Original Article

Effect of Estrogen, Progesterone, LH, and FSH on Oxidant and Antioxidant Parameters in Rat Uterine Tissue

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Abstract

Background: To attain whether the effects of low and high doses of estrogen, progesterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) in acute and chronic administration were related to oxidant and antioxidant parameters in rat uterine tissue as well as cyclooxygenase-1 (COX-1) activity.

Materials and Methods: Acute and chronic administration of estrogen (1 and 5 mg/kg), progesterone (1 and 5 mg/kg), LH (20 U/kg), and FSH (250 U/kg). A combination of mifepristone (50 mg/kg) with progesterone (5 mg/kg) and FSH (250 U/kg); and yohimbine (10 mg/kg) with estrogen (5 mg/kg) and LH (20 U/kg). Measurement total glutathione, nitric oxide levels and malondialdehyde, myeloperoxidase and COX-1 activities.

Results: Acute and chronic administration of progesterone 5 mg/kg and FSH 250 U/kg; and chronic administration of estrogen 1 mg/kg decreased antioxidants and increased oxidants. Combined administration of yohimbine with estrogen and LH showed the effect on these parameters.

Conclusion: While LH had a protective effect, low chronic dose estrogen caused oxidative stress. Because low doses could not stimulate alpha-2 receptors and it inhibited LH, an antioxidant hormone. High doses of estrogen that stimulated alpha-2 receptors showed a stable trend in oxidant and antioxidant levels in both acute and chronic administration. High doses of progesterone had an oxidant effect when it stimulated its own receptor in acute and chronic administration. In low acute and chronic doses, though progesterone could not stimulate its receptors but could inhibit FSH, it showed no effect. The oxidant effects of progesterone and FSH were blocked by mifepristone.

Keywords: Sex Hormones, Oxidant / Antioxidant Parameters, Cyclooxygenase-1, Uterine, Rat

Introduction

While female sex hormone secretion in physiologic levels is very important in the continued normal function of uterine tissue, chronic irregular secretion or oversecretion is detrimental. For example, the depletion of estrogen in mammals causes menopause (1). In this case hormone replacement therapy (HRT) is prescribed for women, which consists of estrogen and progesterone administration, especially during the postmenopausal term (2). Despite beneficial effects such as reducing the symptoms of menopause, it is well documented that HRT can cause an increased incidence of breast cancer, myocardial infarction, stroke, and deep vein thrombosis (3). Therefore, in recent years, studies have been conducted on endogenous and exogenous estrogens and progestins in the uterine tissue of various female species (4, 5).

Endometrial tissue is the most suitable tissue for egg implantation, as this tissue is built up as a result of estrogen and progesterone which are secreted during the ovarian cycle (6). Estrogens have a role in the development of the uterus during pregnancy and in stimulating contractile proteins.

Progesterone facilitates the precise beginning of a pregnancy, implantation of the embryo,

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and the maintenance of pregnancy (7). Hosie and Murphy observed in their experimental study that progesterone caused some negative changes in the mucosal epithelium of the uterus in ovariectomized rats (8). Another study showed that progesterone exerted a gastroprotective effect in a 1 mg/kg dose that not only stimulated its own receptor, but also inhibited follicle stimulating hormone (FSH) production; this study also found that progesterone in 2 and 5mg/kg doses and FSH in 250 and 500 U/kg doses were gastrotoxic (9). Borekci et al. indicated that acute administration of estrogen in ovariectomized rats had a significant anti-ulcerative effect only in the 5-mg/kg dose, but at lower doses, the anti-ulcerative effect was insignificant; these investigations demonstrated that estrogen and luteinizing hormone (LH) show their anti-ulcerative activity via alpha-2 adrenergic receptors (10).

