Effects of progesterone and estradiol on the inflammatory and apoptotic markers of ovariectomized rats challenged with acute septic systemic inflammation

Sinan Subhi Farhan1, Samir Saad Mahgoub2, Saad Abdulrahman Hussain3,*

1Faculty of Pharmacy, Al Rafidain University College, Baghdad, Iraq.
2Department of Biochemistry and Molecular Biology, Faculty of Medicine, Al Minia University, Minya, Egypt.
3Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al Rafidain University College, Baghdad, Iraq.

ARTICLE INFO
Received on: 12/07/2019
Accepted on: 17/10/2019
Available online: 03/12/2019

Key words:
Septic systemic inflammation, liver, estradiol, progesterone, apoptosis.

ABSTRACT
The inflammatory responses during septic inflammation were affected by the differential role of progesterone and estrogen that demonstrated pro-inflammatory and anti-inflammatory roles. This study was designed to evaluate the differential effects of estradiol and progesterone supplementation on the inflammatory and apoptotic responses in an ovariectomized (OVX) rat model of acute systemic septic inflammation (SSI). This study was conducted on 60 female Wistar rats. 40 mg/kg estradiol and 5 mg/kg progesterone were given subcutaneous (s.c.) to OVX rats, after the induction of SSI through caecum puncture with a 21-gauge needle. Serum levels of tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), Alanine transaminase (ALT), estradiol, and progesterone were evaluated; additionally, Inducible nitric oxide synthase (iNOS), Cyclooxygenase (COX)-II, and caspase-3 were evacuated in liver tissue homogenates using the Enzyme-linked immunosorbent assay (ELISA) method. In OVX rats challenged with SSI, serum TNF-α, CRP, and ALT levels were significantly increased associated with a decrease in serum estradiol levels. They also showed overexpression of iNOS and increased the activity of COX-II and caspase-3 in the liver compared to non-OVX rats subjected to SSI. Supplementation with estradiol significantly decreases all serum and liver tissue markers of inflammation and decreased apoptosis. In contrast, in OVX rats supplemented with progesterone, SSI resulted in a significant increase in the studied markers. In conclusion, the supplementation of estradiol in OVX rats challenged with SSI significantly attenuated the systemic and liver inflammatory and apoptotic markers. Meanwhile, the supplementation with progesterone exacerbates the effects of the inflammatory markers and increases the tendency of apoptosis in the liver tissue.

INTRODUCTION
The inflammatory response during sepsis was associated with overexpression of different inflammatory markers including interleukin-6 (IL-6) and TNF-α, which are involved early in the pathogenesis of septic shock, apoptosis, and tissue damage (Bohannon et al., 2012; Jedynak et al., 2012; Lowes et al., 2013). During the advanced stage of septic shock, the increase in hepatic functions results in excessive hepatocellular apoptosis, a critical step in acute hepatic failure that complicates systemic sepsis (Marshall, 2001). Estradiol and progesterone have various immunomodulatory impacts that may be effectively involved in the pathophysiology of sepsis (Dellinger et al., 2008). However, the effects of elevated levels of both hormones in the prediction of the severity and outcomes of acute hepatic injury during septic shock remain controversial (Angstwurm et al., 2005; Szalay et al., 2006). Several studies have investigated the differential effects of estradiol and progesterone on the systemic inflammatory responses of various organs and tissues. It has been reported that estrogen therapy in postmenopausal females increases the hepatic production of C-reactive protein (Cushman et al., 1999) and the expression of IL-6, suggesting a pro-inflammatory role (Herrington et al., 2001). Meanwhile, other
investigators demonstrated contradictory results for estradiol in this regard (Sunday et al., 2006; Wakatsuki et al., 2004). Moreover, the effects of progesterone in systemic inflammation represent a lot of controversies. In one study, progesterone augments the outcome of experimental stroke (Rosano et al., 2000), while another study indicates that progesterone resolves oxidative stress, reduces the production of IL-6 and TNF-α, and ameliorates the sepsis syndrome (Aksoy et al., 2014). This study was designed to evaluate the differential effects of estradiol and progesterone supplementation on the inflammatory and apoptotic responses in an OVX rat model of acute septic inflammation.

MATERIALS AND METHODS

Animals

Sixty female Wistar rats (200–250 gm) were used in the study. The animals were housed in the animal house, Faculty of Medicine, Mu'tah University under standardized conditions of temperature and humidity with 12:12 hour light/dark cycle and fed standard rodent chow and tap water ad libitum. The study protocol was approved by the local Committee of Research Ethics (REC-345-2017) in compliance with the international standard care of experimental animals (Canadian Council on Animal care, updated in 2017).

