Effect of Ovariohysterectomy on Some Oxidative Stress Markers in the Rat

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Abstract: The purpose of the present study was to evaluate the effect of ovariohysterectomy (OHE) on oxidative stress in rats. A total of 12 female Sprague Dawley rats weighing 200-250 g were included in the study. Six rats underwent OHE (group I) and the other 6 rats sham operation (group II) under xylazine (5 mg/kg, i.m.) and ketamine (45 mg/kg, i.m.) anesthesia. In both groups, blood samples were collected preoperatively, day 1 and 10th days after OHE. Serum concentration of malondialdehyde (MDA) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured by spectrophotometric methods. The concentration of MDA increased while activities of SOD and GPx decreased in group I compared to group II on day 1 after the surgery. Similarly, the concentration of MDA was higher while activities of SOD and GPx appeared to be lower in group I than that of group II, although the differences were not statistically significant on the 10th day after surgery. In conclusion, our findings indicated that ovariohysterectomy is related with oxidative stress in the rat.
Keywords: *Oxidative stress, Ovariohysterectomy, Rat*

Ovaryohisterektominin Ratlarda Bazı Oksidatif Stres Parametreleri Üzerine Etkisi

Özet: Bu çalışmanın amacı, ovaryohisterektominin (OHE) ratlarda oksidatif stres üzerine etkilerinin araştırılmasıdır. Bu çalışmada ağırlıkları 200-250 gr, toplam 12 dişi Sprague Dawley rat kullanıldı. Ksilazin (5 mg/kg, i.m.) ve ketamin (45 mg/kg, i.m.) anestezisi altındaki 6 rata OHE (grup I) ve diğer 6 rata da sham operasyonu (grup II) yapıldı. Her iki gruptan da preoperatif dönemde ve cerrahi sonrası 1. ve 10. günlerde kan örnekleri alındı. Serum malondialdehit (MDA) konsantrasyonu ile süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GPx) aktiviteleri spektrofotometrik yöntem ile ölçüldü. Cerrahi sonrası 1. gün, grup I'in MDA konsantrasyonu artarken, SOD ve GPx aktiviteleri grup II'ye göre azaldı. Benzer olarak, grup I'in MDA konsantrasyonu artarken, SOD ve GPx aktiviteleri grup II'ye göre azaldı, ancak cerrahi sonrası 10. gündeki bu fark istatistiksel olarak anlamlı değildi. Sonuç olarak, bulgularımız ovaryohisterektominin oksidatif stresle ilişkili olduğunu gösterdi.
Anahtar Kelimeler: *Oksidatif stres, Ovaryohisterektomi, Rat*

Introduction

Oxidative stress has been identified as an imbalance between oxidative and antioxidative status that increased reactive oxygen species production which initiates lipid peroxidation. Antioxidant defence system prevents molecular and cellular damage by reducing free radicals (Halliwell, 2007; Gunay et al., 2011; Morrone et al.,2015). The evaluate of antioxidant enzyme activities are useful indicator of the antioxidant status in most mammals (Serin et al., 2008; Halliwell, 2012; Kozlik et al., 2015; Tang et al., 2016). Many studies on the evaluation of oxidative/antioxidative status in women and female rodents after ovariectomy or OHE have been demonstrated (Kankofer et al., 2007; Serin et al., 2008; Gunay et al., 2011; Szczubial et al., 2015).

It has been known that postmenopausal life affects oxidative status and causes metabolic diseases like osteoporosis and cardiovascular disorders (Gurdol et al., 1997; Kankofer et al., 2007; Castelao et al., 2008; Yang et al., 2014; Tang et al., 2016). Similarly, ovariectomy in rats has long-term effects on several organs such as liver, intestines and myocardium due to deficiency of ovarian hormones, particularly estrogens following the surgery (Morrone et al., 2015; Tang et al., 2016; Barp et al., 2012; Gomez et al., 2012; Murphy, 2011). Estrogens have been demonstrated to protect the liver and intestines from oxidative damage due to its antioxidative properties (Sener et al., 2005). Kim et al. (2012) also postulated that estrogen deficiency may develop cytokine production in peripheral blood mononuclear cells and increase interleukin-6 (IL-6) concentrations associated with oxidative stress after menapause in women. The aim of this experimental study was to demonstrate the effect of OHE on serum oxidative stress markers.

Materials and Methods

Animals and experimental design: Twelve adult female rats weighing 200 to 250 g were used in this study. Rats were supplied from Harlan Laboratories

B.V. (The Netherlands). The present study was approved by Ethical Committee for Animal Studies of Gazi University (Approval no: 15.31). Rats were kept in cages at room temperature (22-25ºC) with a 12/12 h light/dark cycle and fed a standard chow diet as *adlibitum*. OHE was performed by medial laparotomy in group I (n=6) and sham operation was performed in group II (n=6) under ketamine/xylazine anesthesia. The surgery dura-tions of both groups were recorded. Blood samples were obtained from tail vein at day 0 before surgery and at day 1 and 10 following surgery in both groups. Blood samples were centrifuged at 1550 g for 10 min to obtain the serum. Serum samples were stored at -80°C until analyses.

Determination of MDA concentration: Serum MDA concentration was determined by the method suggested by Yoshioka et al. (1979). Briefly, 0.5 ml of serum samples were mixed with 2.5 ml of 20% trichloroacetic acid and 1 ml of 0.67% thiobarbituric acid and heated for 30 min at 95 °C. The content was then cooled, 4 ml of n-butanol was added and vortexed vigorously. The butanol phase was separated by centrifugation at 1550 g for 10 min. The absorbance of the clear supernatant was measured against n-butanol at 535 nm by spectrophotometer (Thermo Scientific, Genesys 10S UV-VIS, Madison, WI, USA). MDA concentration of serum samples were evaluated from the standard curve of 1,1,3,3 tetraethoxypropane and results were presented as µmol/l.

Determination of GPx activity: Serum GPx activity was determined according to the procedure of Paglia and Valentine (1967). In brief, 20 µl of serum samples were mixed with 800 µl of reaction mixture (pH 7.0) containing 0.1 M phosphate buffer, 10 mM GSH, 1 mM sodium azide, 1 mM ethylene diamine tetra-acetic acid, 1 unit of glutathione reductase, 1.5 mM nicotinamide adenine dinucleotide phosphate and incubated for 5 min at 37 °C. The mixture was treated with 10 µl of the 30 mM tert-butyl hydroperoxide. The absorbance change was spectrophotometrically monitored at 340 nm for 5 min at 37 °C and GPx activity was expressed as U/l.

Determination of SOD activity: SOD activity was measured by the method as described by Sun et al. (1988). Briefly, 100 µl of serum samples were mixed with 2.45 ml of assay reagent (0.3 mM xanthine, 0.6 mM disodium ethylene diamine tetraacetic acid, 0.15 mM nitroblue tetrazolium, 0.4 M sodium carbonate, 1 g/l bovine serum albumin). Then, 20 µl of xanthine oxidase was added to the mixture and incubated at 25 °C for 20 min. After incubation, the reaction was stopped by the addition of 1 ml of 0.8 mM copper (II) chloride. The SOD activity was monitored at 560 nm by detecting the inhibition of the nitroblue tetrazolium reduction rate. SOD activity was expressed as U/l.

Statistical analysis: Statistical analysis was carried out using SPSS version 13.0 software program (SPSS Inc., Chicago, Illinois USA). Comparisons between two groups for continuous variables were perfor-med using Mann-Whitney *U* test. The multiple comparisons were performed by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test. Data are expressed as means ± standard deviation and P values less than 0.05 were considered to be statistically significant.

Results

The mean concentrations of analyzed parame-ters and differences in study groups were presented in Figure 1, 2 and 3. In group I, there were significant differences in the MDA concen-trations on post-operative day 1 and 10 compared with the results of day 0 ($P<0.05$) (Figure 1). In addition, the activitiy of SOD and GPx were significantly different on postoperative day 1 compared with day 0 in group I ($P<0.05$) while there were no significant differences in SOD and GPx activities between day 0 and postoperative day 10 in group I ($P>0.05$). On the other hand, serum MDA concentrations and the activity of SOD and GPx were not significantly different among the days in group II ($P>0.05$) (Figure 2 and 3).Furthermore, the duration of sur-gery since OHE was 13 minutes whereas, duration of the sham operation was about 6 minutes.

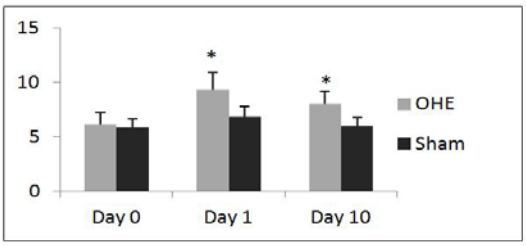

Figure 1. Mean serum concentrations of MDA (µmol/l) at day 0, 1 and 10 in group I (OHE) and II (sham). *P<0.05 (Mann-Whitney *U*-test).

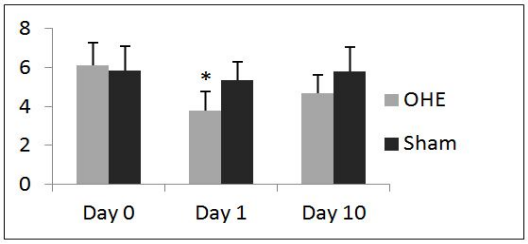

Figure 2. Mean serum activities of SOD (U/l) at day 0, 1 and 10 in group I (OHE) and II (sham). *P<0.05 (Mann-Whitney *U*-test).

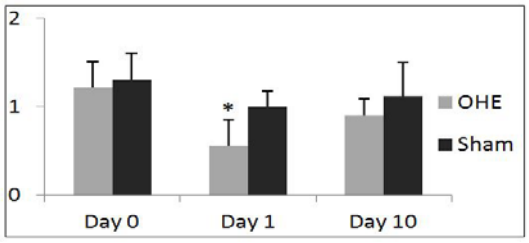

Figure 3. Mean serum activities of GPx (U/l) at day 0, 1 and 10 in group I (OHE) and II (sham). *P<0.05 (Mann-Whitney *U*-test).

Discussion

Several studies indicated that ovariectomy or ovariohysterectomy resulted in antioxidative/oxidative imbalance in most mammals (Muthusami et al., 2005; Kankofer et al., 2007; Serin et al., 2008; Günay et al., 2011; Tang et al., 2016). Some researchers presented that MDA concentration increased at 24 hour after OHE in bitches (Serin et al., 2008; Günay et al., 2011). The present study showed that serum MDA concentration significantly increased while SOD and GPx activities decreased on day 1 after surgery in group I. Furthermore, Gomez-Zubeldia et al. (2002) also determined that SOD activity is slightly increased at day 15 after ovariectomy, whereas reduced MDA concentrations were found in rat uterus. On the contrary, another study revealed that SOD and GPx activities did not alter on day 30 after ovariectomy in rats (Azevedo et al., 2001). In this study, the concentration of MDA increased and activity of GPx appeared to be lower in group I than that of group II though the differences were not significant at day 10 (P>0.05).

It was reported that surgical intervention reduces concentrations of lipid peroxides which contributes oxidative stress (Baines and Shenkin, 2002; Mudron et al., 2007; Kozlik et al., 2015). In addition, Anup et al. (1999) reported that the induction of surgical stress by opening the abdominal wall and handling the intestines leads to oxidative stress in enterocytes. However, these changes were significant at 60 minutes after laparotomy and stabile by 24 hours. Szymczyk et al. (2003) compared serum lipid peroxide concentration and antioxidant status comparing sutured peritoneum and non-closed peritoneum after hysterectomy in women and found that levels of lipid peroxidation products were lower in the study group (non-closed peritoneum) after surgery, whereas total antioxidant values was the same in both groups. Additionally, it has been stated that serous inflammation in the peritoneum resulting from surgical intervention may cause oxidative stress (Cronauer et al., 1999). In the present study, the fact that there is no statistical difference among the concentrations of MDA at day 0, 1 and 10 following sham operation made us thought that lipid peroxidation might not be affected by laparotomy.

Many researchers demonstrated that the oxidative/antioxidative status might be influenced by laparoscopic or open technique of the operation as well as the duration (Anup et al., 1999; Kozlik et al., 2015). It was determined that the laparoscopic tecnique is less traumatic and related to less oxidative stress than open tecnique (Bukan et al., 2004). Furthermore, statistically significant increase in MDA concentration and GPx activity in patients operated over 40 minutes was found compared with the short-term laparoscopy (Kozlik et al., 2015). We thought that the severity of oxidative stress may be related to the duration of surgery since OHE (13 minutes) was longer than the sham operation (6 minutes) in our study. Ovariohysterectomy is also prefered to prevent unwanted pregnancy, vaginal discharge during oestrus and pyometra in the cat and dog. (Johnston et al., 2001). However, it has been indicated that OHE causes to alterations in oxidative/antioxidative balance because of anaesthetic agents in bitches (Naziroglu and Gunay, 1999; Serin et al., 2008; Gunay et al. 2011; Szczubial et al., 2015). Gunay et al. (2011) demonstrated that MDA concentration increased significantly and GSH concentration decreased 24 hours after OHE performed under xylazine-ketamine anaesthesia in dogs. According to Naziroglu and Gunay (1999), serum MDA concentration significantly increased during enflurane anaesthesia of dogs. Furthermore, Alva et al. (2006) revealed that ketamine leads to reduced plasmatic nitric oxide concentrations, generates metabolic asidosis and causes oxidative stress in rats. The present study indicated that OHE performed under xylazine-mine anesthesia concluded with oxidative/antioxidative imbalance on day 1 after OHE, whereas oxidative/antioxidative imbalance did not occur on day 1 after sham-operation. It was thought that oxidative/ antioxidative imbalance may be caused by ligature for removing the genital tract, though both OHE and sham-operation were performed by medial laparotomy with the same anesthetic agents.

In conclusion, our study revealed that lipid peroxidation increased significantly, whereas activities of antioxidant enzymes decreased in rats after OHE under xylazine-ketamine anaesthesia. These results demonstrate that OHE leads to oxidative stress indicating oxidative/antioxidative imbalance in rats.

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