Alpha-2 adrenergic receptors are involved in the cytoprotective, gastroprotective, and neuroprotective effects gastric and hippocampal tissues (11-13). These protective effects are antagonized by yohimbine, an alpha-2 adrenergic receptor blocker (13). Yohimbine is a selective inhibitor of alpha-2 adrenergic receptors (14). This indicates that a cytoprotective effect is observed via alpha-2 adrenergic receptors. In a previous study, we showed that the gastroprotective effect of estrogen and LH disappeared via yohimbine. In this experiment, we also reported that these hormones showed a gastroprotective effect via alpha-2 receptors, not the hormones' own receptors (10). In this investigation, by using yohimbine, we examined whether the effects of estrogen and LH on rat uterine tissue were showed via the alpha-2 receptors or not.

It was also reported that the toxic effects of progesterone and FSH were due to progesterone receptors (PR), and these toxic effects were inhibited by mifepristone, a PR inhibitor (9, 15). By using mifepristone, we investigated whether the effect of progesterone and FSH on rat uterine tissue was due to PR or not.

It was reported that oxidative stress plays a role in female reproduction system diseases such as polycystic ovarian disease and also in the pathogenesis of endometriosis and tubal factor infertility (16). A serial experiment was conducted to investigate if low and high doses of estrogen, progesterone, FSH, and LH in acute and chronic intake were related to oxidant and antioxidant parameters (total glutathione (GSH), nitric oxide (NO), malondialdehyde (MDA), and myeloperoxidase (MPO)) in rat uterine tissue, and to examine the effects of these sex hormones on the cyclooxygenase-1 (COX-1) enzyme level.

Materials and Methods

Animals

A total of 102 female Wistar albino rats weighing 180-200g were obtained from the Ataturk University Medicinal and Experimental Application and Research Center for use in this study. The animals were allocated to treatment groups before experimental procedures were initiated. The animals were housed and fed under standard conditions in a laboratory where the temperature was kept at 22°C. The rats were four and five months old. The experiments were conducted in accordance with the National Guidelines for the Use and Care of Laboratory Animals and were approved by the local Animal Care Committee of Ataturk University.

Chemicals

Progesterone (P 3277), estrogen (E 1024), FSH (F 2293), LH (L 9773), yohimbine (Y 3125), and mifepristone (M 8046) were purchased from SIGMA, Germany.

The acute (single-dose) administration of progesterone, estrogen, FSH, and LH on the uterine tissues of rats on oxidant and antioxidant parameters

In this series of experiments, progesterone (1 and 5 mg/kg) and estrogen (1 and 5 mg/kg) were applied to defined rat groups by oral gavage (p.o.). In addition, FSH (250 U/kg) and LH (20 U/kg) were intraperitoneally (i.p.) injected to other rat groups. Distilled water was given to the intact control group as a vehicle. Six hours after drug administration, the rats were euthanized by an overdose of a general anesthetic (thiopental sodium, 50 mg/kg). The rats' uterine tissues were removed and sent to the biochemistry laboratory for measurement of GSH and NO levels, as well as MPO, MDA, and COX-1 activity. A breakdown of the groups and treatments is listed below:

Group 1: 1 mg/kg estrogen (p.o., single dose)
Group 2: 5 mg/kg estrogen (p.o., single dose)
Group 3: 1 mg/kg progesterone (p.o., single dose)
Group 4: 5 mg/kg progesterone (p.o., single dose)
Group 5: 250 U/kg FSH (i.p., single dose)
Group 6: 20 U/kg LH (i.p., single dose)
Group 7: distilled water (p.o., single dose)

The chronic administration of progesterone, estrogen, FSH, and LH on uterine tissues of rats on oxidant and antioxidant parameters

In this series of experiments, daily administration of progesterone, estrogen, FSH, and LH were given to another six groups of rats in the doses as defined below, for ten days. Six hours after the last dose was administered, the rats' uterine tissues were removed according to the previously mentioned procedure and sent to the biochemistry laboratory for measurement of GSH and NO levels, as well as MPO, MDA, and COX-1 activity. A breakdown of the groups and treatments is listed below:

