cell proliferation, and apoptosis was evaluated by immunohystochemical examination and its effects on the extend of endometriotic tissue and ovarian reserve was assessed by visual observation and histological examination, respectively.

Materials and methods

Adult female Wistar-Albino rats (220–240 g) were obtained from Cumhuriyet University Animal Reproduction Centre (Sivas, Turkey). They were fed with standard feed and water, housed in a controlled environment (22 ± 2 °C) and exposed to with 12:12 h light:dark cycles. The rats were weighed before and after the procedures. All experiments in this study were performed according to the guidelines of Cumhuriyet University for the Care and Use of Laboratory Animals. This study was approved by the Animal Research Ethics Committee of the Cumhuriyet University (approval ID: 394). Every effort was made to minimize the number of animals used and their suffering. The mean weights of the rats were similar before and after the study procedures in all of the groups.

First laparotomy: induction of endometriosis

Endometriosis was induced by autotransplantation of uterine tissue into the peritoneal cavity as described by Vernon and Wilson [25] Intraperitoneally administered Ketamine, 60 mg/kg (Ketalar®; Eczacibasi Warner-Lambert, Istanbul, Turkey) and xylazine, 7 mg/kg (Rompun®, Bayer, Istanbul, Turkey) were used for anesthesia. All rats underwent a 3-cm midline vertical incision under aseptic conditions. After laparotomy, the left uterine horn was excised, immediately immersed in physiological serum, longitudinally opened, and sectioned into a 5 mm × 5 mm piece. This endometrial tissue was sutured to the peritoneum of the anterior right abdominal wall near to a vascular area without removing the myometrium, and then the abdominal incision was closed. All rats were observed for 21 days without medication.

Second laparotomy: evaluation of the endometriotic tissue

Twenty-one days after the first surgical procedure, the rats underwent a second laparotomy to evaluate the occurrence of endometriosis. Twenty-four rats had visually confirmed endometriotic implants and were randomized into three groups to receive imatinib (25 mg/kg/day, p.o.), anastrozole (0.004 mg/day, p.o.), or normal saline (0.1 mL, i.p.) for 14 days.

Third laparotomy: collection of tissue samples

Fourteen days after the second laparotomy, a third laparotomy was performed to obtain endometriotic tissue samples and ovaries. The rats were sacrificed by pentobarbital sodium anesthesia at the end of the procedure. All of the surgical procedures were performed by the same author (C.Y.).

Histopathological examinations

At first, the endometriotic implants and ovaries were stored in 10% formalin solution. Hematoxylin and eosin (H & E) staining was used for histological examination. Immunostaining with primary antibodies including VEGF, CD117, and Bax were used for immunohistochemical (IHC) examination. The histological and IHC examinations were evaluated by same author who was blinded for the study drugs (H.O.).

Histological examination of endometriotic implants

After (H & E) staining, endometriosis score was determined according to the semiquantitative histological classification proposed by Keenan et al. [26] Briefly, this classification is based on the preservation status of the epithelial layer of the endometrium as following: well-preserved epithelial layer = score 3, moderately preserved epithelium with leukocyte infiltrate = -score 2, poorly-preserved epithelium (occasional epithelial cells only) = score 1, no epithelium = score zero.

Immunohistochemical examination of endometriotic implants

The immunohistochemical staining was performed with the BenchMark XT (Ventana Medical Systems, Roche) system. The primary antibodies against VEGF (Ab-4, Clone BFD31, Lab Vision, USA), CD117 (K69, Lab Vision, USA), and Bax (Ab-2, Clone 5B7, Lab Vision, USA) were used. Immunohistochemical staining for VEGF A was evaluated as described by Donnez et al. [27] The histological scores (H) for VEGF were calculated using formula $H = \sum Pi$, where is the intensity ranging from 0 (negative cells) to 3 (intensely stained cells), and P is the percentage of stained cells for each given i, with P values of 1, 2, 3, 4, and 5 indicating $<15\%$, 15–50%, 50–85%, $>85\%$, and 100% positive-staining cells, respectively.

For the analysis of CD117 and Bax, an immunoreactive score (IRS) that was calculated by multiplication of the intensity (0–3) with the percentage of stained cells (0–4) was used [28]. Staining was scored for statistical analysis as following: 0 points = negative, 1–4 points = weak, and 6, 8, 9 or 12 points = strong.

Histological examination of ovaries

Both ovaries of each rat were removed and fixed in 10% formalin, then dehydrated with different grades of ethanol, cleared in xylene and embedded in paraffin. Ovarian sections of 6-μm thickness were prepared and stained with H&E. The numbers of primordial, primary and secondary, and antral follicles were assessed in 5 sections per ovary, with a distance of 120 μm to avoid counting same follicles twice.