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RESEARCH ARTICLE

Effects of ovarian steroids on oxidative stress in overiectomized rats

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Özet

Bulbul A, Aslan R, Celik HA, Avci G, Fidan F. Ovariektomize ratlarda ovaryum steroidlerinin oksidatif stres üzerine etkisi. **Eurasian J Vet Sci, 2012, 28, 2, 94-98**

Amaç: Ovariektomi yapılmış ratlarda östrojen ve progesteron hormonlarının beraber veya ayrı uygulanmasının lipid peroksidasyon, antioksidatif aktivite (AOA) ile β-karoten, vitamin A ve C düzeyine olan etkisinin belirlenmesi amaçlandı.

Gereç ve Yöntem: Araştırma overioktemi yapılmış ve her biri 10 rattan oluşan kontrol ve 3 deneme grubunda yürütüldü. Kontrol grubuna susam yağı, progesteron grubuna progesteron 2 mg/rat/gün, östrojen grubuna 17β-östradiol 10 µg/rat/gün, östrojen + progesteron grubuna 17β-östradiol 10 µg/rat/gün + progestagen 2 mg/rat/gün olarak 10 gün uygulandı. En son uygulamadan 2 saat sonra genel anestezi altındaki ratlardan alınan kan örneklerinde malondialdehit (MDA), AOA, β-karoten, vitamin A ve C düzeyleri ölçüldü.

Bulgular: Tam kan MDA düzeylerinin kontrol gruba göre östrojen grubunda azaldığı, ancak diğer deneme gruplarında fark bulunmadığı tespit edildi. Plazma AOA düzeyinin östrojen ve östrojen + progesteron gruplarında kontrol ve progesteron gruplarına göre yüksek ($p<0.05$) olduğu belirlendi. Plazma β-karoten düzeyinin tüm deneme gruplarında kontrol grubuna göre azaldığı görüldü. Vitamin C düzeylerinin kontrole göre tüm deneme gruplarında yüksek olduğu bulunurken en yüksek artış östrojen ve östrojen + progesteron gruplarında saptandı.

Öneri: Ovariektomi yapılmış ratlarda östrojenin antioksidan etkinliği artırarak lipid peroksidasyonunu azalttığı, progesteronun ise vitamin A ve C düzeyini artırmasına karşın lipid peroksidsyonuna etkisi olmadığı ifade edilebilir.

Abstract

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Aim: Determining the effects of combined or separate estrogen and progesterone administration on lipid peroxidation, antioxidative activity (AOA), β-carotene, vitamin A and C levels in overiectomized rats.

Materials and Methods: The study was conducted on 1 control and 3 trial groups each consisted of 10 overiectomized rats. Control group received sesame oil while progesterone group received 2 mg/rat/day progesterone, estrogen group 10 µg/rat/day 17β-estradiol and estrogen + progesteron group 10 µg/rat/day 17β-estradiol+ 2 mg/rat/day progesterone for 10 days. Blood samples of rats were collected under general anesthesia after 2 hours following last administration and checked for malondialdehyde (MDA), AOA, β-carotene, vitamin A, and C levels.

Results: Whole blood MDA levels in estrogen group were decreased as compared to control group while remained same in others. Plasma AOA levels of estrogen and estrogen + progesterone groups were higher ($p<0.05$) than control and progesterone groups. Plasma β-carotene levels were decreased in all trial groups when compared to control. Vitamin C levels of all groups were higher as compared to control group and were highest in estrogen + progesterone group.

Conclusion: It can be concluded that estrogen decreases lipid peroxidation and increases antioxidant capacity besides effects positively the levels of antioxidant vitamins, particularly showing that mentioned level of estrogen act as an antioxidant.

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Keywords: Lipid peroxidation, estrogen, progesterone, vitamin A, vitamin C

► Introduction

Free radicals are molecules with unpaired electron, composed by redox reactions during cell metabolism (Halliwell 1997). Enzymatic and non-enzymatic antioxidant system regulates control of increased free radicals. Oxidative stress is described as corruption of oxidant/antioxidant balance by increased oxidant or decreased antioxidant (Soprano et al 1986, Wefers and Sies 1988). Increased oxidative stress causes many diseases such as myocardial infarctus, diabetes, cancer, cataract, rheumatoid arthritis, infertility and diseases of respiratory, nervous and urinary systems (Yuce and Aksakal, 2005).

It is known that lipid peroxidation in liver and brain has increased after overiectomy (Borras et al 2003, Ozgonul et al 2003) and insufficient levels of ovarian hormones increases levels of reactive oxygen radical (ROS) varieties thus causing oxidative stress (Strehlow et al 2003, Ha et al 2006). Similarly, oxidative stress increases after menopause hence particularly increases cardiovascular diseases in humans (Subbiah et al 1993, Sack et al 1994).

Estrogen is an antioxidant (Yagi and Komura 1986, Mukai et al 1990, Subbiah et al 1993). Because of that feature, prevents oxidation of lipoproteins like low density lipoprotein (LDL), inhibits their deposition in arterial walls and prevents arteriosclerosis (Rifici and Khachadurian 1992, Kinlay et al 1996). Similarly estrogen decreases superoxide production in arterial wall but nonetheless increases superoxide dismutase (SOD) level. Besides it is reported that estrogen increases nitric oxide (NO) level which is the sweeper of superoxide molecule thus decreasing lipid peroxidation. Also in our former studies, NO levels were high either in estrogen administration (Bulbul et al 2007) or during estrus and proestrus in which estrus levels are high (Bulbul et al 2008). There are different reports about effect of progesterone on antioxidant enzymes. It increases uterine tissue levels of MnSOD and Cu/Zn SOD while estrogen levels increase moderately, on the other hand, when administrated combined with estrogen, decreases estrogen elevated SOD levels in vascular smooth muscle cell culture (Wassmann et al 2005).

It is reported that there is a positive correlation between estrogen and Vitamin A, which is another antioxidant (Schweigert et al 1986). Similarly, in our research, Vitamin A level was highest in follicular period in which also the estrogen level is highest (Celik et al 2009).

Detailed researches about effects of ovarian hormones and particularly estrogen on oxidative stress are not available however, mentioned ones are postmenopausal human studies (Bhavnani 2003, Lutoslawska et al 2003, Bednarek-Tupikowska et al 2004).

In this study, it was aimed to investigate the effects of female genital hormones on oxidative stress, Vitamin A, Vitamin C and some biochemical parameters in overiectomized rats.

► Materials and Methods

In the current research, 3 months aged 40 female Sprague Dawley rat are used. Animals were divided into 4 equal groups as 3 trials and 1 control. Study protocol was approved by Ethic Committee of Afyonkocatepe University. Animals were fed ad libitum with commercial rat feed. Rats in control and trial groups were anesthetized by IP injection of 21.1 mg/kg ketamine + 4.2 mg/kg xylazine overiectomized. After 2 weeks overiectomy, control group (Ov group) received sesame oil while progesterone group received 2 mg/rat/day progesterone, estrogen group received 10 μg/rat/day 17β-estradiol and estrogen + progesterone group received 10 μg/rat/day 17β-estradiol+ 2 mg/rat/day progestagen for 10 days. After 2 hours following last administration, blood samples of the rats were collected into heparinized tubes. MDA levels of heparinized blood were evaluated, and remaining portion was centrifuged to obtain plasma for 15 minutes in +4 ^{0}C and 3000 rpm. Whole blood MDA (Draper and Hardley (1990) and plasma antioxidative activity (AOA) (Korecevic et al 2001), vitamin A, β-carotene (Suzuki and Katoh 1990) and vitamin C (Kway 1978) levels were measured with ELISA (Thermo Multiscan FC) by previously reported methods.

Data were evaluated by ANOVA and Tukey test. $p<0.05$ level was accepted as statistically significant.

► Results

MDA, AOA, vitamin A, β-carotene and vitamin C levels are shown in Table 1. Blood MDA levels decreased ($p<0.05$) in estrogen group. Plasma AOA levels of estrogen and estrogen + progesterone groups were higher ($p<0.05$) then control and progesterone groups. Plasma vitamin A levels of control and estrogen + progesterone groups were higher ($p<0.05$) than progesterone and estrogen groups, although plasma β-carotene levels were higher ($p<0.05$) than all groups. Highest vitamin C level was determined ($p<0.05$) in estrogen + progesterone group while lowest vitamin C level measured ($p<0.05$) in control group.

► Discussion

Lipid peroxidation consists of harmful chain reactions which severely alters structure and functions of biological membranes. The levels of extremely toxic by products such as MDA and 4-hydroxyalkenals formed following these reaction show the oxidative stress in tissues (Halliwell 1997). Malondialdehyde is formed as a result of non-enzymatic oxidative destruction of polyunsaturated fatty acids or as a byproduct during oxygenation of arachidonic acid and accepted as an indicator of damage due to radicals (Katz et al 1996). Whole blood MDA levels of progesterone and

Table 1. Malondialdehyde (MDA), antioxidative activity (AOA), vitamin A, β-carotene and vitamin C levels of groups (mean±SE).

	Control	Progesterone	Estrogen	Estrogen + Progesterone
MDA nmol/L	4.97±0.18^{ab}	5.23±0.25^{a}	3.45±0.24^{c}	4.08±0.16^{b}
AOA mmol/L	7.97±0.31^{b}	7.80±0.27^{b}	9.32±0.42^{a}	9.08±0.26^{a}
Vitamin A µg/dL	67.0±1.81^{b}	160 ± 9.03^{a}	182 ± 12.1^{a}	108±8.14^{b}
ß-carotene µg/dL	172±7.29^{a}	63.0±1.00^{b}	64.2±3.47^{b}	71.2±9.06^{b}
Vitamin C mg/dL	4.86±0.24^{c}	5.56±0.26^{b}	5.96±0.19^{ab}	6.63±0.08^{a}

$^{a, b, c}$: Different letters in the same column indicate statistical significance (Tukey test, $p<0.05$).

estrogen + progesterone groups were not different to those of control while they were significantly lower in estrogen group in the study (Table 1), showing that in vitro presence of 17β-estradiol and 2-hydroxyesterodiol inhibit lipid peroxidation in accordance with reports of Ruiz-Larrea et al (2000). However, reports (Ayers et al 1998, Lutoslawska et al 2003) showing either in vitro or in vivo presence of estrogen decreases production of ROS and free radicals and increased oxidative stress in women due to postmenopausal suppression of estrogen synthesis support that result.

It was reported that estrogen and estrogen + medroxyprogesterone administration in postmenopausal women increased the total antioxidant status (Bednarek-Tupikowska et al 2004) besides there was a positive correlation between estrogen level and total antioxidant status during menstrual cycle (Michos et al 2006). Delibasi et al (2006) report that estrogen administration in postmenopausal women for 3 weeks increased in the antioxidant capacity. Plasma AOA levels of estrogen and estrogen + progesterone groups were increased as compared to those of control group (Table 1) which is in conformation with reports of the researchers (Bednarek-Tupikowska et al 2004, Delibasi et al 2006, Michos et al 2006).

Estrogen stimulates some pro-oxidants in microsome and liposomes, thus it inhibits lipid peroxidation (Dlugosz et al 2009). It has been reported that estrogen increases levels of antioxidant enzymes such as SOD and also increases level of NO which is not an antioxidant but considered so because it sweeps superoxide, a more dangerous oxidant. On the other hand, there is a positive correlation between estrogen and vitamin A which has antioxidant characteristics (Maenpaa et al 1988). Similarly, it is reported that vitamin A level in cows during proestrus and estrus period is higher in which the estrogen level is high (Haliloglu et al 2002). Retinol binding protein (RBP), responsible of vitamin A transportation, is synthesized primarily in liver and adipose tissue (Soprano et al 1986, Makover et al 1989). Estrogen administration does not affect liver RBP synthesis in overiectomized rats while its synthesis in kidney is increased (Whitman et al 1990). Higher vitamin A levels in progesterone and estrogen groups as compared to those of control group in this study (Table 1) is in accordance with former researches (Maenpaa et al 1988, Haliloglu et al 2002) suggests that mentioned increase may be due to increase in synthesis of RBP. Lower β-carotene level in trial groups than that of control group (Table 1) suggests that carotene is converted to vitamin A in liver (Maenpaa et al 1988, Whitman et al 1990, Haliloglu et al 2002).

Ascorbic acid is a primary antioxidant such as α-tocopherol and β-carotene besides it is also a secondary antioxidant as reduces tocopheroxyl radical (Wefers and Sies 1998). Ascorbic acid level is high in teca interna, teca granulosa and luteal compartments of ovarium and has role in biosynthesis of steroid and peptide hormones (Luck et al 1995). Increased plasma vitamin C level in all trial groups as compared to that of control and significant increase in estrogen + progesterone group as compared to progesterone group in the study (Table 1) supports the reports (Michos et al 2006) informing the presence of positive correlation between ascorbic acid levels and alterations in estrogen levels during menstrual cycle. However, collagen synthesis is necessary for follicle growth during follicular and luteal period, repairing ovuled follicles and corpus luteum growth. Ascorbic acid is the cofactor of enzymes in charge of hydroxylation during formation of procollagen and provides secretion of proteoglycans and collagen into follicular fluid (Luck et al 1995).

► Conclusions

Eventually, is was found that estrogen increases antioxidant activity thus it decreases lipid peroxidation, besides progesterone increases vitamin A and vitamin C levels however it does not affect lipid peroxidation in overiectomized rats.

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