Group 1: 1 mg/kg estrogen (p.o., 10 doses, daily)
Group 2: 5 mg/kg estrogen (p.o., 10 doses, daily)
Group 3: 1 mg/kg progesterone (p.o., 10 doses, daily)
Group 4: 5 mg/kg progesterone (p.o., 10 doses, daily)
Group 5: 250 U/kg FSH (i.p., 10 doses, daily)
Group 6: 20 U/kg LH (i.p., 10 doses, daily)
Group 7: distilled water (p.o., 10 doses, daily)

The effects of FSH and progesterone on yohimbine-treated rats' uterine tissues, and the effect of estrogen and LH on mifepristone-treated rats' uterine tissues in terms of oxidant and antioxidant parameters

In this experiment, mifepristone (50 mg/kg) was injected i.p. into the first and second rat groups, and yohimbine (10 mg/kg) was injected i.p. into the third and fourth groups. Thirty minutes after mifepristone injection, progesterone (5 mg/kg, p.o.) and FSH (250 U/kg, i.p.) were administered to the first two groups. Thirty minutes after yohimbine injection, estrogen (5 mg/kg, p.o.) was administered orally, and LH (20 U/kg, i.p.) was administrated intra-peritoneally to the last two groups, as well. Six hours after hormone treatment (progesterone, estrogen, FSH, and LH), the rats' uterine tissues were removed according to the same procedure and sent to the biochemistry laboratory for measurement of GSH and NO levels, as well as MPO, MDA, and COX-1 activity. A breakdown of the groups and treatments is listed below:

Group 1: 5 mg/kg progesterone (p.o.) + 50 mg/kg mifepristone (i.p.)
Group 2: 250 U/kg FSH (i.p.) + 50 mg/kg mifepristone (i.p.)
Group 3: 5 mg/kg estrogen (p.o.) + 10 mg/kg yohimbine (i.p.)
Group 4: 20 U/kg LH (i.p.) + 10 mg/kg yohimbine (i.p.)
Group 5: distilled water

Biochemical Analyses

Biochemical investigation of rats' uterine tissues

GSH and NO levels in addition to MPO and MDA enzyme activities in rat uterine tissues were determined. To prepare the tissue homogenates, uterine tissues were macerated with liquid nitrogen in a mortar. The tissues (0.5 g each) were then treated with 4.5 mL of the appropriate buffer. The mixtures were homogenized on ice using an Ultra-Turrax homogenizer for 15 minutes. Subsequently, these supernatants were used for enzymatic activity determination. All assays were conducted at room temperature in triplicate.

Determination of total GSH

The amount of GSH in uterine mucosa was measured according to Sedlak and Lindsay's method (17). The sample was weighed and homogenized in 2 mL of 50 mM Tris–HCl buffer containing 20 mM EDTA and 0.2 mM sucrose at pH 7.5. The homogenate was immediately precipitated with 0.1 mL of 25% trichloroacetic acid, and the precipitate was removed after centrifugation at 4200 rpm for 40 minutes at 4 °C. The supernatant was used to determine GSH using 5,5'-dithiobis (2-nitrobenzoic acid). Absorbance was measured at 412 nm using a spectrophotometer. The results of the GSH level in uterine mucosa were expressed as nanomoles per milligram of tissue (nmol/mg tissue).

Determination of NO

Tissue NO levels were measured as total nitrite plus nitrate levels by using the Griess reagent in Bories and Bories and Moshage et al. method "after" as previously described (18, 19). The Griess reagent consists of sulfanilamide and N-(1-napthyl)-ethylenediamine. The method is based on a two-step process, of which the first step is the conversion of nitrate into nitrite using a nitrate reductase. In the second step, the Griess reagent is added which converts nitrite into a deep purple azo compound; Photometric absorbance at 540 nm of the azo compound can accurately determine nitrite concentrations. NO levels were expressed as μmol/g wet tissue.

Determination of MPO activity

MPO activity was measured according to Bradley et al. modified method (20). The homogenized samples were frozen and thawed three times and subsequently centrifuged at 1500 g for

10 minutes at 4 °C. MPO activity in the supernatant was determined by adding 100 mL of the supernatant to 1.9 mL of 10 mmol/L phosphate buffer (pH 6.0) and 1 mL of 1.5 mmol/L o-dianisidine hydrochloride containing 0.0005% (wt/vol) hydrogen peroxide. The changes in absorbance at 450 nm of each sample were recorded on a UV–vis spectrophotometer. The MPO activity in uterine tissues was expressed as micromoles per minute per milligram of tissue (mmol/min/mg tissue).

Determination of lipid peroxidation or MDA formation

The concentrations of uterine tissue lipid peroxidation were determined by estimating MDA by using the thiobarbituric acid test (21). The rats' uterine tissues were promptly excised and rinsed with cold saline. To minimize the possibility of hemoglobin interference with free radicals, any blood adhering to the mucosa was carefully removed. The uterine tissue was weighed, and homogenized in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added to a solution containing 0.2 mL of 80 g/L sodium lauryl sulfate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate, and 0.3 mL distilled water. The mixture was incubated at 98 °C for 1 hour. After the mixture had cooled, 5 mL of n-butanol:pyridine (15 : l) was added. The mixture was vortexed for 1 minute and centrifuged for 30 minutes at 4000 rpm. The absorbance of the supernatant was measured at 532 nm. The standard curve was obtained by using 1, 1, 3, 3-tetramethoxypropane. The recovery was greater than 90%. The results were expressed as nanomoles of MDA per gram of wet tissue (nmol/mg tissue).

Determination of COX activity

For this part of our experiment, COX activity of the rats' uterine tissues was measured via a COX activity assay kit (Cayman, Ann Arbor, MI, USA). Uterine tissues were collected and washed thoroughly with ice-cold Tris buffer, pH 7.4, containing 0.16 mg/ml of heparin to remove any red blood cells and clots. A sample of uterine tissue was homogenized in 5 ml of cold buffer (0.1 M Tris-HCl, pH 7.8, containing 1mM EDTA) per gram of tissue and centrifuged at 10,000xg for 15 minutes at 4°C. The supernatant was removed for the assay and stored on ice. Protein concentration in the supernatant was measured by the Bradford method (22). The COX kit measured the peroxidase activity of COX. The peroxidase activity was assayed colorimetrically by monitoring the appearance of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine at 590 nm. Results are given as units per milligram of protein for COX-1 activity.

Statistical analyses

The enzyme activity data were subjected to one-way ANOVA using SPSS software (Version 13.0, Chicago, IL). Differences among groups were attained using the least significant difference (LSD) option, and significance was declared at $p<0.05$.

Results

Acute administration of progesterone, estrogen, FSH, and LH in rats' uterine tissues

As can be seen in Table 1, low doses (1 mg/kg) of neither progesterone nor estrogen exerted significant effects on oxidant and antioxidant parameters when compared to the intact control group.

Table 1: The effect of acute (single dose) administration of progesterone, estrogen, FSH and LH in uterine tissue of rats on oxidant and anti oxidant parameters (n=6 per group).

Drugs	Dose	GSH (nmol/mg tissue)	NO (µmol/g wet tissue)	MPO (mmol/min/mg tissue)	MDA (nmol/mg tissue)
Estrogen	1 mg kg^{-1}	6.4 ± 1.4	68.5 ± 11.6	5.2 ± 0.9	2.3 ± 0.8
Estrogen	5 mg kg^{-1}	7.2 ± 0.8	69.9 ± 6.1	4.1 ± 1.2	1.9 ± 0.8
Progesterone	1 mg kg^{-1}	7.1 ± 1.1	69.2 ± 6.5	5.6 ± 1.7	2.8 ± 0.9
Progesterone	5 mg kg^{-1}	2.3 ± 0.5**	32.3 ± 7.4**	9.1 ± 0.8**	4.4 ± 0.7**
FSH	250 U kg^{-1}	1.6 ± 0.4**	22.5 ± 7.3**	12.2 ± 2.5**	5.2 ± 0.7**
LH	20 U kg^{-1}	7.4 ± 0.9	72.0 ± 6.1	3.6 ± 0.7*	1.7 ± 0.8
Intact (Healthy)	-	6.8 ± 0.4	70.6 ± 4.3	5.4 ± 0.8	2.5 ± 0.5