Study design

The rats were randomly allocated to six groups (10 rats each) as follows: group I, served as a negative control group and subjected to Sham ovariectomy procedure (Sh-OVX); group II, a positive control where the Sh-OVX was followed by the induction of a septic systemic inflammation (SSI) 2 weeks later; group III, exposed to OVX followed by the induction of SSI 2 weeks later (Fink and Heard, 1990); group IV, after OVX, each rat was administered a daily subcutaneous (s.c.) estradiol dose of 40 mg/kg body weight followed by the induction of SSI 2 weeks later; group V, after OVX, each rat received 5 mg/kg/day progesterone s.c. followed by SSI induction 2 weeks later (Tsai and Legan, 2002); group VI, OVX is followed by the administration of a combination of estradiol and progesterone doses (as mentioned previously) followed by SSI induction 2 weeks later.

Surgical intervention and induction of septic systemic inflammation

After short anesthesia with diethyl ether, the rats in groups I and II were subjected to Sham OVX; while the rats in groups I–IV were subjected to bilateral ventral OVX followed by the hormonal supplementation (as mentioned above). In all rats (except group I), SSI was induced after 2 weeks. The induction of inflammation was performed by ileocecal ligation and punctured using a 21-gauge needle. After 24 hours of sepsis induction, a blood sample was obtained through a direct cardiac puncture under mild anesthesia; then, the animals were euthanized to extract livers for the assay of the tissue markers of inflammation and apoptosis according to standard procedures (Aksoy et al., 2014).

Measurements of markers

The obtained blood samples were kept in plain tubes and left to clot. The collected sera were used for the assay of estradiol, progesterone, TNF-α (Mizutani et al., 2003), Alanine transaminase (ALT) activity, and C-reactive protein levels (Gewurz et al., 1982) using ready-made kits (Biomatic, Ontario, Canada). The rate of expression of Inducible nitric oxide synthase (iNOS) and the levels of Caspase-3 and Cyclooxygenase (COX)-II in the liver tissue homogenates were analyzed using Enzyme-linked immunosorbent assay (ELISA) kits (MyBiosource, USA) according to the specifications of the manufacturer.

Statistical analysis

The results were expressed as mean ± standard deviation (SD), unpaired Student’s t-test and analysis of variance (ANOVA) confirmed with Bonferroni’s post hoc test were utilized to evaluate the differences between groups. A p-value < 0.05 was considered for significant differences.

RESULTS

Table 1 shows that serum levels of progesterone were significantly increased in groups IV, V, and VI (72%, 225%, and 268%, respectively) compared to the corresponding levels in group I; while progesterone levels were not significantly changed in groups II and III (p > 0.05) versus controls. Moreover, serum levels of estradiol in groups III and V were found to be significantly lower than those obtained in group I (79% and 43%). Meanwhile, group VI demonstrates a significantly higher level of serum estradiol compared to all other groups (p < 0.05). Additionally, Table 1 shows that the level of TNF-α was increased in groups II, III, V, and VI (636%, 726%, 795%, and 213%, respectively) compared to group I. However, serum TNF-α level in group IV was not significantly changed versus the controls (p > 0.05) and found to be significantly lower than that reported in other groups. A similar pattern of changes was reported in serum C-reactive protein (CRP) levels, where groups IV and VI demonstrated significantly lower levels compared to the other groups, but they are still significantly higher than that of the control group (43% and 36%, respectively). In Figure 1, serum ALT activity was significantly elevated in all test groups compared to the controls. However, serum ALT levels in groups IV and VI were

Table 1. Effects of estradiol and progesterone supplementation on their serum levels and the concentrations of CRP and TNF-α in OVX rat challenged with SSI.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Animal groups (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
</tr>
<tr>
<td>S. Progesterone (ng/ml)</td>
<td>11.6 ± 1.4b</td>
</tr>
<tr>
<td>S. Estrogen (pg/ml)</td>
<td>49.6 ± 5.8a</td>
</tr>
<tr>
<td>S. CRP (μg/ml)</td>
<td>1.4 ± 0.4a</td>
</tr>
<tr>
<td>S. TNF-α (pg/ml)</td>
<td>11.5 ± 1.2a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD; n: number of rats in each group; * significantly different compared to the controls (unpaired t-test, p < 0.05); values with non-identical superscripts (a,b,c,d,e) among different groups are significantly different (ANOVA, p < 0.05).
found to be significantly lower than the other test groups (II, III, and V). Figure 2 indicates that the activity of caspase-3 was significantly elevated in all test groups compared with the sham-operated without the SSI group of rats ($p < 0.05$). The highest levels of caspase-3 were reported in groups II and III, and the lowest degree of elevation in caspase-3 level was reported in group III. There was no significant difference in the liver tissue level of caspase-3 between groups V and VI ($p > 0.05$). Regarding the influence of estradiol and progesterone supplementation to OVX rats challenged with SSI on the expression of COX-II in the liver, Figure 3 shows that COX-II expression was significantly elevated in all test groups compared to the control group, and the highest levels of expression were reported in groups II and III. Meanwhile, the levels of COX-II expression in groups V and VI were found non-significantly different ($p > 0.05$). Additionally, the expression of iNOS in the liver tissue was significantly elevated in groups II, III, and V versus the controls (Fig. 4). Meanwhile, iNOS expression in groups IV and VI was not significantly different from that reported in the control group and found to be comparable when compared with each other ($p > 0.05$).

