

Regression of endometrial explants in a rat model of endometriosis treated with melatonin

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Objective: To determine the antioxidant, antiinflammatory, and immunomodulatory effects of melatonin on endometrial explants, the distribution of cyclooxygenase-2 (COX-2), the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), and levels of malondialdehyde (MDA) in the rat endometriosis model.

Design: Prospective, placebo-controlled experimental study.

Setting: Experimental surgery laboratory in a university department.

Animal(s): Twenty-five rats with experimentally induced endometriosis.

Intervention(s): Endometriosis was surgically induced in 25 rats by transplanting an autologous fragment of endometrial tissue onto the inner surface of the abdominal wall. Four weeks later, three rats were killed and the remaining 22 rats given second-look laparotomies to identify and measure ectopic uterine tissue in three dimensions. After the second laparotomy, 4 weeks of vehicle and melatonin treatment were administered, then all of the rats were given a third laparotomy and killed.

Main Outcome Measure(s): The volume and weight of the implants were measured. The remaining rats were randomly divided into two groups. In control group (group 1; $n = 11$) no medication was given. To the rats in melatonin-treated group (group 2; $n = 11$), 10 mg/kg a day of melatonin was administered intraperitoneally. Four weeks later, after the second laparotomy, the endometrial explants were reevaluated morphologically, and COX-2 expression was evaluated immunohistochemically and histologically. In addition, endometrial explants were analyzed for the antioxidant enzymes SOD, CAT, and MDA, a marker of lipid peroxidation. A scoring system was used to evaluate expression of COX-2 and preservation of epithelia.

Result(s): The pretreatment and posttreatment volumes within the control group were 135.9 ± 31.5 and 129.4 ± 28.7 , respectively. The mean explant volume was 141.4 ± 34.4 within the melatonin group before the treatment and 42.9 ± 14.0 after 4 weeks of treatment. There was a statistically significant difference in spherical volumes (129.4 ± 28.7 versus 42.9 ± 14.0 mm³) of explant weights (155.8 ± 27.1 versus 49.6 ± 19.5 mg) and COX-2 positivity (91% versus 18.1%) between groups after the third laparotomy. In the melatonin-treated group, the endometrial explant levels of MDA statistically significantly decreased and activities of SOD and CAT significantly increased when compared with the control group. The epithelia showed statistically significantly better preservation in the control group when compared with the melatonin-treated group (2.54 ± 0.52 versus 0.63 ± 0.50).

Conclusion(s): Melatonin causes regression and atrophy of the endometriotic lesions in rats. (Fertil Steril® 2008;89:934–42. ©2008 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, cyclooxygenase type 2 inhibitor, malondialdehyde, melatonin, rat model

Human endometriosis is an estrogen-dependent condition characterized by implantation and growth of endometrial tissue outside the uterine cavity (1). Its cause remains unknown, but factors such as retrograde menstruation, heredity, impaired immune function, and environmental toxins have been implicated. Endometriosis is a multifactorial disease associated with a general inflammatory response in the peritoneal cavity. Oxidative stress has been proposed as a potential factor in the pathophysiology of the disease (2). Inducers of

oxidative stress may include erythrocytes, apoptotic endometrial cells, and undigested endometrial cells in the menstrual effluent (3). Activated macrophages induce oxidative stress, lipid peroxide formation, and other byproducts resulting from the interaction of apolipoproteins with peroxides. Peritoneal fluid (PF) volume in women with endometriosis is increased, and the content significantly differs from normal women (1).

The main reactive oxygen species (ROS) that must be considered are superoxide anion (O_2^-), which is predominantly generated by the mitochondria; hydrogen peroxide (H_2O_2), produced from O_2^- by the action of superoxide dismutase (SOD); and peroxynitrite ($ONOO^-$), formed when O_2^-

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couples with nitric oxide (NO). These continuously produced ROS are scavenged by superoxide dismutase (SOD) and catalase (CAT). Under some circumstances, these endogenous antioxidant defenses are likely to be perturbed due to overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue. Several studies have indicated that antioxidant defenses may be altered in endometriosis, as suggested by aberrant expression of endometrial antioxidant enzymes and lower levels of the antioxidant vitamin E in peritoneal fluid (4–7). Altered expression of these antioxidant enzymes may constitute further evidence for pronounced oxidative stress in endometriosis. Levels of these endogenous indexes of oxidative stress have been reported in the PF of infertile women (8).