GSH: Reduced glutathione; NO: Nitric oxide; MPO: Myeloperoxidase; MDA: Malondialdehyde; FSH: Follicle stimulating hormone; LH: Luteinizing hormone.
The same experiment has been performed three times. Data determined are expressed as Mean ± SEM of three measurements. All groups were statistically compared with intact group. * Means significant at <0.05 and ** Means significant at <0.0001.

However in the group that received a 5 mg/kg dose of progesterone, GSH and NO levels were reduced, whereas MPO and MDA levels increased significantly in comparison with the control group. In rats receiving the 5 mg/kg dose of estrogen no change was observed in oxidant and antioxidant parameters versus the control group. FSH was found to increase the levels of oxidant parameters (MPO and MDA) and decrease that of antioxidant parameters (GSH and NO). Acute administration of 20 U/kg LH exerted a significant decrease only in MPO levels.

Chronic administration of progesterone, estrogen, FSH, and LH in rats' uterine tissues

As can be concluded from the data in Table 2, rats that received 1mg/kg estrogen demonstrated reduced GSH and NO levels and increased MPO and MDA levels versus the control group. Both 5 mg/kg estrogen and 1 mg/kg progesterone did not exert significant effects on oxidant and antioxidant parameters when compared to the intact control group. In the group receiving a 5-mg/kg dose of progesterone, the GSH and NO levels were reduced, whereas the MPO and MDA levels increased significantly in comparison with the control group. FSH was found to increase the levels of oxidant parameters (MPO and MDA) and decrease that of antioxidant parameters (GSH and NO). LH did not change these parameters except for MPO. Administration of 20 U/kg LH reduced the MPO level versus the control group.

Table 2: The effect of chronic administration of progesterone, estrogen, FSH and LH in uterine tissue of rats on oxidant and anti oxidant parameters (n=6 per group).

Drugs	Dose	GSH (nmol/mg tissue)	NO (µmol/g wet tissue)	MPO (mmol/min/mg tissue)	MDA (nmol/mg tissue)
Estrogen	1 mg kg^{-1}	4.0 ± 0.9**	34.2 ± 6.3**	7.7 ± 0.8*	4.5 ± 1.0**
Estrogen	5 mg kg^{-1}	6.4 ± 0.9	72.0 ± 11.1	4.8 ± 0.6	2.7 ± 0.8
Progesterone	1 mg kg^{-1}	7.2 ± 0.7	73.5 ± 6.5	4.9 ± 0.8	2.3 ± 0.8
Progesterone	5 mg kg^{-1}	2.6 ± 0.5**	30.8 ± 5.1**	8.1 ± 0.9**	4.6 ± 1.1**
FSH	250 U kg^{-1}	2.2 ± 0.8**	25.3 ± 6.6**	9.8 ± 1.5**	5.0 ± 0.6**
LH	20 U kg^{-1}	7.7 ± 0.6	75.1 ± 5.3	3.7 ± 0.8*	2.1 ± 0.6
Intact (Healthy)	-	6.8 ± 0.4	70.6 ± 4.3	5.4 ± 0.8	2.5 ± 0.5

GSH: Reduced glutathione; NO: Nitric oxide; MPO: Myeloperoxidase; MDA: Malondialdehyde; FSH: Follicle stimulating hormone; LH: Luteinizing hormone.
The same experiment has been performed three times. Data determined are expressed as Mean ± SEM of three measurements. All groups were statistically compared with intact group. * Means significant at <0.05 and ** means significant at <0.0001.

Table 3: The effects of FSH and estrogen on uterine tissues of yohimbine administered rats as compared with the effects of progesterone and LH on uterine tissues of mifepristone administered rats on oxidant and anti-oxidant parameters (n = 6 per group)

Drugs	Dose	GSH (nmol/mg tissue)	NO (µmol/g wet tissue)	MPO (mmol/min/mg tissue)	MDA (nmol/mg tissue)
Estrogen + Yohimbine	5 mg kg^{-1} + 10 mg kg^{-1}	2.9 ± 0.3*	34.7 ± 2.2*	8.3 ± 0.3*	4.4 ± 0.5*
Progesterone + Mifepristone	5 mg kg^{-1} + 50 mg kg^{-1}	6.4 ± 0.4	64.1 ± 2.4	4.4 ± 0.3	2.3 ± 0.4
FSH + Mifepristone	250 U/kg + 50 mg/kg	6.1 ± 0.4	61.4 ± 2.6	4.7 ± 0.4	2.2 ± 0.2
LH + Yohimbine	20 U/kg + 10 mg/kg	3.5 ± 0.3*	36.7 ± 1.7*	7.7 ± 0.5*	3.9 ± 0.5*
Intact (Healthy)	-	6.8 ± 0.4	70.6 ± 4.3	5.4 ± 0.8	2.5 ± 0.5

GSH: Reduced glutathione; NO: Nitric oxide; MPO: Myeloperoxidase; MDA: Malondialdehyde; FSH: Follicle stimulating hormone; LH: Luteinizing hormone.
The same experiment has been performed three times. Data determined are expressed as Mean ± SEM of three measurements. All groups were statistically compared with intact group. * Means significant at <0.05 and ** Means significant at <0.0001.

The effect of progesterone and FSH on mifepristone-treated rats' uterine tissues and the effect of estrogen and LH on yohimbine-treated rats' uterine tissues

In the uterine tissues of the mifepristone-treated rats, progesterone (5 mg/kg) and FSH (250 U/kg) did not cause a significant difference in comparison with the control group on oxidant and antioxidant parameters. In the yohimbine-treated rat uterine tissues, estrogen and LH significantly reduced GSH and NO levels whereas it increased MPO and MDA activities (Table 3).

Effect of progesterone, estrogen, FSH, and LH on COX-1 levels in rat uterine tissues

In the group of rats that received a 5 mg/kg dose of acute and chronic progesterone and 250 U/kg acute and chronic FSH, COX-1 activity was reduced significantly in comparison with the control group. This reduction was also observed in the chronic 1-mg/kg estrogen group. In combined administration, the estrogen + yohimbine and LH + yohimbine groups displayed significantly diminished COX-1 activity in comparison with the control group (Table 4).

Table 4: COX-1 activity of progesterone, estrogen, FSH and LH in acute and chronic administration and in combined administration of FSH and estrogen with yohimbine and progesterone and LH with mifepristone in rat uterine tissue (n=6 per group).

Drugs	Dose	COX-1 activity (u/mg protein)
In acute administration		
Estrogen	1 mg kg^{-1}	459.6 ± 100.5
Estrogen	5 mg kg^{-1}	508.0 ± 56.4
Progesterone	1 mg kg^{-1}	433.8 ± 63.6
Progesterone	5 mg kg^{-1}	166.4 ± 36.1**
FSH	250 U kg^{-1}	112.2 ± 28.0**
LH	20 U kg^{-1}	530.3 ± 64.4
In chronic administration		
Estrogen	1 mg kg^{-1}	283.8 ± 63.8**
Estrogen	5 mg kg^{-1}	455.2 ± 64.0
Progesterone	1 mg kg^{-1}	513.4 ± 49.9
Progesterone	5 mg kg^{-1}	185.6 ± 35.7**
FSH	250 U kg^{-1}	153.2 ± 55.7**
LH	20 U kg^{-1}	548.4 ± 42.7
In combined administration		
Estrogen + Yohimbine	5 mg kg^{-1} + 10mg kg^{-1}	208.9 ± 21.6*
Progesterone + Mifepristone	5 mg kg^{-1} + 50mg kg^{-1}	455.2 ± 28.45
FSH + Mipepristone	250 U/kg + 50 mg/kg	438.7 ± 28.7
LH + Yohimbine	20 U/kg + 10 mg/kg	246.2 ± 21.1*
Intact (Healthy)	-	482.6 ± 28.3

GSH: Reduced glutathione; NO: Nitric oxide; MPO: Myeloperoxidase; MDA: Malondialdehyde; FSH: Follicle stimulating hormone; LH: Luteinizing hormone.
The same experiment has been performed three times. Data determined are expressed as Mean ± SEM of three measurements. All groups were statistically compared with intact group. * Means significant at <0.05 and ** Means significant at <0.0001.

Discussion

This study investigated whether or not the effects of low and high doses of estrogen, progesterone, LH, and FSH in acute and chronic administration were related to oxidant and antioxidant parameters in the uterine tissue of rats. In addition, the effects of acute and chronic administration of these hormones on COX-1 activity were measured.

A decrease in ovarian hormones is an important oxidative stress source (23, 24). Reactive oxygen species (ROS) cause an imbalance between oxidants and antioxidants; this imbalance reveals that oxidants are a predominant cause of damage via oxidative stress in the epithelial cell spaces (25). Clinical hormone replacement therapy can be performed by estrogen and progesterone administration for hormone depletion, which is noted in the postmenopausal term (2). However, the literature has shown that this therapy can have both harmful (26, 27) and beneficial effects (28, 29). The beneficial effects are a result of its antioxidant characteristics, which originate from its phenolic structure (30). In both human and animal investigations, it has been observed that estrogen therapy reduced oxidative stress, which was apparent in ovariectomy, and for this reason, estrogen was described as an "antioxidant" (31, 32). It also supported the theory that estrogen increased antioxidant enzyme expression (33). In a study performed on gastric tissue, it was demonstrated that chronic estrogen (1 mg/kg) removed the gastroprotective effect of LH by inhibiting it; in higher doses, acute and chronic administration of this hormone caused a protective effect via alpha-2 receptor stimulation (10). In this study, when estrogen was given in acute low and high doses, no alterations were observed in GSH and NO levels. In low dose (1 mg/kg) chronic administration, estrogen reduced GSH and NO levels and the 5 mg/kg dosage treatment prevented the reduction in these antioxidant parameters in uterine tissue. Also in this study LH, whose secretion is closely related to estrogen, protected uterine tissue. These results might suggest that, when 1 mg/kg estrogen was given chronically, the protective effect of LH was abolished by inhibiting its secretion. Also, in a previous study, when 1 mg/kg estrogen was administrated chronically the antioxidant effect of LH was abolished by the same manner (34). Conversely, administration of high-dose estrogen with the alpha-2 blocker yohimbine significantly decreased GSH and NO levels. Namely, yohimbine negated the protective effect of high-dose estrogen. Therefore, high-dose estrogen may be gastro-protective via alpha-2 adrenergic receptors. It is known that estrogen shows its effect via both genomic (by bounding its receptor) and non-genomic (without bounding its receptor) mechanisms (35, 36). It has been reported that estrogen can cause an antioxidant effect by non-genomic mechanisms (37, 38). GSH and other antioxidant substances (melatonin, vitamins) prevent tissue damage by stabilizing levels of ROS in normal concentration in cells (39, 40). While a reduction in the GSH level causes damage in tissues, an increase causes protective effects (41). NO regulates mucosal blood flow (42) and protects membrane lipids from peroxidation (43). It was observed that NO and NO releasing substances such as glycerin trinitrate and nitroprusside prevented mucosal damage and accelerated healing (44).

Toxic oxygen radicals that are formed highly in tissues exposed to oxidative stress stimulate lipid peroxidation, which causes MDA formation (45). MDA is used as an oxidative stress marker (25). An increased MPO level is accepted as a messenger of neutrophil infiltration in various tissues experimentally and is used as a model for research (46). An increase in the MPO level shows a parallel to tissue damage (41). In this investigation, administration of acute low and high doses of estrogen did not cause a significant alteration in the MDA and MPO levels. In low dose (1 mg/kg) chronic administration, estrogen increased MDA and MPO levels and the 5 mg/kg high dose treatment prevented an increase in these oxidant parameters in uterine tissue. Also, these results may support our hypothesis that 1 mg/kg dose of estrogen destroys uterine tissue via inhibiting LH secretion. However, a combination of estrogen and yohimbine significantly enhanced the levels of these oxidants.

COX-1 is an enzyme that is widely produced in the uterus and has an important role in beneficial actions such as regulation of blood flow and maintenance of mucosal integrity (47, 48). Our study showed no changes in COX-1 activity in response to acute and chronic administrations of high-dose estrogen. COX-1 activity was attenuated with a combination of estrogen and yohimbine. Our data suggest that a reduction in COX-1 activity can be evaluated as an indicator of tissue damage.

Hosie et al. have pointed out that, in ovariectomized animals, progesterone causes some negative changes in the surface morphology of uterine mucosal epithelia (8). We also observed

that, when progesterone was administered to rats in acute and chronic high doses, it diminished GSH and NO levels and significantly increased MPO and MDA levels. In low doses, it did not affect the levels of these antioxidants and oxidants. When high dose progesterone was combined with mifepristone, a progesterone receptor antagonist, the antioxidant and oxidant levels did not change. This outcome might lead us to consider that progesterone caused a decrease in antioxidant levels by stimulating progesterone receptors in high doses. A study has mentioned that low doses of progesterone produced gastroprotective effects by inhibiting FSH (10). Also in uterine tissue, chronic application of low-dose progesterone protected the uterus from a decrease in antioxidant levels by inhibiting FSH. Low-dose single acute progesterone administration did not affect FSH and did not stimulate the progesterone receptor. For this reason, this acute administration did not affect these parameters either negatively or positively. Progesterone doses that affected oxidant and antioxidant levels changed COX-1 activity, too.

In this investigation, in acute and chronic applications, FSH minimized GSH and NO levels and enhanced MDA and MPO levels. The damaging feature of FSH on uterine tissue supported the idea that progesterone caused protective characteristics by inhibiting FSH. This damaging feature of FSH was sublimated when combined with mifepristone. Changes in COX-1 activity have a parallel to the data provided above.

Results have shown that, opposite to FSH, LH blocked reduction of antioxidant activity in acute and chronic administration. In acute and chronic applications, LH significantly decreased MPO activity. The inhibitor effect of LH on MDA was insignificant statistically. LH could not prevent a decrease in antioxidants and an increase in oxidants in the rats that received yohimbine.

Conclusion

LH had a protective effect on antioxidant and oxidant levels on uterine tissue. Estrogen caused oxidative stress on uterine tissue when given in low doses chronically that could not stimulate alpha-2 receptors and inhibited LH, an antioxidant hormone. High doses of estrogen that stimulated alpha-2 receptors showed a stable trend in oxidant and antioxidant levels in both acute and chronic administration. High doses of progesterone had an oxidant effect when it stimulated its own receptor in both acute and chronic administration. Yet, in low acute and chronic doses, though progesterone could not stimulate its receptors but could inhibit FSH, there was no effect on the oxidant and antioxidant levels. The oxidant effects of progesterone and FSH were blocked by mifepristone.

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