ACUTE EFFECT OF OVARIOHYSTERECTOMY ON LIPID PEROXIDATION AND SOME ANTIOXIDANT LEVELS IN DOGS

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Abstract

The aim of this study was to investigate the acute effect of spaying on antioxidant defence mechanism and lipid peroxidation in healthy bitches. Thirty-one healthy bitches were used in the study. All the animals were anaesthetised with xylazine-ketamine combination and subjected to total ovariohysterectomy (OVH). The blood glutathione (GSH), plasma malondialdehyde (MDA), ceruloplasmin, β-carotene, and retinol levels were determined in the preoperative and early postoperative periods. There was a significant decrease in blood GSH levels and an increase in plasma MDA activity at early postoperative stage as compared to the stage before the operation. There were no significant differences in the concentration of β-carotene, retinol, and ceruloplasmin levels between the preoperative and early postoperative periods. In conclusion, these findings indicate some alterations in the antioxidant defence system in the early postoperative stage. The assessment of GSH and MDA levels can be useful for the evaluation of oxidative stress after OVH in bitches.

Key words: bitches, ovariohysterectomy, oxidative stress, antioxidants.

The trauma of a surgical procedure may contribute to oxidative stress. Ischaemia-reperfusion process, the increasing of free iron and copper released from tissues, and activation of the inflammatory response increase the oxidation and lipid peroxidation in patients (2). Serous inflammation in the peritoneum caused by surgery may also lead to the oxidative stress (6).

Antioxidant defence mechanism includes natural antioxidant molecules, such as glutathione, ceruloplasmin, β-carotene, and retinol. Glutathione (GSH) is a major non-enzymatic antioxidant, involved in several reactions in vitamin metabolism. It also plays an important role in cell membrane protection against lipid peroxidation by free radicals (12). Ceruloplasmin (Cp) is a plasma metalloprotein synthesised and involved in peroxidation of Fe(II) to Fe(III). Ceruloplasmin is a multifunctional protein that behaves as an acute-phase reactant. It tends to be raised after tissue damage or inflammation, and it is a marker of severity of the inflammatory process (9). Retinol works as chain-breaking and preventive antioxidant (10). The β-carotene can function as an effective radical-trapping antioxidant. However, β-carotene represents an unknown class of biological antioxidants. There is ample evidence that β-carotene is a very effective quencher of singlet oxygen (5, 8, 13). Malondialdehyde (MDA) is a low molecular weight aldehyde that results from free radical attacks on polyunsaturated fatty acids (PUFA) localised in the cell membrane. The amount of MDA is used to evaluate the severity of lipid peroxidation (11).

Ovariohysterectomy (OVH) is the most common contraception method in pets. Many bitches are exposed to spaying every year. Preventing postoperative complications largely depends on proper monitoring of the health condition of bitches. However, there is the lack of data in literature concerning the oxidative stress in canine surgery. Therefore, the purpose of this research was to compare the effect of OVH on the levels of GSH, MDA, Cp, β-carotene, and retinol in bitches at the preoperative and postoperative periods.

Material and Methods

Animals. Thirty-one mongrel clinically healthy bitches, aged one to five years, weighing between 15 and 28 kg were used in this study. All the animals were kept at the same place and under identical feeding conditions. Prior to the operation, the animals were examined generally for any infection and tumoural disease. No image indicating uterine pathology or pregnancy was observed via ultrasonographic examinations.

OVH operation. The bitches were anaesthetised by an injection of xylazine (2 mg/kg b.w., Alfazyne-Alfasan, the Netherlands) and ketamin HCl (15 mg/kg b.w., Alfamine-Alfasan, the Netherlands) after a 24 h fasting period. OVH were performed by medial laparotomy, according to the routine methods
The time of operations did not exceed 60 min including the application of anaesthesia.

**Plasma and serum samples collection.**
Venous blood samples were taken just prior to the beginning of anaesthesia and 24 h after the operation. The samples were transferred into tubes containing lithium heparin as anticoagulant and were used for the determination of serum and plasma parameters. The sera were separated from blood and all the samples were stored at -20°C until analyses.

**Biochemical analyses.** The blood GSH level was determined by the measurement of products of peroxidative processes (23). Various methods are used to determine the level of lipid peroxidation in the organism and the most frequently used method is the determination of MDA levels (11).

The blood oestrogen level decreases gradually and reaches a baseline level in several days after OVH (14). It is well-known that a decrease in serum oestrogen levels in surgical menopause is responsible for a change in the oxidant and antioxidant status six weeks after operation. (15). With regard to the data obtained in this study at the 24 h after the operation, changes in anti-oxidative/oxidative balance that depend on oestrogen depletion are unexpected.

In this study, we demonstrated the increase in lipid peroxidation and decrease in GSH function after OVH in bitches under xylazine-ketamine anaesthesia. Our data is consistent with the data of other authors, who showed the increase in lipid peroxidation at the early stage after major abdominal surgery (20), total abdominal hysterectomy (22, 27), and laparoscopic surgery to remove uterine myomas or ovarian cysts (7). Our data also agrees with the data of Luo et al. (17), who reported that the GSH concentration in plasma was by 20% lower 24 h after elective abdominal surgery.