**DISCUSSION**

In this study, Table 1 shows that SSI induction was associated with a significant reduction in serum estradiol level in group III, which can be attributed to the excessive production of NO that leads to direct inhibition of Gonadotropin-releasing hormone (GnRH) synthesis (Herrington et al., 2001). Moreover, previous reports suggested that the pro-inflammatory mediators directly inhibited the process of steroidogenesis in the ovaries (Son and Roby,

![Figure 1](image1.png)

**Figure 1.** Effects of estradiol and progesterone supplementation on serum ALT levels in OVX rat challenged with SSI. Number of rats: 10 in each group; values with non-identical letters (a,b,c,d,e) are significantly different (ANOVA, $p < 0.05$).

![Figure 2](image2.png)

**Figure 2.** Effects of estradiol and progesterone supplementation on liver tissue caspase-3 activity in OVX rat challenged with SSI. Number of rats: 10 in each group; values with non-identical letters (a,b,c,d,e) are significantly different (ANOVA, $p < 0.05$).

![Figure 3](image3.png)

**Figure 3.** Effects of estradiol and progesterone supplementation on liver tissue COX-II levels in OVX rat challenged with SSI. Number of rats: 10 in each group; values with non-identical letters (a,b,c,d) are significantly different (ANOVA, $p < 0.05$).

![Figure 4](image4.png)

**Figure 4.** Effects of estradiol and progesterone supplementation on liver tissue iNOS levels in OVX rat challenged with SSI. Number of rats: 10 in each group; values with non-identical letters (a,b,c,d) are significantly different (ANOVA, $p < 0.05$).
Those hormones in the regulation of iNOS expression (Yilmaz et al., 2013) have been mentioned that both progesterone- and estradiol-enhanced iNOS expression supporting the involvement of those hormones in the regulation of iNOS expression (Ogando et al., 2003). Our finding in this respect was in tune with the many data reported by other researchers (Buhimschi et al., 2000; Hassouna et al., 2014) regarding the negative effect of progesterone on the hepatic NO levels, whereas Al-Hijji et al. (2001) reported the stimulatory effect of progesterone administration on uterine NOS activity (Al-Hijji et al., 2001). These variable effects on iNOS expression could be attributed to the use of different types of cell preparations or animal models. In experimental animals, previous data indicated that the administration of estradiol to OVX rats causes overexpression of uterine COX-II mRNA compared to the use of progesterone alone (Engstrom, 2001). Regarding the impacts on COX-II, the presented data were in tune with the previous finding; where lowest COX-II expression was observed in group V supplemented with progesterone alone and a little bit higher in group VI after supplementation with estradiol/ progesterone combination. However, both findings conflicted with that reported by Hassouna et al. (2014) that suggested a generalized decrease in COX-II expression in the liver tissues during estradiol supplementation in physiological doses. This study revealed the reduction of liver tissue caspase-3 expression in the groups treated with estradiol, progesterone, or their combination. A similar finding mentioned that treatment with progesterone was associated with a decreased caspase-3 activity (Karatepe et al., 2012). Moreover, Da et al. showed that estradiol downregulates the expression of caspase-3 in the lacrimal and submandibular glands of OVX rats due to the anti-apoptotic and antioxidant effects (Da et al., 2015). In this regard, Xue et al. (2017) attributed the progesterone-induced reduction of caspase-3 expression to the inhibition of the NF-kB pathway in the OVX rats (Xue et al., 2017).

CONCLUSION

The supplementation of estradiol in OVX rats challenged with SSI significantly attenuated the systemic and liver inflammatory and apoptotic markers of acute systemic inflammation. Meanwhile, the supplementation with progesterone doses exacerbates the effects of the inflammatory mediators and increases the tendency of apoptosis in the liver tissues.

ACKNOWLEDGMENT

The authors thank Al-Rafidain University College for supporting the project.

CONFLICT OF INTEREST

The authors have declared that no competing interest exists.

FUNDING

No specific fund received.

REFERENCES


Buhimschi IA, Yallampalli C, Buhimschi CS, Saade GR, Garfield RE. Distinct regulation of nitric oxide and cyclic guanosine monophosphate


How to cite this article: