ACTIVITY OF ANTIOXIDANT ENZYMES IN UTERINE TISSUES OF BITCHES WITH PYOMETRA

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Abstract

The aim of the study was to determine the activities of glutathione peroxidase (GSH-Px), superoxide dismutase, and catalase in the uterine tissues of bitches with pyometra. The uterus from 11 bitches with pyometra and from 18 healthy bitches, which had undergone sterilisation (controls), were used. The activities of enzymes were determined in uterine tissue homogenisates using spectrophotometric methods. Lower GSH-Px activities in the uterine tissues of bitches with pyometra indicate deteriorated capacities of cells to protect against ROS and suggest possible involvement of oxidative stress in the aetiology and pathogenesis of pyometra. Further studies, however, are required to thoroughly elucidate this issue.

Key words: bitches, uterus, pyometra, antioxidant enzymes.

Although reactive oxygen species (ROS) are unavoidable intermediates during metabolism, and may have also beneficial effects, their level and balance between their production and degradation must be controlled. Small amounts of ROS are necessary for numerous vital processes in the organism (16, 29, 30). However, their uncontrollable increased generation results in oxidative damage to almost all cellular components, which, in turn, leads to the injury of cell membranes, degradation of cell structures, cell lysis, and tissue damage (12).

Human beings and animals are equipped with defensive mechanisms to protect them against harmful effects of ROS by maintaining the production of ROS at a level safe for cells (11, 13). The essential elements of antioxidative protection include antioxidant enzymes: glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) (11, 13, 36).

Glutathione peroxidase (GSH-Px) is a tetramer, whose each subunit contains one selenium atom bound with cysteine (24). Its main function is to remove hydrogen peroxide (34). GSH-Px catalyses the hydrogen peroxide-glutathione reaction, which results in the oxidation of glutathione. Moreover, GSH-Px reduces organic peroxides, mainly lipid peroxides, to alcohols (34). In cases of lipid peroxides, this discontinues the process of lipid peroxidation.

In mammals, superoxide dismutase (SOD) occurs in three distinct forms varying in a cofactor required for their proper functioning and location (10). The dismutase dependent on zinc and copper ions (ZnCu-SOD) occurs in the cytoplasm; its activity constitutes about 70% of the total SOD activity. The manganese-dependent dismutase (Mn-SOD) occurs in the mitochondria. The third form of SOD present in the plasma is the extracellular superoxide dismutase (EC-SOD). The mechanism of SOD-catalysed reactions consists of the reduction and oxidation of metal ions in the active centres. The enzyme catalyses the reaction of dismutation of superoxide anion radicals to hydrogen peroxides (10).

Catalase (CAT) is a tetramer composed of four subunits, about 60 kDa, each. In mammalian cells, CAT occurs predominantly in peroxysomes (24). Together with glutathione peroxidase, it prevents the accumulation of hydrogen peroxide in the cell, degrading it to water and molecular oxygen (34). CAT is believed to play an essential role at high concentrations of hydrogen peroxide. At low concentrations of hydrogen peroxide, GSH-Px is considered more relevant (10).

At present, ROS are implicated in the aetiology and pathogenesis of many diseases (1, 2), including gynaecological diseases (6, 19, 25, 27, 31). Studies in humans have demonstrated that the mechanisms of antioxidative protection are impaired in many uterine diseases (6, 19, 25, 27, 31).

Pyometra is the most common gynaecological disease observed in bitches (5, 32). Generally, it occurs from 4 weeks to 4 months after oestrus and is characterised by the accumulation of purulent secretion in the enlarged uterus; in some cases, vaginal purulent discharge (open-cervix pyometra), and general clinical symptoms (polyuria, polydipsia, apathy, inappetite, vomiting, and enlarged abdominal integuments) are observed (4, 32). The aetiology of pyometra in bitches is
complex and has not been fully elucidated. The disease is believed to be primarily caused by hormonal disorders, resulting in cystic proliferation of the uterine mucosa glands; bacterial infections induced mainly by *Escherichia coli* are considered the secondary cause (4). The available literature lacks data concerning the antioxidative status in bitches with pyometra.

The aim of the present study was to determine the activities of antioxidative enzymes: glutathione peroxidase, superoxide dismutase, and catalase in the uterine tissues of bitches with pyometra.