Numerous studies have demonstrated that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues. Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of lipid peroxidation (LPO) mediated by oxidative stress (9).

Melatonin, or *N*-acetyl-5-methoxytryptamine, is an indole mainly produced in the mammalian pineal gland during the dark phase. Melatonin has been shown to possess anti-inflammatory effects, among a number of other actions. Melatonin reduces tissue destruction during inflammatory reactions by a number of means. Melatonin also has been shown to act as a potent antioxidant and free radical scavenger, protecting against a number of radical species in both in vivo and in vitro models of oxidative stress (10). Finally, there is indirect evidence that melatonin inhibits the production of adhesion molecules that promote the sticking of leukocytes to endothelial cells (11, 12). Thus, we hypothesize that it can be effective in inducing regression of endometriotic implants and reduction in LPO.

The autotransplantation of uterine pieces into the peritoneal cavity is a well-established method for induction of endometriosis in rats (13, 14). These autologous endometrial explants are similar to human lesions in vivo, and they react in a similar manner as human endometriotic tissues and cells in isolated cell cultures (15, 16). This rat model of endometriosis has served well in previous studies on pathophysiology of endometriosis and therapeutic modalities (13, 14).

Our study evaluated by immunohistochemistry the effect of melatonin on regression of endometriotic explants and the expression of cyclooxygenase-2 (COX-2) of endometriotic explants in a rat endometriosis model. In addition, effect of melatonin on ectopic endometrial tissue MDA levels and activities of CAT and SOD were investigated. To our knowledge, this experimental study is the first to test the effect of melatonin on regression and oxidative stress markers of endometriotic explants in a rat endometriosis model.

MATERIALS AND METHODS

Chemicals

All test materials were obtained from Sigma Chemical Inc. (St. Louis, MO); all organic solvents were obtained from Merck Chemical Inc. (Darmstadt, Germany). All reagents were of analytical grade. All reagents, except the phosphate buffers, were prepared daily before use and stored in a refrigerator at 4°C. The reagents were equilibrated at room temperature for 0.5 hour before use, when the analysis was initiated or reagent containers were refilled.

Animal Model

Twenty-five female Wistar albino rats (aged 10 to 12 weeks) weighing 180 to 200 g obtained from the Laboratory Animal Production Unit of Suleyman Demirel University were used in the experiment. The rats were sexually mature and had had at least three consecutive regular estrous cycles. The animals were procured, maintained, and used in accordance with the “Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals” prepared by Suleyman Demirel University’s Animal Ethics Committee. The study was approved by the institutional review board of Suleyman Demirel University. The rats were placed in a temperature (21 ± 2°C) and humidity (60 ± 5%) controlled room in which a 12 hour/12 hour light/dark cycle, which was maintained for 1 week before the start of the experiment. A commercially balanced diet (Hasyem Ltd., Isparta, Turkey) and tap water were provided ad libitum.

Experimental Design and Treatment

Endometriosis was surgically induced in the rats by transplanting an autologous fragment of uterine tissue onto the inner surface of the abdominal wall, as proposed by Vernon and Wilson (13) with modifications by Uygur et al. (17). Briefly, each rat was anesthetized with an intramuscular injection of ketamine (60 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 37°C, 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 a single nonabsorbable 6-0 polypropylene suture at the middle to the abdominal wall. The peritoneal cavity was kept moist with copious amounts of saline solution throughout the surgery. The abdominal incision was closed using 4-0 chromic gut. The operation was limited to 20 minutes for each rat to control the effect of room-air tissue drying. All of the operations were performed by the first author.

The animals were individually caged after the operation and were left for a recovery period of 4 weeks. To investigate

the success of the technique in inducing endometriosis, 4 weeks after autotransplantation of uterine tissues, three rats that were in estrous state were killed. The remaining 22 rats were given second-look laparotomy by the first author to identify and measure the ectopic uterine tissue in three dimensions (length \times width \times height in millimeters) using a caliper. The spherical volume of each ectopic uterine tissue was calculated using the prolate ellipsoid formula: V (mm³) = $0.52 \times A \times B \times C$, where A , B , and C denote width, length, and height, respectively (17). These rats were randomly divided into two groups. The first group was designated the control group ($n = 11$) and was given no medication except isotonic saline solution (an equal volume of melatonin) intraperitoneally for 4 weeks. Rats comprising group 2 ($n = 11$) were injected with 10 mg/kg each day of melatonin intraperitoneally for 4 weeks (18, 19). The melatonin was dissolved in 1:90 ethanol/saline for this procedure.

Starting 4 weeks after the second operation and lasting for 5 days, daily vaginal smears were performed. Four weeks after the beginning of the treatments (24 hours after the last dose of melatonin), a third laparotomy was performed, and all rats that were in estrus were killed by ketamine anesthesia. The sizes of the implants were measured again with the same caliber method by the same investigator, who was blinded to the groups.

Specimen Collection

The endometrial explants and the uteri were quickly excised and weighed (in milligrams). The endometrial explants were divided equally into two longitudinal sections. One half was placed in formaldehyde solution for routine histopathologic and immunohistochemical examination by light microscopy. The other half of the endometrial explant was washed with physiologic saline for biochemical analyses of MDA, CAT, and SOD; tissue samples then were suspended in 3 mL (pH 7.3) of Tris-HCl buffers containing 0.25 M sucrose, and were stored at -80°C until analysis.

Two hundred and fifty milligrams of endometrial explant tissue was homogenized in a motor-driven tissue homogenizer (IKA Ultra-Turrax T 25 Basic; Sigma-Aldrich, St. Louis, MO) with phosphate buffer (pH 7.4). Unbroken cells, cell debris, and nuclei were sedimented at $5000 \times g$ force for 30 minutes, and the supernatant was pipetted into plastic tubes and stored at -80°C until assayed.

Biochemical Analysis

Malondialdehyde determination Malondialdehyde levels, an indicator of free radical generation that increases at the end of the LPO, were estimated by the double heating method of Draper and Hadley (20). The principle of the method is a spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL of supernatant in each centrifuge tube, and the tubes were placed in a boiling water bath for

15 minutes. After cooling in tap water, the tubes were centrifuged at $1000 \times g$ for 10 minutes, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L TBA solution in a test tube. The tube was placed in a boiling water bath for 15 minutes. The solution was then cooled in tap water, and its absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 532-nm wavelength. The concentration of MDA was calculated by the absorbance coefficient of the MDA-TBA complex (absorbance coefficient = 1.56×10^5 cm/M) and was expressed as nanomoles per gram protein (nmol/g protein). In our study, the thiobarbituric-acid reaction method was used to determine lipid peroxidation. Although the method is not specific for lipid peroxidation, measurement of the thiobarbituric-acid reaction is an easy and reliable method, which is used as an indicator of lipid peroxidation and ROS activity in biological samples (9).

Superoxide dismutase activity determination Total (Cu-Zn and Mn) SOD activity was determined according to the method of Sun et al. (21) with a slight modification by Durak et al. (22). The principle of the method is based, briefly, on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 mL of ethanol/chloroform mixture (5/3, v/v) had been added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed as units per gram (U/g) protein.

Catalase activity determination Catalase activity was determined according to the method of Aebi (23). The principle of the assay is based on the determination of the rate constant k (dimension: s^{-1} , k) of hydrogen peroxide decomposition. By measuring the absorbance change per minute, the rate constant of the enzyme was determined. Activities were expressed as k (rate constant) per gram (k/g) protein. The protein content of the endometriosis tissues was determined using the method of Lowry et al. (24).

Histopathologic Analysis

The explants and the uteri were fixed in 10% neutral buffered formaldehyde solution. After dehydration procedures, the samples were blocked in paraffin, and 4- μm sections were cut by a microtome and stained with hematoxylin and eosin. Mounted slides were examined under a light microscope. The pathologist assessing the treatment effects was blinded to the treatment groups.