During the general anaesthesia, the metabolism of anaesthetic agents may be toxic and may cause degenerations in tissues and blood cells. Naziroglu and Gunay (19) reported that the significant oxidative effect in dogs was caused by enflurane anaesthesia. In their study, an increase in serum MDA, retinol levels, and a decrease in the GSH levels were observed after anaesthesia. However, Alva et al. (1) observed no changes in the serum MDA level; however, there was a decrease in the GSH levels in rats after xylazine-ketamine anaesthesia. Our data shows a significant increase in MDA and decrease in glutathione levels in serum 24 h after anaesthesia, compared to the levels before operation. This change of GSH and MDA levels could be connected with perioperative hyperthermia. Anaesthetic agents and bleeding may induce hyperthermia in dogs during operation. To maintain the thermoregulation, skeleton muscle activity increases in the early postoperative stage. The response to hyperthermia is variable from mild shivering to convulsions. Shivering can increase tissue oxygen demands by as much as 400%-500% (3). In this respect, the increasing oxygen consumption at early postoperative stage could be responsible for oxidative stress in bitches.

### Table 1

Mean levels of preoperative and postoperative blood and plasma parameters in bitches subjected to OVH (n=31)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre OVH</th>
<th>Post OVH</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (mg/dL)</td>
<td>26.80±0.99</td>
<td>20.16±0.81</td>
<td>***</td>
</tr>
<tr>
<td>Cp (mg/dL)</td>
<td>7.70±0.73</td>
<td>7.45±0.81</td>
<td></td>
</tr>
<tr>
<td>MDA (µmol/L)</td>
<td>16.61±1.80</td>
<td>30.52±2.56</td>
<td>***</td>
</tr>
<tr>
<td>β-carotene (µg/dL)</td>
<td>14.00±0.95</td>
<td>13.87±0.95</td>
<td></td>
</tr>
<tr>
<td>Retinol (µg/dL)</td>
<td>37.53±2.16</td>
<td>35.76±2.04</td>
<td></td>
</tr>
</tbody>
</table>

*** P<0.001.

**Statistical analysis.** The biochemical data are expressed as the mean ± standard error. The Student’s t test was carried out for a comparison between the preoperative and postoperative data, with P<0.05 set as the limit of significance.

**Results**

The mean values of the investigated parameters and differences between them before and after OVH operation are presented in Table 1. As can be seen from the Table, a highly statistically significant increase in MDA and a decrease in the GSH levels were observed at the 24 h after OVH (P<0.001). However, there were no significant differences between the preoperative and postoperative stage in plasma levels of Cp, β-carotene, and retinol.

**Discussion**

The postoperative state is accompanied by a series of metabolic changes, involving a compensatory response to the stress of surgery. It is well-known that anaesthesia, hypothermia, serous inflammation, ischaemia of tissues, and postoperative pain induce oxidation and lipid peroxidation (1, 2, 6, 16, 17, 19, 29). The corruption of the balance between production and neutralisation of reactive oxygen species leads to activating enzymatic and non-enzymatic defence systems. There are among them glutathione peroxidase, superoxide dismutase, catalase, as well as water and lipid soluble vitamins, glutathione, and urates. Ceruloplasmin and GSH function as major preventive antioxidants, while retinol and β-caroten function as chain-breaking antioxidants (10). Any disturbance in the anti-oxidative/oxidative balance can be detected by the determination of products of peroxidative processes (23).
In this study, there were no significant differences between preoperative and postoperative serum β-carotene and retinol levels. This condition could be associated with the situation that ketamine anaesthesia has no effect on hepatic function. Alva et al. (1) reported that ketamine led to a moderate oxidative stress without risking hepatic toxicity.

Ceruloplasmin is an acute phase protein in dogs and is responsible for the translating of copper in plasma. Although it is a well-known major preventive antioxidant, it is not used in the diagnosis of dogs as commonly as other acute phase proteins (18). Pinoeiro et al. (21) reported that serum Cp level was increasing within 24 h after operation in dogs. In contrast to this, there was no significant difference in plasma levels of Cp at the 24 h after OVH in our study (Table 1). The lack of differences in serum Cp levels in our study could be explained by no major postoperative complications (intraoperative and/or postoperative haemorrhage, peritonitis, and anaesthetic complication) detected in the bitches.

In conclusion, the OVH used as a primary contraception method leads to alterations in anti-oxidative/oxidative balance in bitches anaesthetised with xylazine-ketamine. Within 24 h after OVH, blood GSH and plasma MDA levels were changed significantly. Thus, evaluation of GSH and MDA levels gives valuable information about the response to operation and may serve to monitor the early postoperative stage for preventing surgical and anaesthetic complications.

References