Effects of Gender on the Severity of Sepsis

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Abstract

**Purpose.** To investigate the differences between male and female rats and the effects of sex hormones on tissue changes in the lung and liver in a sepsis model.

**Methods.** Sixty Sprague-Dawley rats were divided into six groups of ten. Groups 1 and 2 were the control male and female groups, respectively, subjected only to sepsis; groups 3 and 4 were the male and female groups, respectively, subjected to sepsis, then given 0.04 mg/kg estrogen + progesterone (E-P) intramuscularly (i.m.); and groups 5 and 6 were the male and female groups, respectively, subjected to sepsis, then given 0.5 mg/kg testosterone (T) i.m. The rats were killed and the histopathological changes in the lung and liver were examined, and plasma endotoxin levels were measured.

**Results.** Histopathological examination revealed less congestion, portal inflammation, and focal necrosis of the liver, and less congestion, edema, and emphysematous and inflammatory changes in the lung in the E-P groups than in the other groups. Moreover, signs of systemic endotoxemia in plasma were proportionally less in the female rats and in the E-P groups than in the male rats and the T groups.

**Conclusion.** Female rats subjected to sepsis showed less liver and lung tissue damage and less systemic endotoxemia than male rats, because of the effects of female sex hormones.

**Key words** Sepsis · Gender · Lung · Liver · Endotoxemia

Introduction

Sepsis has always been a major health problem because it is difficult to treat and is associated with high mortality. Despite aggressive treatment and a better understanding of the molecular aspects of septic shock, the mortality rate remains as high as 40%–70%.1–4 Therefore, determining the factors affecting prognosis and identifying the population of patients with a bad prognosis is very important. Determining the factors affecting prognosis and the application of new management strategies are rational approaches.4

Many studies have been published on the effect of gender and sex hormones on the severity of sepsis,5–7 but few studies have examined the effect of gender and sex hormones on the histopathological changes in the lung and liver, and systemic endotoxemia in severe sepsis. Pathological investigations suggest that lipopolysaccharide may cause pulmonary alveolar congestion, edema, exudation, capillary dilatation, and inflammatory cell infiltration in liver and lung tissue.8–10 However, the relationship between these changes and gender has never been reported. The lung and liver have a rich macrophage population and are considered to be the first gate for endotoxins.11 Subsequently, most tissue damage is expected to occur in these two organs. Thus, we examined the histopathological changes in these organs. This study was done to investigate the differences between male and female rats and the effects of sex hormones on tissue changes in the lung and liver in an experimental sepsis model.

Materials and Methods

This study was done at the Experimental Research Centre of Selçuk University with the approval of the Ethical Committee. We used 60 Sprague-Dawley rats weighing 250–290g (range: 275 ± 17g) and divided...
them into six groups of ten. Sepsis was induced in all six groups by cecal ligation and puncture (CLP).

Group 1: Control male group, subjected to CLP only
Group 2: Control female group, subjected to CLP only
Group 3: E-P male group, subjected to CLP, then given 0.04 mg/kg estrogen + progesterone (E-P; Dipro, Organon, Oss, the Netherlands) intramuscularly (i.m.)
Group 4: E-P female group, subjected to CLP, then given 0.04 mg/kg E-P (Dipro) i.m.
Group 5: T-male group, subjected to CLP, then given 0.5 mg/kg testosterone (T; Sustanon, Organon) i.m.
Group 6: T-female group, subjected to CLP, then given 0.5 mg/kg T (Sustanon) i.m.

Sepsis Model

Sepsis was induced by using the CLP model. After anesthesia with ketamine hydrochloride (60 mg/kg), all the animals were restrained in the supine position, shaved, and a 2-cm midline incision was made under sterile conditions. The cecum was isolated and tied with 5-0 silk, ligating just below the ileocecal valve without interrupting continuation of the small bowel and colon. The cecum was then punctured twice with a 22-gauge needle and a small amount of bowel content was extruded through the puncture holes. The ligated and punctured cecum was returned to the peritoneal cavity, and the abdominal cavity was closed in two layers with 4-0 silk sutures. Normal saline (20 mg/kg body weight) was then administered subcutaneously to replace insensible losses.

Following abdominal closure, 0.04 mg/kg E-P or 0.5 mg/kg T were given by i.m. injection to the respective groups. The drug doses were calculated per kilogram body weight according to the manufacturer’s recommended dose for humans. After the above procedures the rats were separated into groups and placed in special cages under controlled temperature, moisture, and lighting conditions. All rats were fed standard rat chow and allowed water ad libitum. Blood estrogen levels were measured after the rats were killed. The basal estrogen level was 43.2 ± 8.6 pg/ml in the control female group and 61.9 ± 11.6 pg/ml in the E-P female group.

Endotoxin Measurement

All rats were anesthetized again 24 h after the above operation and 7 ml blood was taken by cardiac puncture, 5 ml of which was used for the plasma endotoxin level measurement and centrifuged at 3000 cycles/min for 5 min. The isolated plasma was kept at −80°C until the day of the study.

Endotoxemia was determined by the Limulus Amebosit Lisat (LAL) method using an E Toxate (Sigma, St. Louis, MO, USA) kit. While interpreting the tests, experimental tubes were evaluated for gel formation. Hard gel formation was regarded as positive, whereas soft gel formation, turbidity, slight increase in viscosity, and clear liquid were regarded as negative. Accordingly, graduated changes in the tubes were interpreted as follows: soft gel: (−), (+), (++); hard gel: (+++), (+++).

Histopathological Examination

After relaparotomy, tissue specimens were taken from the lung and liver for pathological examination. Tissue specimens were fixed in a 10% formaldehyde solution with a phosphate tampon. The materials were fixed in 10% buffered paraformaldehyde, prepared with Autotechnicon, and then embedded in paraffin. Two slices were obtained from each sample with a microtome and stained with hematoxylin–eosin. The specimens were obtained and photographed using a photomicroscope (Nikon Eclipse E400, Nikon Coolpix 5000; Nikon, Tokyo, Japan). Congestion and portal inflammation were examined in the liver, and congestion, edema, and emphysematous and inflammatory changes were examined in the lung.

Statistical Analysis

Statistical analysis among the groups was done by the chi-square ($\chi^2$) test, and a P value of less than 0.05 was regarded as significant.

Results

One rat from group 1 and one rat from group 6 died before the completion of the study, and new rats were instated and subjected to the same procedure. Tables 1 and 2 summarize the histopathological changes observed in the liver and lung, respectively, and Table 3 shows the plasma endotoxin levels in the six groups of rats just after they were killed.

Liver

Histopathological examination of the liver tissue samples revealed significantly less congestion, portal inflammation, and focal necrosis in the E-P groups than in the T groups and control groups (Figs. 1 and 2).