ORIGINAL RESEARCH

Effects of estrogens on oxidative protein damage in plasma and tissues in ovariectomised rats

Topçuoglu A¹

Uzun H²

Balci H³

Karakus M⁴

Çoban I⁵

Altug T⁵

Aydin S²

Topçuoglu D⁴

Çakatay U⁶

¹ Department of Obstetrics and Gynecology, Izzet Baysal University, Faculty of Medicine, Bolu, Turkey.

² Department of Biochemistry,

³ Fikret Biyal Central Research Laboratory,

⁴ Department of Obstetrics and Gynecology,

⁵ Experimental Animal-Research and Breeding Laboratory, Cerrahpasa Medical Faculty,

⁶ Central Laboratory of Clinical Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Turkey.

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Abstract

Purpose: To assess estrogen-related changes in the redox status of the brain and liver proteins as well as the systemic oxidative stress in ovariectomised (OVX) rats

Methods: Twelve-week-old, sexually mature female Sprague-Dawley rats (200-250g) were randomly divided into four groups: The following treatment combinations were administrated daily to all in 0.05 ml 96% ethanol solution by gastric gavage. (1) Sham operation (2) OVX rats (3) OVX rats [0.02 mg/kg/day of 17 β -estradiol (E2) and 0.01 mg/kg/day of norethisterone acetate] (4) OVX rats [E2 (0.01 mg/kg/day) and drospirenon (0.02 mg/kg/day)]. Estrogen levels were determined using routine clinical-chemistry methods. We also measured protein oxidation parameters such as protein carbonyl (PCO), total thiol (T-SH) and the other oxidative stress markers malondialdehyde (MDA) and glutathione (GSH).

Results: Ovariectomy resulted in abnormal elevation of plasma and tissue oxidative stress markers and changes in redox status of the proteins in tissue dependent manner. Supplementation of various estrogens combinations partially alleviated these abnormalities and restored redox homeostasis of proteins after the ovariectomy. Among the

studied protein oxidation parameters, plasma and tissue PCO levels of the OVX rats were higher than those of the control groups ($P<0.01$). Hormone replacement therapies (HRT) caused a decrease in PCO and MDA in both plasma and tissue of the OVX rats ($P<0.01$). HRT in OVX rats decreased plasma MDA and increased liver and brain GSH ($P<0.01$). Liver MDA levels of the Drospirenon-treated rats were lower than in the norethisterone acetate group ($P<0.01$). On the other hand, Drospirenon increases brain GSH s more effectively than norethisterone acetate ($P<0.01$). After bilateral oophorectomy, plasma and tissue T-SH levels decreased in the OVX group compared with control ($P<0.01$). Norethisterone acetate increased plasma T-SH more effectively than Drospirenon ($P<0.05$)

Conclusions: The study showed the extent of oxidative protein damage (OPD) in this model of estrogen deficiency. The protective effect of estrogens against tissue specific OPD suggests that estrogens play an important role within the antioxidant defense systems in plasma, liver and brain. The exact molecular mechanisms leading to these findings are not yet completely known. Meanwhile, hormone replacement therapy for the prevention of OPD in a tissue specific manner may be required.

Aging is the progressive accumulation of oxidative changes with time that are associated with the reduction of antioxidant defense and increase in the extent of oxidative protein damage (OPD).^{1,2} Menopause is a natural condition of aging and can also occur when the ovaries are surgically removed.³

It is well known that various estrogens scavenge reactive oxygen species (ROS) efficiently in both aqueous and lipophilic cellular components. Estrogens, acting as free radical scavengers, break the free radical chain formation produced from membrane oxidation processes and hence inhibit lipid and protein oxidation.^{4,5} In support of this, it has been postulated that estrogens may be able to sequester metal ions or donate a proton to reduce peroxy-free radicals.⁶ Although the exact mechanisms involved in the antioxidant properties of estrogens have not been fully defined, a number of hypotheses have been put forth.^{6,7} The antioxidant activity of estrogens depends not only on the hydrophilic or lipophilic nature of the scavenged radical, but also phenolic structure of estrogens.⁶ Estrogens may also influence the activity of cellular antioxidant enzyme systems, but the exact antioxidant role in antioxidant defense remains controversial.⁷ It is well known that estrogen administration increases the risk of endometrial cancer during and after treatment, unless it is taken with adequate progesterone.⁸ Norethisterone acetate is a 19-nortestosterone-derived progestin, with potent effects on minimizing estrogen-induced endometrial stimulation, even at very low doses.⁹ Drospirenon is the first synthetic progestin with antimineralocorticoid activity, has a similar effect to progesterone itself and is structurally related to 17 α -spiro lactone used in contraception and in post-menopausal hormone replacement therapy (HRT). Drospirenon has anti-mineralocorticoid activity in the kidney as reflected by elevated natriuresis.¹⁰

In women, the loss of estrogen may cause changes in redox homeostasis of proteins.⁵ Proteins are highly sensitive to OPD by ROS and reactive nitrogen species (RNS). All amino acid protein residues are sub-

ject to oxidation by ROS and metal ion-catalyzed oxidation of some residues lead to the formation of PCO derivatives.¹¹ One or more reduced thiol (-SH) groups are essential for the function of many proteins. The thiol group on the side chain of the amino acid cysteine is particularly sensitive to redox reactions and is an established redox sensor. Proteins containing Cys thiol groups are particularly susceptible to oxidation by ROS.¹² Glutathione (GSH) can be reversibly bound to protein thiol groups by a mechanism called S-glutathionylation and leading to the formation of S-glutathionylated proteins.¹³ Malondialdehyde (MDA) is a physiological ketoaldehyde produced by peroxidative decomposition of unsaturated lipids as a by-product of arachidonic acid metabolism. Excessive production of MDA, as a result of tissue injury, may combine with free amino groups of proteins. MDA reacts mainly with Lys residues by Michael type addition reaction and forms MDA-modified protein adducts.¹³

The gradual decline of estrogen causes a wide variety of changes in tissues that respond to estrogen, including the heart, blood vessels, and brain at natural menopause. Estrogen treatment may prevent some but not all of the negative outcomes.³ On the other hand, inactivation of estrogens in the body occurs mainly in the liver. During the cyclic passage through the liver, estrogens are degraded to less active metabolites.¹⁴ In experiments with ovariectomized (OVX) rats, it has been suggested that endogenous conversion of certain progestogens to biologically significant amounts of estrogen metabolites may occur in the gastrointestinal tract or in the liver.¹⁴ Post-mitotic tissues of high-energy demand are at greater risk of damage by free radicals, consistent with the notion where signs of oxidative damage usually start to appear at these body sites.¹⁵ Due to the differing efficacy of redox homeostasis mechanisms, post-mitotic tissues are generally much more vulnerable to OPD than are mitotic cells. While the liver is the organ with a high mitotic rate, the major organ of antioxidant enzyme release, the brain, which is of post-mitotic nature, produces more

ROS per gram of tissue than any other organ, because of its high lipid content, high oxygen consumption (20% of all oxygen) and relatively poor antioxidant defense.^{16,17}

There is a lack of literature addressing the changes in redox homeostasis of post-mitotic tissue proteins, such as brain and liver, in humans and in experimental animals. The current study was conducted to assess estrogen-related changes in the redox status of the brain and liver proteins as well as the systemic oxidative stress in OVX rats. Administration of estrogens to castrated females seems to reduce oxidative changes in the liver of old OVX rats.¹⁸ Thus, it seems more clinically relevant to examine liver tissue as the liver is the first metabolic target for estrogen therapy.¹⁹ On the other hand, some regions of the brain are endowed with estrogen receptors alpha and beta. Complex interactions with different harmful biochemical reactions occur during the brain ischemia. Estrogen, to a great degree prevents the formation of ROS. Thus, some authors have suggested the use of exogenous estrogen as a therapeutical neuroprotector.^{20,21}

Treatment of OVX rats with estrogen-progestin combinations may slow down the OPD, opening new avenues for pharmacological prevention of OVX-induced protein oxidation. In the current study, our aim was to evaluate the redox status of plasma, brain and liver proteins as well as the protective role and dose of estrogens-progestin combinations in OVX-induced menopause.

Materials and Methods

Chemicals and apparatuses

Chemicals and solvents used in the experiments were of highest purity and analytical grade. Deionized water was used in analytical procedures. Reagents were stored at +4 °C. The reagents were maintained in equilibrium at room temperature for 0.5 hour before use. Centrifugation procedures were performed with a Jouan G 412 centrifuge. Protein oxidation and other oxidation parameters were measured with a spectro-

photometer (Heraeus 400, Kendro Laboratory Product, Osterode, Germany).

Experimental procedure

The experimental protocols were approved by Istanbul University Animal Care and Use Committee (27 March 2007/ 7676). Efforts were made to minimize the number of animals. Twelve-week-old, sexually mature female Sprague–Dawley rats (200–250g) under study were randomly divided into four groups: The following treatment combinations were administered daily to the all groups in 0.05 ml 96% ethanol solution by gastric gavage: (1) Sham operated (n=8) (2) OVX rats (n=8) (3) OVX rats (n=8) [0.02 mg/kg/day of 17 β -estradiol (E2) and 0.01 mg/kg/day of norethisterone acetate (Kliogest, Novo Nordisk, Bagsv&rd, Denmark)] (4) OVX rats (n=8) [E2 (0.01 mg/kg/day) and drospirenon (0.02 mg/kg/day) (Angeliq, Schering AG, Berlin)]. Animals were kept in conventional wire-mesh cages, four rats per cage, in a room with the temperature regulated at 21 $\pm$ 1 °C, humidity 45–50%, and light/dark cycles (12h). Sprague–Dawley rats were given *ad libitum* access to conventional food (Eris Chow Industry, Istanbul, Turkey) and water from a drinking bottle throughout the course of the experiment.

Animals were anaesthetized by the administration of ketamine (50 mg/kg) and xylazine (10 mg/ kg) into the marginal tail veins. The rats underwent either a bilateral ovariectomy via a dorsal incision or a sham surgical procedure, and incisions were closed.

Blood-sample collection and processing

Blood samples were drawn in the fasting state and processed within 1 hr of collection. Samples were collected in tubes containing lithium heparin, EDTA or no additive depending on the nature of the analysis. For analysis of protein-oxidation parameters and MDA, plasma samples containing lithium heparin were stored at –80 °C until analysis. Other routine clinical-chemistry variables were determined on the

day of collection using the Hitachi 704 auto-analyzer (Boehringer Mannheim, Tokyo, Japan).

Preparation of tissue samples

Brain tissue samples, except cerebellum, pons, medulla oblongata and liver, were quickly removed, washed in cooled 0.15 M NaCl, and placed on ice-cold plate. Tissue samples were then immediately frozen in liquid N₂ until experimentation. Tissue (200 mg) samples were homogenized manually in 2 ml homogenizing buffer (100 mM KH₂PO₄-K₂HPO₄, pH 7.4, plus 0.1% (w/v) digitonin) in a glass homogenizer to avoid disruption of nuclear membranes. In this way, contamination by nucleic acids was minimized.²² Homogenates obtained from rats were centrifuged at 5000 x g for 10 minutes, and determination of various analytes was performed in the supernatant fraction.

Determination of liver tissue and plasma protein carbonyl levels

Protein carbonyls (PCO) were measured spectrophotometrically using the method of Reznick and Packer.²³ PCO groups react with 2,4-dinitrophenylhydrazine (DNPH) to generate chromophoric dinitrophenylhydrazones. DNPH was dissolved in HCl, and after the DNPH reaction, proteins were precipitated with an equal volume of 20% (w/v) trichloroacetic acid and washed three times with 4 ml of an ethanol/ethyl acetate mixture (1:1). Washings were achieved by mechanical disruption of pellets in the washing solution using a small spatula, and re-pelleting by centrifugation at 6000 g for 5 min. Finally, the precipitates were dissolved in 6 M-guanidine-HCl solution and the absorbance values were measured at 360 nm, using the molar extinction coefficient of DNPH, $\epsilon = 2.2 \times 10^4 \text{ M}^{-1} \times \text{cm}^{-1}$. Protein contents were determined on the HCl blank pellets spectrophotometrically using a Folin kit (Sigma Diagnostics, St. Louis, MO, USA). The coefficients of intra- and inter-assay variations for PCO assay were 4.2% (n=8) and 8.2% (n=8), respectively.

Determination of brain protein carbonyl levels

The PCO levels in the homogenates of the brain frontal section taken from aged rats were treated following the method of Evans et al.²⁴ The supernatant was transferred to a plastic tube, left for 15 min. at room temperature, and then streptomycin sulphate solution (10% w/v) was added to a final concentration of 1% to precipitate any extracted DNA which could react with DNPH (2,4-dinitrophenylhydrazine) and contribute to the carbonyl level. The solution was mixed and left to stand a further 15 min at room temperature, after which it was centrifuged at 2,800g for 10 min. at room temperature. The supernatant was removed and divided equally between two 10 ml plastic centrifuge tubes with the remaining supernatant being reserved for other assays. DNPH (1.6 ml, 10 mM in 2 M HCl) was added to one tube and 1.6 ml of 2 M HCl to the other tube (ratio of supernatant to DNPH solution should be 1:4, v/v). The tubes were then incubated for 1 hr on a rotator at room temperature and then the protein was precipitated by adding an equal volume of 20% (w/v) trichloroacetic acid (TCA) to the tubes and leaving them for 15 min. The protein was spun down at 3400g (10 min, room temperature), the supernatant was discarded, and the pellet was washed with 1.5 ml of an ethyl acetate: ethanol mixture (1: 1, v/v) to remove excess DNPH. This procedure was repeated three times. The final protein pellet was dissolved in 1.25 ml of 6 M guanidine hydrochloride and the absorbance values of both solutions (DNPH and HCl) were measured at 370 nm from which the PCO content could be evaluated (PCO concentration in nmol/ml: $\Delta A_{370} \times 45.45$, where ΔA_{370} equals A_{370} of DNPH solution – A_{370} of HCl solution). Protein contents were determined on the HCl blank pellets spectrophotometrically using a Folin kit (Sigma Diagnostics, St. Louis, MO, USA). The coefficients of intra- and inter-assay variations for PCO assay were 4.2% (n=12) and 9.6% (n=10), respectively.

Determination of plasma total thiol concentrations

Plasma total thiol (T-SH) concentration was determined by using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) as described by Hu.²⁵ The coefficients of intra- and inter-assay variations were 1.6% (n=8), and 4.5% (n=10), respectively.

Determination of tissue total thiol concentrations

T-SH groups were measured spectrophotometrically using the method of Sedlak and Lindsay.²⁶ Aliquots of 250 µl of the supernatant fraction of the tissue homogenate were mixed in 5 ml test tubes with 750 µl of 0.2 M Tris buffer, pH 8.2, and 50 µl of 0.01 M 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). The mixture was brought to 5 ml with 3950 µl of absolute methanol. A reagent blank (without sample) and a sample blank (without DTNB) were prepared in a similar manner. The test tubes were stoppered with rubber caps, colour was developed for 15 min and the reaction mixtures were centrifuged at approximately 3,000g at room temperature for 15 min. The absorbance of supernatant fractions was read in a spectrophotometer at 412 nm. The molar extinction coefficient at 412 nm was 13100 l.mol⁻¹cm⁻¹.

Determination of reduced glutathione concentrations

Whole blood and tissue reduced glutathione (GSH) levels were measured by a commercially available GSH assay kit (Cayman Chemical, Ann Arbor, MI, USA). The GSH assay kit utilizes an enzymatic recycling method, using glutathione reductase, for the quantification of GSH. The thiol group of GSH reacts with DTNB (5-5'-dithiobis-2-nitrobenzoic acid, Ellman's reagent) and produces a yellow coloured 5-thio-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB that is concomitantly produced, is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to the concentration of GSH in the deproteinized sample. Measurement of the absorbance of

TNB at 414 nm provides an accurate estimation of GSH in the sample.

Determination of lipid peroxidation

The rate of lipid peroxidation was determined by the procedure of Beuge and Aust.²⁷ One of the major secondary products of lipid peroxidation is malondialdehyde (MDA). MDA along with other by-products react with thiobarbituric acid (TBA) to generate a coloured product which absorbs at 535 nm representing the color produced by all the thiobarbituric acid reactive substances (TBARS). The coefficients of intra- and inter-assay variations for MDA assay were 3.5% (n = 10) and 5.4% (n = 10), respectively.

Statistical analyses

Data are expressed as mean ± SEM for eight animals in each group. Differences among groups were assessed by one way analysis of variance (ANOVA) using the SPSS software package for Windows. Post-hoc testing was performed for inter-group comparisons using the least significance difference (LSD) test. Post hoc tests were conducted with the Bonferroni-Dunn test. A probability value of < 0.05 was considered statistically significant for all comparisons.

Results

Routine clinical-chemistry parameters of the female rat groups subjected to OVX alone and in combination with estrogens-progestins of two doses, and their respective controls (sham operated) are summarized in Table 1. No allergic or gastrointestinal complications or hepatotoxicity were observed during the experimental period. There was neither intraoperative death nor any death in the experimental period. The group natriuretic drospirenon group lost more weight than those in the norhisterone acetate group (Table 1).

Five weeks after surgery, E2 levels of sham operated rats had not changed. On the other hand, E2 levels decreased ($P < 0.001$) in OVX rats (Table 1).

TABLE 1. Body weight and plasma 17 β -estradiol concentrations.

	Body weight (g)	E2 (pg/ml)
Sham operated rats		
Initial (n=8)	201.00 $\pm$ 17.23	298.67 $\pm$ 106.60
Sham (n=8)	204.50 $\pm$ 16.60	299.50 $\pm$ 104.89
OVX rats		
Initial (n=8)	198.50 $\pm$ 25	256.00 $\pm$ 108.16
OVX (n=8)	208.17 $\pm$ 21.24 (a*)	13.67 $\pm$ 2.73 (a***)
Rats receiving HRT Drospirenon (Angeliq)		
Initial (n=8)	201.25 $\pm$ 17.00	257.88 $\pm$ 102.43
OVX (n=8)	216.25 $\pm$ 19.91 (a**)	14.75 $\pm$ 3.85 (a***)
OVX + Angeliq (n=8)	184.38 $\pm$ 16.73 (a***,b***)	220.13 $\pm$ 77.56 (a*b***)
Rats receiving HRT Norethisterone acetate (Kliogest)		
Initial (n=8)	205.50 $\pm$ 23.75	251.50 $\pm$ 111.24
OVX (n=8)	211.50 $\pm$ 24.19 (a**)	16.38 $\pm$ 3.66 (a***)
OVX + Kliogest (n=8)	201.88 $\pm$ 16.93 (b***)	162.38 $\pm$ 34.99 (a***b***)

E2, 17 β -estradiol; OVX, ovariectomized; HRT, Hormone replacement therapyMean $\pm$ SEM: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. ^a different from initial, ^b different from OVX

These results confirm successful ovariectomy. The effects of 5 weeks of administration of drospirenon (Angeliq) and norethisterone acetate (Kliogest) on serum E2 levels were determined as 220.13 $\pm$ 77.56 pg/ml; 162.38 $\pm$ 34.99 pg/ml, respectively (Table 1). Current data suggest that ovariectomy successfully promoted E2-deficiency and E2 replacement therapy was effective in OVX rats. In sham-operated rats, no changes were observed in all of the parameters compared to initial values (Table 1).

Plasma and tissue levels of the protein oxidation markers in the study groups are given in Figure 1. Among the studied protein oxidation variables, plasma and tissue PCO levels of the OVX rats were higher than those of the control groups. The current PCO results indicate that hormone replacement therapy (HRT) caused a decrease in protein oxidation rates in both plasma and tissue of the OVX rats. After bilateral oophorectomy, plasma and tissue T-SH levels decreased in the OVX group compared with control groups, whereas the levels in groups treated with Angeliq and Kliogest lost much of the steepness (Figure 1: D,E,F). Thus, Kliogest increases plasma T-SH levels more effectively than Angeliq (Figure 1D).

The effects of Kliogest and Angeliq on malondialdehyde (MDA) and glutathione (GSH) levels in plasma, liver, and brain are given in Figure 2. Plasma and tissue MDA levels were found to be higher in OVX rats than in the control groups. Liver MDA levels of the Angeliq-treated rats were lower than in the Kliogest group (Figure 2B) Hormone replacement therapy in OVX rats caused a decrease in plasma MDA levels and an increase in liver and brain GSH levels. On the other hand, Angeliq increased brain GSH levels more effectively than do Kliogest (Figure 2F).

Discussion

The existence of increased extra- and intra-cellular oxidative stress in physiological aging¹ and menopause^{4,5} is undergoing extensive discussion. Altered oxidant-antioxidant balance is a risk factor for the development of various pathological states such as cardiovascular disease, cognitive impairment, Parkinsonism, osteoporosis, psychological wellbeing and sexual dysfunction in women who underwent prophylactic bilateral oophorectomy.^{3,28} The goal of postmenopausal hormone therapy is to alleviate clinical symp-

FIGURE 1. Effects of Kliogest and Angeliq on protein carbonyl (PCO) and thiol (T-SH) levels in plasma, liver, and brain. Mean $\pm$ SEM.

A,D- * $P < 0.05$; ** $P < 0.01$; ^a difference with initial, ^b difference with OVX, ^c difference with OVX+KLIO.

B,C,E,F- * $P < 0.05$; ** $P < 0.01$. ^a difference with SHAM, ^b difference with OVX, ^c difference with OVX+ANG, ^d difference with OVX+KLIO.

PCO, protein carbonyl; T-SH, total thiol; pr, protein; OVX, ovariectomized; KLIO, Kliogest; ANG, Angeliq.

FIGURE 2. Effects of Kliogest and Angeliq on malondialdehyde (MDA) and glutathione (GSH) levels in plasma, liver, and brain. Mean $\pm$ SEM.

A,D- * $P < 0.05$; ** $P < 0.01$; ^a difference with initial, ^b difference with OVX, ^c difference with OVX+KLIO.

B,C,E,F- * $P < 0.05$; ** $P < 0.01$. ^a difference with SHAM, ^b difference with OVX, ^c difference with OVX+ANG, ^d difference with OVX+KLIO.

MDA, malondialdehyde; GSH, glutathione; OVX, ovariectomized; KLIO, Kliogest; ANG, Angeliq.

toms that are associated with the loss of estrogen. Many formulations of estrogen and progestin are available depending on the needs and circumstances of each woman. Ovarian hormone depletion in ovariectomized experimental animals is a useful model for studying the physiopathological consequences of menopause in women.²⁹ We searched the literature to find the effective dose of estrogens and decided on current recommended doses. The use of moderate estrogen doses and the means of administration have been described previously.^{9,30} The effective doses of 17 β -estradiol (E2) and norethisterone acetate we selected were based on the concentration capable of inhibiting oxidative stress.^{9,30} The current study shows the extent of OPD in this model of estrogen deficiency. The protective effects of estrogen-progestin combinations against tissue specific OPD suggest that estrogens play an important role within the antioxidant defense systems in plasma, liver and brain.

For many years lipid oxidation has been the focus of investigation but, due to their relatively high abundance, it is now recognized that proteins are the main targets for ROS. Measurement of oxidized protein concentrations in biological materials may have some advantages in comparison with lipid peroxidation products because of the relatively early formation and relative stability of oxidized proteins.¹³ On the other hand, PCO groups may be introduced by secondary reaction of the nucleophilic side chains of cysteine, histidine, and lysine residues and reactive aldehydes produced during lipid peroxidation process.¹³ Recently, markers of protein oxidation such as PCO and protein thiol groups have begun to attract the attention of various investigators.^{4,5,24,25} Telci and colleagues have shown that hormone replacement therapy leads to decrease in plasma PCO levels in postmenopausal women.⁵ Others have suggested that an important component of the mechanism underlying this redox interaction may depend on estrogen's antioxidant effect and its preventive role in OPD. There is a lack of

literature addressing the changes in redox homeostasis of the tissue proteins such as brain and liver in humans and experimental animals.

In current study, we found that PCO and MDA levels in the whole series of OVX rat groups were higher than those of the control groups. The decrease in plasma and tissue PCO levels has been attributed to the antioxidant activity of estrogen-progestin combinations. We also observed a marked decrease in MDA levels in current study. MDA levels show a similar trend to that in the PCO levels in our study groups. In the current study, liver MDA levels of the Angelique-treated rats were lower than Kliogest group. Metabolism of 17-beta-estradiol is carried out in brain, kidney or liver, and triggers different products such as 2- and 4- hydroxyestradiol (2OH and 4OH).³¹ These products have antioxidant properties against oxidative stress in several experimental models. Drug-dependent variations in liver MDA may be due to altered regulation of drug-metabolizing pathways in our study. In the present study, the occurrence of OPD in plasma and tissues of OVX rats was also confirmed by a marker (T-SH assay) that provides information on the degree of oxidative damage to proteins, and the data obtained support those found with detection of PCO. The presence of a reactive thiol (-SH) group in proteins and amino thiols such as GSH not only predisposes these molecules to oxidative modification but also confers potent antioxidant properties.¹² The assessment of total or reduced, oxidized, and protein bound thiols could both provide an estimate of the antioxidant potential, and could also provide insight into the redox-status.¹² T-SH status in menopause has predominantly been investigated in whole blood and plasma.^{4,5} The total amount of thiol containing molecules or the amount of specific thiols has been reported to be lower in plasma or erythrocyte lysate of women with menopause as comparing the corresponding values in women receiving hormone replacement therapy.^{4,5} The increase in plasma T-SH and erythrocyte GSH levels might also contribute to the beneficial effects of hormone replacement therapy. After bi-

lateral oophorectomy, plasma and tissue T-SH decreased in the OVX group compared with control groups, whereas the levels in groups treated with Angelix and Kliogest lost much of its steepness. Our results show that Kliogest-estrogen combination increases plasma T-SH levels more effectively than Angelix. There are no established data or explanation why plasma T-SH levels cannot be balanced in OVX rats by different estrogen-progestin combinations. These changes are probably related to different drug interactions and/or homeostatic regulations in such tissues. Accumulation of oxidized protein reflects not only the rate of protein oxidation but also the rate of oxidized protein degradation, which is also dependent upon variables, including the concentrations of proteases that preferentially degrade oxidized proteins and numerous factors (metal ions, inhibitors, activators, and regulatory proteins) that affect their proteolytic activity.^{11,13} However, in this study, there was no relationship between estrogens and oxidized protein degradation.

An efficient clinical laboratory diagnosis of altered redox status of the plasma and tissue proteins in menopausal stage is very important in order to control the degenerative conditions associated with OPD.⁵ On the other hand, an effective preventive and therapeutic strategy against oophorectomy-induced OPD has not been developed. In fact, the relationship between molecular events, such as control of redox regulation, and intracellular signaling pathways are still under investigation. Molecular mechanisms that control redox-regulation systems related to cellular proteins, and variation in the regulation of these controlling systems will contribute to the susceptibility of post-mitotic tissues to oxidative stress during aging and menopause. As redox regulation mechanisms in aging and menopause become clearer, new therapeutic approaches and prospective solutions are coming into view. A major research and development effort is required to produce novel therapies related to redox regulation of proteins and make them available to elderly women. The findings of our study will lead to new insight into the ex-

tent of oxidative protein damage in this model of estrogen deficiency. On the other hand inhibition of protein oxidation and lipid peroxidation by estrogens may be one of the anti-atherosclerotic effects of estrogens. The underlying mechanisms need clarification. The molecular mechanisms are not clear and further study is required. Meanwhile, hormone replacement therapy to prevent OPD in a tissue specific manner may be required.

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Correspondence to:

Ufuk Çakatay
Şenesenevler, Abdüleziz Paşa Sk.
Çiçek Apt. No: 9/7 Bostancı-Istanbul- Turke
e-mail: cakatay@yahoo.com