Effect of pinealectomy on homocysteine levels in ovariectomized rats

Esma Oztekin [1], Bahadır Ozturk [1], Rasim Mogulkoc [2], Abdulkerim Kasim Baltaci [2] & Ali Muhtar Tiftik [1]

1. Department of Biochemistry, Meram Medical School of Selcuk University, Konya, Turkey
2. Department of Physiology, Meram Medical School of Selcuk University, Konya, Turkey

Correspondence to: Dr. Esma Oztekin
Deparment of Biochemistry,
Meram Medical School, Selcuk University, 42080, Konya, Turkey
PHONE: +90 332 223 67 92
FAX: +90 332 223 61 81
EMAIL: eoztekin@selcuk.edu.tr

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Abstract

OBJECTIVE: Hyperhomocysteinemia plays a role in obstetric and gynecological diseases and such diseases are seen as a consequence of an impaired methionine cycle. In the present study, it was aimed to determine the effect of estradiol and progesterone on homocysteine levels in pinealectomized and ovariectomized rats.
MATERIALS AND METHODS: The study was carried out on 24 adult Spraque-Dawley female rats. Rats were divided into 4 groups as follows: Group 1: Sham ovariectomy group (Sham-Ovx), Group 2: Ovariectomized and sham pinealectomized group (Ovx-Sham-Px), Group 3: Ovariectomized and pinealectomized group (Ovx-Px), Group 4: Group which were supplemented with estradiol and progesterone after ovariectomy and pinealectomy (Ovx-Px-E+P). Serum homocysteine levels were determined after experimental period.
RESULTS: Homocysteine levels in Group 3 were higher than those in Groups 1, 2 and 4 ($p<0.05$). Homocysteine levels in Groups 2 and 4 were higher than those in Group 1 ($p<0.05$). Homocysteine levels in Groups 2 and 4 were not different.
CONCLUSION: The findings of the present study demonstrate that ovariectomy lead to increases homocysteine levels. Pinealectomy in addition to ovariectomy increases homocysteine levels. However, administration of estradiol and progesterone following pinealectomy prevents the increase in homocysteine levels in ovariectomized rats.

O R I G I N A L A R T I C L E

INTRODUCTION

Homocysteine, a product of the methionine cycle, plays a role in obstetric and gynecological diseases and such diseases are seen as a consequence of an impaired methionine cycle (Obsweger *et al*., 1994). Plasma homocysteine levels are influenced by non-genetic and genetic factors. It was postulated that estrogen status appears to be a non-genetic factor affecting homocysteine metabolism (Pines *et al*., 1997; Wouters *et al*., 1995). Although homocysteine levels appear as a result of the change in hormone condition after menopause, they are considered a risk factor for hyperhomocysteinemia, arteriosclerosis and osteoporosis (El-Sewefy *et al*., 2002; Mijatovic *et al*., 1999). Thus, changes in lipoprotein a, coagulation, fibrinolysis and homocysteine metabolism are regarded related with hormone treatment (Mijatovic *et al*., 1999).

It has been reported that high homocysteine levels in ovariectomized rats decreased with estradiol and folic acid supplementation (El-Sewefy *et al*., 2002). It was concluded that high homocysteine levels in ovariectomized rats was related to the significant increments in atherogenic index (total cholesterol/high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol/HDL cholesterol).

However, high homocysteine levels accelerate endothelial cell damage by producing hydrogen peroxides (Dimitrova *et al*., 2002a). It was stated that estradiol prevents acceleration of endothelial cell damage and reduction in cell viability by increasing the intracellular content of glutathione (GSH) (Dimitrova *et al*., 2002b). Estrogen and especially estradiol have been reported to function like antioxidants (Mijatovic *et al*., 1999). It is believed that estrogen may be among the mechanisms that protect the heart by reducing LDL cholesterol, apolipoprotein B and lipoprotein (a), while increasing HDL cholesterol and apolipoprotein A1. Therefore, the effect of homocysteine on sex hormones is important in assessing cardiovascular risk (Dimitrova *et al*., 2002b).

Melatonin is a hormone secreted by pineal gland (Reiter, 1993a). It plays a role in the control of the reproductive system and affects sexual maturation age, periodicity of estrous cycle and gonadal steroid genesis (Dardes *et al*., 2000). Melatonin has an important role in regulating a variety of functions synchronized light-dark rhythm. It is also known that has important effects on determination of onset of puberty via its effect on hypothalamus-pituitary-gonadal axis (Reiter, 1993a). Melatonin also regulates reproductive physiology of animals in according to seasonal changes (Reiter, 1993b). Photoperiodic animals spontaneously have seasonal fluctuations in body mass, reproductive activity and energy metabolism (Gunduz 2002). Melatonin receptors, which have a suppressive effect on GnRH, are also localized in pars tuberalis cells of anterior pituitary gland (Reiter, 1993b). The discovery of melatonin as a scavenger of oxygen radicals has opened up a new area of biological and medical investigation (Mogulkoc *et al*., 2005; Reiter, 1993a). Studies on the relation of homocysteine with antioxidants demonstrate that high concentrations of melatonin can protect cardiovascular system by decreasing plasma homocysteine levels (Baydas *et al*., 2002).

The aim of present study was to determine pinealectomy and estradiol-progesterone supplementation on serum homocysteine levels in ovariectomized rats.

MATERIALS AND METHODS

This study was carried out at the Selcuk University Experimental Medicine Research and Application Center and was approved by ethics committee of research center. The study included 24 adult female rats of Sprague-Dawley strain, which were 6 months old and weighed 200–250 g. The rats were kept at 18–21 °C room temperature, under 12 hours light/dark cycle (07.00 am–07.00 pm) and fed with standard rat pellet in all groups. The groups were formed as follows:

Group 1 (n=6): Sham ovariectomy (Sham-Ovx)
Group 2 (n=6): Ovariectomy and sham pinealectomy (Ovx-Sham Px)
Group 3 (n=6): Ovariectomy and pinealectomy (Ovx-Px)
Group 4 (n=6): Supplemented with estradiol and progesterone after ovariectomy and pinealectomy (Ovx-Px-E+P)

The rats were ovariectomized under general anesthesia (60 mg/kg ketamine and 5 mg/kg rompune) (Waynfort and Flacknell, 1994). Pinealectomy was performed as described by Kuszack and Rodin under general anaesthesia (Kuszack and Rodin, 1977).

Injections started one week after the operations and continued for 4 weeks. The hormone preparations used were 450 μg/kg Acropholline ampule (estradiol propionate, Biofarma Ilaç) and 15 mg/kg Depoprovera ampule (medroxyprogesterone acetate, Eczacibasi) in the 1st and 3rd weeks and 15 mg/kg Depoprovera ampule in the 2nd and 4th weeks (Acs *et al*., 2000). The injections were intramuscular.

Twenty-four hours after the last injection, rats in all groups were decapitated and blood samples were collected at 09.00 p.m. for homocysteine analysis. Serums were kept at −85 °C until analysis. Serum homocysteine levels (catalogue no: LKH01) were carried out in Immulite 2000 analyser using Immulite commercial kit. Values were calculated as μmol/l.

Statistical Analysis

The SPSS program was used for statistical analysis. Kruskal-Wallis variance analysis and Mann-Whitney U test were conducted. The level of significance was set at $p<0.05$. Homocysteine levels in the study groups were presented as mean ± SD.

RESULTS

The examination of the present revealed that serum homocysteine levels in Group 3 (Ovx-Px) were significantly higher than those in Groups 1, 2 and 4 ($p<0.05$) (Table 1). Homocysteine levels in Groups 2 (Ovx-sham Px) and 4 (Ovx-Px-E+P) were significantly higher than those in Group 1 ($p<0.05$) (Table 1). Homocysteine levels in Group 2 and Group 4 were not different.

Table 1. Serum homocysteine levels in the study groups.