**Material and Methods**

The study was performed on 29 bitches of various breeds and mongrels. The bitches were divided into two groups. Group I comprised bitches suspected of suffering from pyometra (*n*=11), aged 5-12 years. All dogs of this group showed polydipsia and a reduced or complete lack of appetite (anorexia). Physical examinations revealed apathy, enlarged abdominal integuments, and an increased pulse and respiration rates in most of the bitches. In all animals, morphological observations indicated leukocytosis with a left image shift. Abdominal ultrasound (Pie Medical Scanner 200) and the linear head 5/7.5 MHz revealed an enlarged uterus of diameter ranging from 2 to 4 cm with a hypoechochogenic content.

Vaginoscopy indicated congested serous membranes of the vaginal vestibule and vagina, which could be seen to be covered with purulent secretions in bitches with patent pyometra (*n*=3). On the basis of these findings, the bitches were assigned to the pyometra group. Group II comprised 18 bitches, aged 2-7 years, brought for sterilisation. On the basis of abdominal ultrasound and the results of routine haematological and biochemical examinations revealed apathy, enlarged abdominal integuments, and an increased pulse and respiration rates in all animals, morphological observations indicated leukocytosis with a left image shift. Abdominal ultrasound (Pie Medical Scanner 200) and the linear head 5/7.5 MHz revealed an enlarged uterus of diameter ranging from 2 to 4 cm with a hypoechochogenic content.

The fragments of the entire wall of the uterine body, about 2×2 cm in size, were collected immediately after surgery, washed with 0.9% NaCl, frozen at -76°C, and kept until laboratory tests. In pyometra cases, the samples of pus were collected from the uterus. The pus was cultured on the agar medium with 5% ram blood. After a 24 h incubation at 37°C under oxygen conditions, the microorganisms were initially identified by evaluating the morphology of colonies and microscopic examination of Gram-stained specimens. The isolated bacterial strains were finally identified using commercial API tests (API-Staph, and API-20E, BioMérieux).

The activities of enzymes were determined in homogenisates of uterine tissue and expressed as units per milligram of protein. The samples of uterine tissue were homogenised in the Ultra Turrax T25 apparatus (Ikawerk, Janke and Kunkel Inc., Germany). The enzyme activities were measured spectrophotometrically using Ultrospec 2000 (Pharmacia, Sweden). The activity of GSH-Px was determined using the Paglia and Valentine method (26), the activity of SOD - using the Sun and Zigman method (33) while that of CAT according to Cohen (8). The protein content in supernatants was measured according to Lowry *et al.* (17).

The data were statistically analysed by calculating the mean, standard deviation, and significance of differences between both groups using the Statistica 5.0. The level of significance was set at the P<0.05.

**Results**

The bacteriological tests showed the presence of bacterial flora in nine cases. In seven (63.64%) samples *E. coli* and in two (18.18%) *Staphylococcus aureus* strains were found. No growth was observed in two (18.18%) cases.

The results of glutathione peroxidase, superoxide dismutase, and catalase determinations are presented in Table 1. In cases of pyometra, the activity of GSH-Px in affected uterine tissues was statistically significantly lower (P<0.05) compared to that in healthy tissues, i.e. 3.49 and 5.87 U/mg of protein, respectively. The activity of SOD in affected uterine tissues was on average 6.28 U/mg of protein and did not statistically differ from that in healthy uterus (6.19 U/mg of protein). The activity of CAT in the affected and healthy uterine tissues samples was similar, 108.02 and 105.25 U/mg of protein, respectively.

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bitches with pyometra</th>
<th>Healthy bitches</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=11</td>
<td>n=18</td>
</tr>
<tr>
<td>GSH-Px (U/mg protein)</td>
<td>3.49*</td>
<td>5.87</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>6.28</td>
<td>6.19</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>108.02</td>
<td>105.25</td>
</tr>
</tbody>
</table>

* - P<0.05
Discussion

One of the key mechanisms responsible for tissue damage is oxidative stress caused by overproduction of ROS (15). Once the growth of these metabolites of molecular oxygen is uncontrollable, they are likely to cause oxidative injury to all main constituents of the cell and are involved in the aetiology of numerous diseases (1, 2, 12). The main event related to ROS production is an inflammatory process (2, 3). In pyometra, the inflammation develops in the uterine mucosa and is the protective reaction of the organism, yet simultaneously leads to tissue damage. At the inflammation site, the activating factors such as complement fragments, opsonised bacteria, viruses, immunoglobulins, and chemotactic peptides, induce rapid consumption of oxygen in the phagocytic cells, called the “respiratory burst” and production of ROS used by phagocytes to destroy the absorbed microorganisms (35). However, the lack of proper control of ROS production by antioxidative mechanisms results in their overproduction and damage to the phagocytic cells, as well as adjacent tissue cells. The in vitro studies have demonstrated that ROS released by activated neutrophils and macrophages may be toxic for various somatic cells such as erythrocytes, epithelial cells, fibroblasts, or platelets (13).