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. The persistence of epithelial cells in endometrial explants was evaluated semiquantitatively as follows: 3 = well-preserved epithelial layer; 2 = moderately preserved epithelium with leukocyte infiltrate; 1 = poorly preserved epithelium (occasional

epithelial cells only); and 0 = no epithelium. This evaluation was based on a previous rat endometriosis study (25).

Analysis of COX-2

Immunohistochemical analysis for COX-2 was performed on formalin-fixed, paraffin-embedded, archival tissue using the streptavidin-biotin-peroxidase technique. For all rats, a 4- μ m histologic section was deparaffinized in xylene and dehydrated in a descending dilution of ethanol. For the antigen retrieval, slides were treated by microwave heating in citrate buffer (pH 6.0) for 10 minutes. Endogenous peroxidase activity was blocked by 20 minutes of incubation with 0.3% hydrogen peroxidase. Slides were tested with COX-2 antibody (1:100 Epitope specific rabbit antibody; Lab Vision, Fremont, CA). Sections were tested with streptavidin-biotin-peroxidase kit (Ultra Vision Large Volume Detection System Anti-polyvalent, HRP; Lab Vision); after incubation, the reaction product was detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's hematoxylin, and mounted with mounting medium. Human colon cancer tissue served as the positive control in the COX-2 immunostaining.

Light microscopy analysis of all tissue sections was done by pathologist (N.K.) without prior knowledge of the rats' experimental treatment. Intensity of COX-2 in endometriosis epithelium was subjectively evaluated on a range from 0 (none) through 1 (faint) to 2 (strong) and 3 (very strong). Cases in which the intensity of staining was scored >2 were considered positive (26).

Statistical Analysis

Statistical analysis was performed on a personal computer by using SPSS, version 12.0 (SPSS Inc, Chicago, IL). Lilliefors-adjusted Kolmogorov-Smirnov test was used to test whether the variables used in the study were normally distributed. Data were expressed as mean \pm standard deviation (SD) for volume, weight, and histopathologic score (data were normally distributed); Bonferroni-adjusted Mann-Whitney *U* tests were used to analyze the data. Chi-square test was

used to analyze the distribution of the COX-2-positive rats. $P < .05$ was considered statistically significant.

RESULTS

All laparotomy sites were intact, and none of the animals had an incisional hernia. The general appearance of the rats and daily weights were observed. The treatment had no adverse effects on the general health or estrous cyclicity of the rats; no deaths resulted from the treatment in any of the groups.

Three rats were killed to provide morphologically cystic implants macroscopically. The average volume and weight of the explants were $161.2 \pm 41.3 \text{ mm}^3$ and $182.4 \pm 47.5 \text{ mg}$, respectively. Histologically, the epithelia of the cystic implants were found to be more preserved with a score of 3 for three samples.

Four weeks after the second laparotomy, all of the animals were killed to measure the volume and the weight of the endometrial explants in the third laparotomy. Statistical analysis within and between groups was performed. Pretreatment and posttreatment volumes in the control group were 135.9 ± 31.5 and 129.4 ± 28.7 , respectively ($P > .05$). In the melatonin group, the mean explant volume was 141.4 ± 34.4 before treatment and 42.9 ± 14.0 after 4 weeks of treatment ($P < .01$) (Table 1).

There was a statistically significant difference in the volume measured after the third laparotomy between the control and melatonin treatment groups ($129.4 \pm 28.7 \text{ mm}^3$ vs $42.9 \pm 14.0 \text{ mm}^3$, respectively; $P < .01$) (Table 2). The weight of the implants after the third laparotomy was statistically significantly different between groups: $155.8 \pm 27.1 \text{ mg}$ for the control group and $49.6 \pm 19.5 \text{ mg}$ for melatonin-treated group ($P < .01$).

Histologic Analysis

The eutopic endometria of the control and treatment groups were histologically similar (Fig. 1). The histopathologic finding of endometrial glands and stroma in the surgical site of implantation of endometrial squares allowed the diagnosis

TABLE 1

Comparison of the pretreatment volume and the posttreatment measurements of volume and weight in the control and melatonin-treated groups.

Group	Pretreatment	Posttreatment	
	Volume (mm^3)	Volume (mm^3)	Weight (mg)
Control (n = 11)	135.9 ± 31.5	129.4 ± 28.7	155.8 ± 27.1
Melatonin (n = 11)	141.4 ± 34.4^a	42.9 ± 14.01	49.6 ± 19.5^b

Note: Values are mean \pm standard deviation.
^a Mean $P < .01$, when compared with posttreatment.
^b Mean $P < .01$, when compared with controls.

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TABLE 2

Comparison of the posttreatment measurements of volume and histopathologic score in the control and melatonin-treated groups.

	Control group (n = 11)	Melatonin group (n = 11)	P value
Volume (mm ³)	129.4 ± 28.7	42.9 ± 14.01	< .01
Histologic score	2.54 ± 0.52	0.63 ± 0.50	< .01

Note: Values are mean ± standard deviation.

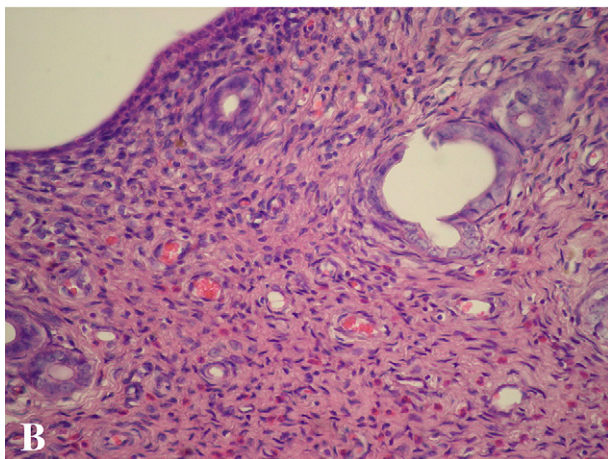
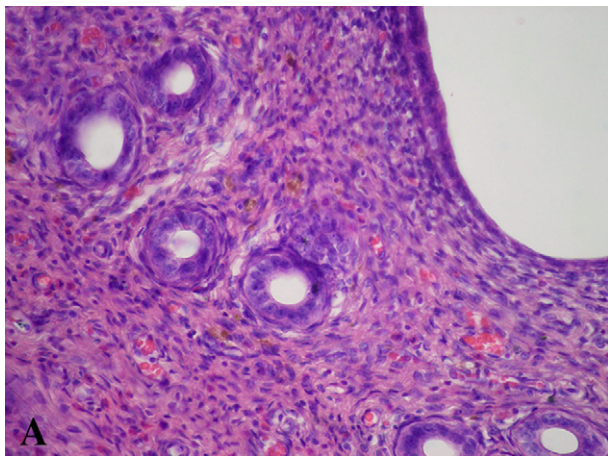
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of experimental endometriosis. Histologically, epithelia of the cystic implants in the control group were found to be more persistent when compared with the melatonin-treated group (Fig. 2). The semiquantitative analysis of the endometriotic explants after the third laparotomy found changes similar to those observed in the endometriotic explant vol-

ume and weights. Histologic evaluation of the uterine explants showed a statistically significantly lower score in the melatonin-treated group when compared with the control group (0.63 ± 0.50 vs 2.54 ± 0.52 , respectively, $P < .01$) (see Table 2).

FIGURE 1

Eutopic endometrium of the (A) control group and (B) melatonin-treated group. Stain: hematoxylin and eosin; magnification: $\times 100$.



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Analysis of COX-2

Immunostaining of COX-2 was observed in both nuclei and cytoplasm of endometriotic cells (Fig. 3). The COX-2 immunostaining was scored as positive in 10 out of 11 specimens (91%) from endometrial explants in the control rats, whereas the percentage of COX-2 positivity was 18.1% (two out of 11 specimens) in the melatonin-treated rats ($P < .01$).

Biochemical Analysis

The level of MDA and activities of CAT and SOD were measured in endometrial explants obtained during the third laparotomy. There were statistically significant reductions in the mean endometrial explants' MDA levels in rats treated with melatonin compared with mean MDA levels of the control group ($P < .01$). The activity of CAT and SOD in the rats treated with melatonin was statistically significantly higher than the mean activity found in rats in the control group ($P < .01$). Table 3 summarizes the results.

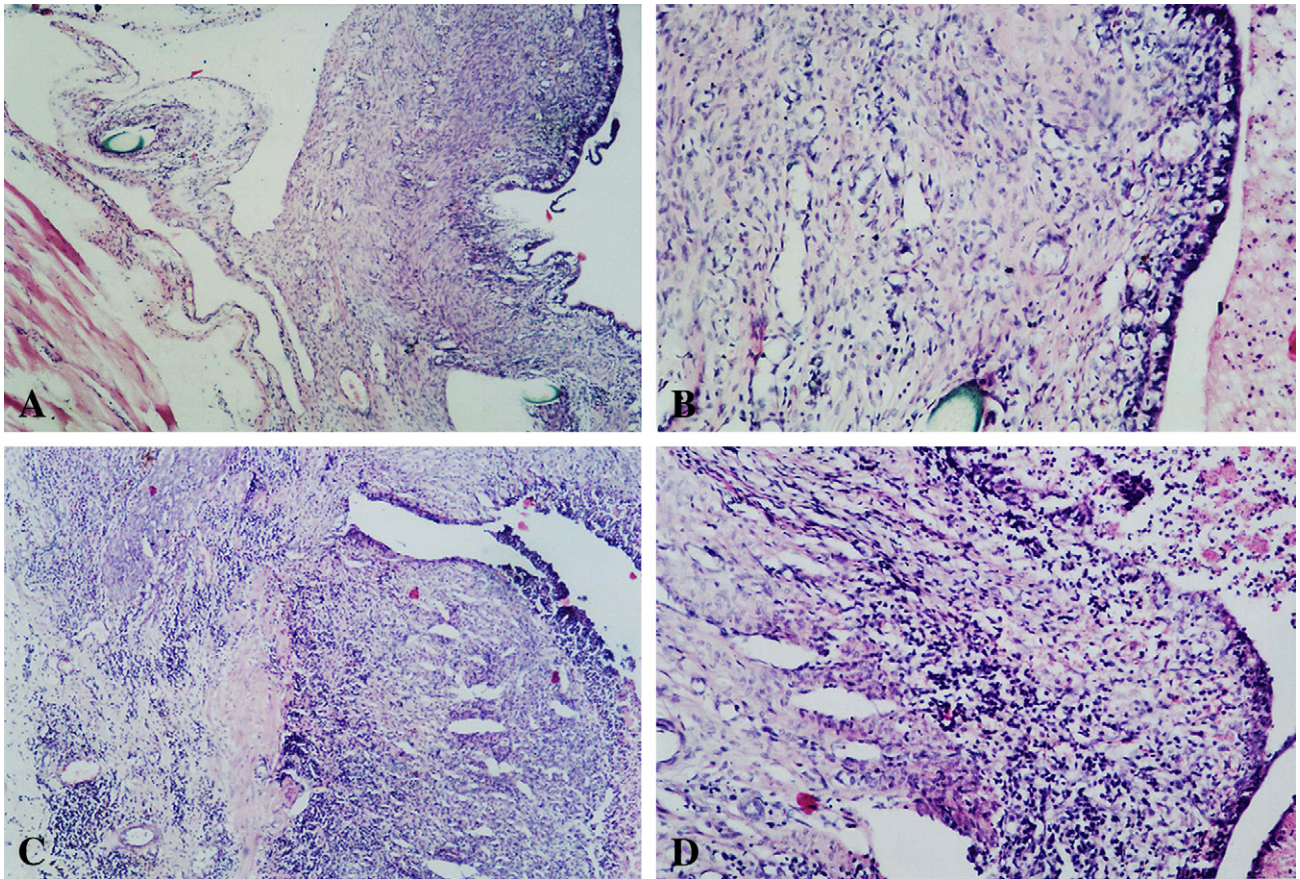
DISCUSSION

Medical treatments for endometriosis are usually aimed at reducing endogenous steroid production. Medroxyprogesterone acetate, danazol, oral contraceptives, and gonadotropin-releasing hormone agonist (GnRH-a) are effective for the pain-associated symptoms of endometriosis as well as the regression of the endometriotic lesions (27). Unfortunately, the adverse effects of these hormone treatments limit their long-term use. Besides, recurrence rates after cessation of therapy are high, and the treatments have had no benefit in endometriosis-associated infertility (28). Therefore, new medical treatments with an improved side effect profile are desired. It is expected that such treatments should be as effective as hormone treatments, with no negative effect on fertility.

Our study showed that treatment with melatonin effectively regressed endometriotic explants in a rat model.

FIGURE 2

Histologic analysis of the endometriotic explants showing striking differences in melatonin-treatment group compared with control. (A, B) Explant from a control rat indicating viable epithelial tissue at 8 weeks after the original implantation. (C, D) Histologic section of the site of the endometriotic explant from a rat treated with melatonin for 4 weeks showing absence of any viable endometrial epithelial tissue. Stain: (A–C) hematoxylin and eosin; magnification (A, C) $\times 40$, (B, D) $\times 100$.



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Furthermore, we observed that melatonin inhibited the decrease of antioxidant enzyme (SOD, CAT) levels in endometriotic explants of the rats and melatonin decreased LPO.

Zeller et al. (29) showed that production of ROS by PF mononuclear cells was increased in endometriosis. They concluded that large amounts of ROS were released after chronic stimulation of PF macrophages in women with endometriosis. Wang et al. (30) found similar levels of ROS in the PF of patients with endometriosis and disease-free controls. An increase in the oxidation of low-density lipoproteins in patients with endometriosis was demonstrated by Murphy et al. (31). The oxidation process appears to be induced via LPO and the subsequent release of aldehydes such as MDA (32).

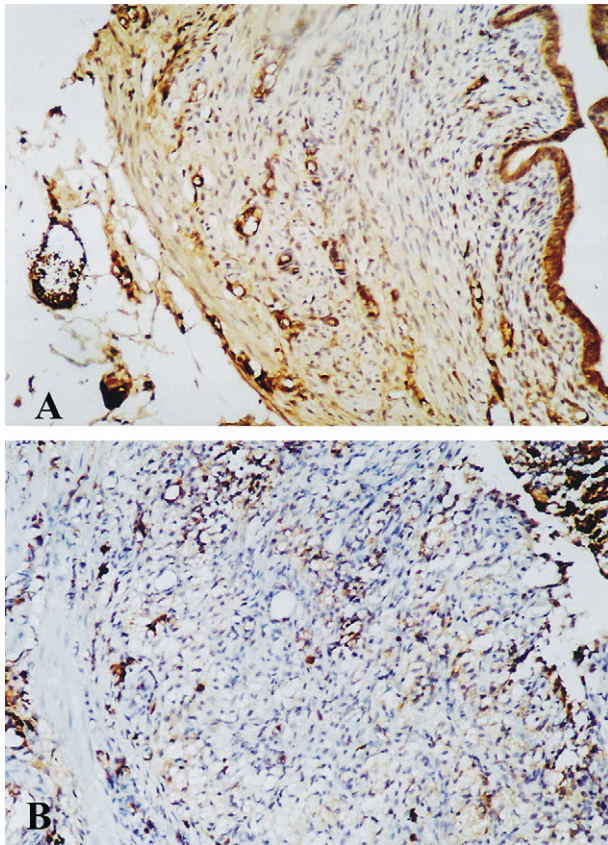
In our study, LPO was monitored by measuring MDA, which results from free radical damage to membrane components of the cells. There was a statistically significant in-

crease in MDA concentration in surgically induced endometriotic explants, which suggests the involvement of LPO induced by oxidative stress. Melatonin statistically significantly attenuated the increase of MDA concentration in endometrial explants. The increased MDA can be modulated by the likelihood fact that LPO can be induced by endometriosis itself or by a possible increase in ROS induced by endometriosis; or endometriosis can inhibit some antioxidant enzymes, and in turn LPO may increase due to reduction in the activities of these protective antioxidant enzymes. Thus, our study demonstrated that melatonin caused regression of the surgically induced endometriosis, which supports the hypothesis that superoxide radicals are involved in its pathogenesis.

Portz et al. (33) found that antioxidants protect against endometriosis. They showed that a combination of enzymes scavenge ROS-reduced endometrioma adhesions. Polak

FIGURE 3

COX-2 immunostaining in the endometriotic explants in (A) control group and (B) melatonin-treatment group. Magnification: $\times 100$.



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et al. (34) also demonstrated a lower total antioxidant potential in the PF of patients with idiopathic infertility. In our study, the decreased activities of antioxidant enzyme in the endometriotic explants after surgically induced endometriosis may have been related to oxidative stress in endometriotic tissue. The distribution of extracellular SOD in the

vessel walls of endometriotic tissue seems ideal for detoxifying superoxide anions produced in the sera of animals exposed to oxidative stress (35, 36). It is possible that endometriosis will affect the mitochondrial membranes, producing the large amounts of oxygen radicals that caused extreme use of SOD in rat endometrial explants in this study. Activation of COX-2 and infiltration of neutrophils may be other sources of ROS under our experimental conditions (37, 38).

Nevertheless, the decreased SOD and CAT activities together in the endometrial explants in our control rats may have been due to an overconsumption of these enzymes related to increased oxidative stress. The increased antioxidant enzyme activities may have been an ameliorating, adaptive response to the endometrial explant in the melatonin-treated rats. Therefore, the decreased enzyme activity in endometrial explants would indicate a high degree of oxidative stress, resulting in the increased endogenous H_2O_2 . There is evidence that melatonin in vitro directly scavenges OH, H_2O_2 , singlet oxygen, and inhibits LPO. Melatonin stimulates a number of antioxidative enzymes including SOD and CAT. Based on the similar proposed mechanism in other studies, we hypothesized that melatonin administration may reduce the volume and weight of endometrial explants in rat by its free-radical trapping activity.

Previous reports have suggested that an effective antioxidant therapy for patients with endometriosis would be helpful; significant clinical improvement has been demonstrated when antioxidants are given as adjuvant therapy (39, 40). Vitamin C and E therapy reduced pelvic pain in women with endometriosis (41). Thus, present results suggest that melatonin may be considered as a choice of adjuvant therapy to reduce oxidative stress and improve the patient outcome in endometriosis and associated diseases.

Another mechanism of action of melatonin is its inhibitory effect on prostaglandin production. Its protective effect was reported to be correlated with suppression of COX-2 expression and COX-2-derived prostaglandins in the inflamed tissues (42). Chishima et al. (43) demonstrated more frequent and denser COX-2 staining in ectopic endometriosis implants when compared with eutopic endometrium. Therefore,

TABLE 3

Levels of MDA and activities of antioxidant enzymes in ectopic endometrium in endometriosis in control and melatonin-treated groups.

	Control group (n = 11)	Melatonin group (n = 11)	P value
MDA (nmol/g protein)	4.70 \pm 0.33	3.56 \pm 0.32	< .01
CAT (k/g protein)	0.68 \pm 0.13	1.25 \pm 0.18	< .01
SOD (U/g protein)	59.63 \pm 15.86	104.0 \pm 17.1	< .01

Note: Values are mean \pm standard deviation.
CAT, catalase; MDA, malondialdehyde; SOD, superoxide dismutase.

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decreasing COX-2 enzyme activity in endometriotic tissues via a COX-2 inhibitor may be a new therapeutic strategy for endometriosis treatment. Our study showed that COX-2 overexpression was immunohistochemically detected in endometriotic lesions of rats and that melatonin decreased COX-2 enzyme activity of endometriotic implants. Therefore, another benefit of melatonin administration in endometriosis could be its inhibitory effect on expression of COX-2.

Secretory products of macrophages such as interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF- α) and growth factors are also found at increased concentration in the PF of women with endometriosis (44). From this point of view, new agents with the ability to inhibit TNF- α may be effective in the elimination of endometriosis. We found that melatonin treatment is effective on regression of established lesions.

Melatonin may reduce oxidative stress, volume, and weight of the endometrial explants. This is clearly supported in our study by the histologic, immunohistochemical, and biochemical findings showing morphologic degenerative changes with melatonin treatment. If confirmed in humans, this finding could be an important step toward a effective hormone treatment of endometriosis. Further experimental and clinical studies are required before this treatment can find a place in clinical practice.

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