Groups	Homocysteine level (μmol/l)
Group 1: Sham Ovx (n=6)	8.68±0.82 [c]
Group 2: Ovx-sham Px (n=6)	12.60±1.45 [b]
Group 3: Ovx-Px (n=6)	16.83±1.25 [a]
Group 4: Ovx-Px-E+P (n=6)	11.63±0.49 [b]

* Letters superscripted on the right show that differences among the groups in terms of the measured parameter is at $p<0.05$
a>b>c for homocysteine levels

DISCUSSION

It is suggested that high homocysteine concentrations could be a marker and a cause of obstetrical and gynecological disorders (Obsweger *et al*., 1994). It has been showed in rats that homocysteine levels that increased as a result of ovariectomy decreased by estradiol and folic acid supplementation. It was postulated that increased levels of homocysteine after ovariectomy were associated with the significant increase in atherogenic index (El-Sewefy *et al* 2002; Kapral *et al*., 2002). High levels of homocysteine we obtained in ovariectomized rats (Group 2) in comparison to Group 1 are consistent with the findings of the above-mentioned researchers. However, these researchers also reported increased homocysteine levels decreased with estradiol supplementation after ovariectomy (El-Sewefy *et al* 2002; Kapral *et al*., 2002). In our study, administration of estradiol and progesterone following pinealectomy in ovariectomized rats (Group 4) led to a significant decrease in homocysteine levels when compared to Group 3. But these levels in Group 4 were higher than the levels in Group 1 and were not different from those in Group 2. From this aspect, administration of estradiol and progesterone in ovariectomized-Px rats reduced homocysteine levels compared to Ovx-Px group. Decreased levels of homocysteine in Group 4 were not different from those in Group 2, and even higher than the levels in Group 1. In this respect, findings of our study are similar by other researchers. However, in our study we supplemented not only estradiol, but also progesterone to ovariectomize and pinealectomized rats. Other researchers obtained a decrease in homocysteine levels by administering only estradiol to ovariectomized rats. The difference between our results and those of other researchers can be attributed to this variation of supplementations.

Recently, attention has been focused on the relation between homocysteine and antioxidants. It was demonstrated in a number of studies that homocysteine increased free radical formation (Okatani et. al, 2001a; Okatani et. al, 2000). Thus, studies investigating the relation between homocysteine and melatonin, a strong free radical scavenger, have begun to attract more interest (Okatani et. al, 2001a; Okatani et. al, 2000; Okatani *et al*., 2001b). It was noted that high levels of homocysteine is a factor increasing free radical formation, whereas melatonin prevents this formation (Baydas *et al*., 2003). It was also reported that exogenous melatonin supplementation for 1 week led to an insignificant decrease in plasma homocysteine levels in control rats, whereas exogenous melatonin administration for 6 weeks brought about a significant decrease (Baydas *et al*., 2002a). It was assumed that high concentrations of melatonin can protect cardiovascular system by reducing plasma homocysteine levels (Baydas *et al*., 2002a). It was reported in another study by the same researchers homocysteine levels in pinealectomized rats were significantly higher than those in sham and control group rats (Baydas *et al*., 2002b). Therefore, low levels of melatonin result from pinealectomy increases homocysteine levels (Reiter, 1993a). In our study, despite the use of different materials and methods, pinealectomy significantly increased homocysteine levels in ovariectomized rats. Our finding to the effect that pinealectomy increased homocysteine levels is parallel to the findings of the aforementioned researchers. However, as opposed to researchers studying the relation between pineal gland and homocysteine, we planned to study how pinealectomy affected homocysteine concentration in ovariectomized rats. In this respect we could not find a study completely comparable with ours. It can be recommended to study the relation between homocysteine levels and administration of melatonin following ovariectomy in addition to ovariectomy and pinealectomy application.

In conclusion, the results of our study show that ovariectomy causes an increase in homocysteine levels, this increase much more after pinealectomy. However, this increase can be normalized by estradiol and progesterone supplementation.

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