The excess of ROS results in the damage and necrosis of cells through various mechanisms including peroxidation of cellular membrane lipids, protein denaturation, and DNA damage (7). Moreover, ROS such as superoxide anion radical and hydrogen peroxide, mediate in the infiltration and accumulation of neutrophils at the inflammation site (14) and mobilise the changes in arachidonic acid (21). ROS are also involved in the activation of transcription factors, such as NF-kB and AP-1. The transcription factors are activated during inflammation in the epithelial and inflammatory cells, in which they induce the expression of many encoding genes, e.g. TNFα, IL-1, IL-6, nitrogen oxide synthesis, MHC complex I antigens, and adhesive particles (3). Furthermore, it is believed that a large group of proteases, called metalloproteinases, is affected by ROS, which exacerbates the tissue damage at the inflammatory focus (35).

The essential element of the antioxidative protection of each cell is the group of antioxidant enzymes: GSH-Px, SOD, and CAT (11, 13). The antioxidative action of these enzymes is to neutralise superoxide anion radicals and hydrogen peroxide – substrates for the production of the most toxic oxygen metabolite – a hydroxyl radical (10, 11, 24).

Superoxide anion radical is relatively poorly active, yet it is generated with high frequency (11). It serves as the substrate for the formation of markedly more toxic oxygen metabolites such as hydrogen peroxide, hypochlorous acid, and hydroxyl radical (20). The majority of superoxide anion radical formed undergoes spontaneous and SOD-catalysed dismutation to hydrogen peroxide (10).

Hydrogen peroxide may oxidise the thiol groups and the ions of transition metals. The latter reaction (the Fenton’s reaction) leads to the formation of hydroxyl radical (24). Compared to superoxide anion radical, hydrogen peroxide considerably more easily permeates the cytoplasmatic membranes infiltrating the extracellular space of lower capacities of anti-ROS protection than the interior of the cell (24). It has been demonstrated that hydrogen peroxide causes irreversible damage to the epithelial cells (23). GSH-Px and CAT are responsible for the neutralisation of hydrogen peroxide formed (34).

Hydroxyl radical is the most reactive form of oxygen and shows high toxicity at the site of its formation. It rapidly reacts with most biological particles (lipids, proteins, nucleic acids, carbohydrates) present at the adjacent places of its synthesis. Due to the extremely high speed of the reaction, the destructive effects of the hydroxyl radical, once formed, are difficult to avoid (24). Therefore, it is essential to prevent its formation by removing superoxide anion radical and hydrogen peroxide by antioxidative enzymes (11).

Antioxidative enzymes show adaptive features enhancing their activity once the production of ROS increases (9). Our findings showed that pyometra did not cause significant changes in the activities of SOD and CAT in uterine tissues; however, the activity of GSH-Px in the affected uteri was found to be markedly lower compared to healthy uteri. This is likely to indicate impaired cellular antioxidative protection in bitches with pyometra, as it is known that proper anti-ROS protection of cells requires strict cooperation of all three enzymes (28). Lower GSH-Px activity in the affected bitches impose the risk of insufficient neutralisation of hydrogen peroxide, the main substrate for the production of hydroxyl radical and other toxic ROS. Hydrogen peroxide, as the main ROS activating transcription factors involved in the expression of genes encoding various factors causing inflammation, is likely to affect the development and course of inflammatory processes. Moreover, lower activity of GSH-Px – involved in the neutralisation of lipid peroxidation products – in bitches with pyometra, indicate their deteriorated protective capacities against the effects of lipid peroxides and toxic aldehydes (18, 34).

It is known that oestrogens increase the activity of GSH-Px, while progesterone does not (22). Since all the bitches used in the study were in the luteal phase of oestrus, the influence of sex hormones on the differences in GSH-Px activity observed between the affected and healthy bitches should be excluded.

In conclusion, lower GSH-Px activity in uterine tissues from bitches with pyometra indicate reduced abilities of cell protection against ROS and suggest possible involvement of oxidative stress in the aetiology and pathogenesis of pyometra. However, this issue requires further studies.
References