OXIDATIVE/ANTIOXIDATIVE STATUS OF BLOOD PLASMA IN BITCHES WITH MAMMARY GLAND TUMOURS

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Received for publication January 02, 2008

Abstract

The aim of the study was the estimation of oxidative/antioxidative status of bitches with mammary gland tumours by the determination of lipid peroxidation intensity, concentration of glutathione and β-carotene as well as total antioxidant capacity (TAC) in blood plasma. The experiment was carried out on 18 bitches with spontaneously occurring mammary gland tumours (12 with malignant tumours and 6 with benign tumours) and 6 clinically healthy controls. The intensity of lipid peroxidation did not differ significantly among the examined groups of animals. The concentration of glutathione was higher in both neoplasm groups than in healthy bitches, but the differences were not significant. The concentration of β-carotene in plasma was similar in cases of malignant and benign tumours, but was significantly lower (P<0.05) than in healthy controls. TAC of plasma was lower in bitches with tumours than in healthy animals. Significant difference was noticed between malignant tumours and controls (P<0.01). In conclusion, the alterations in antioxidative status occur in bitches with mammary gland tumours, suggests the presence of general antioxidative stress. A necessity of more frequent sample collection for the detection of dynamic changes in peroxidation process revealing the intensity of oxidative stress in bitches suffering from mammary gland tumours should be considered.

Key words: bitch, mammary gland tumours, oxidative stress, antioxidants.

Human and animal organisms are provided with defence mechanisms against ROS. The defence mechanisms fall into two systems, including enzymatic and non-enzymatic antioxidants, which control the balance between the production and neutralisation of ROS, which is at a level necessary for proper cell functioning (13).

The imbalance between pro- and antioxidative processes caused by an increase in ROS production and/or the reduction of antioxidative protection may lead to peroxidative damage to macromolecules like lipids, proteins, and nucleic acids (23, 34). The determination of products of peroxidative damage to macromolecules is a useful marker for the estimation of oxidative stress intensity in pathological conditions in humans as well as in animals (5, 14). Lipids are the most susceptible for peroxidative damage due to low energy necessary for the initiation of the process as well as the presence of unsaturated bonds (5). This process is the most often examined one (5, 14, 28).

Antioxidative status can be described as total antioxidant capacity or may be characterised by single elements covering enzymatic and non-enzymatic mechanisms (5, 28). The non-enzymatic antioxidants consist of low molecular weight compounds of endogenous as well as exogenous origin (13, 14). Glutathione and β-carotene are of great importance (24, 31). The measurement of non-enzymatic antioxidant concentration in blood plasma is crucial for the estimation of antioxidative/oxidative status. However, the discrepancies in their chemical properties and possible interactions make the determinations very difficult. That is why several methods quantifying total antioxidative capacity (TAC) in different biological samples trying to cover the sum of almost all antioxidants in the examined sample were established (5, 28). The reaction based on the capacity of antioxidants in plasma to iron reduction at presence of 2,4,6-tri-pyridyl-s-triazine - Ferric Reducing/Antioxi-
Neoplasia is one of the diseases aetiology of which is connected with ROS imbalance and oxidative stress (1, 10). It is known that ROS may influence the development and the progression of neoplasm via oxidative changes in DNA leading to genome damage (1, 34), inactivation of suppressor genes, increase in protooncogene expression (8), and stimulation of angiogenesis (7). It is also known that ROS may deliver electrons to carcinogens causing an increase in their carcinogenic activity (18). Previous research in humans showed that cancer tissues may be a source of ROS, which may damage blood vessel walls and facilitate metastases (30).

Neoplasm, especially its malignant forms, belongs to one of the most dangerous diseases in humans and animals and its frequency exhibits an increasing tendency (4). Among domestic animals, dogs are the species where neoplasms are diagnosed most often. Mammary gland tumour is the second most common neoplasm in bitches, after skin tumours (21, 26). Approximately 50% of mammary neoplasms are malignant (4, 26). However, literature is scarce with regard to the participation of oxidative stress in the development of mammary gland tumours in bitches as well as antioxidative defence mechanisms in these cases.

The aim of present study was to estimate the oxidative/antioxidative status in bitches suffering from mammary gland tumours. The estimation was based on the determinations of lipid peroxidation intensity (LPI), concentration of glutathione, β-carotene, as well as TAC in blood plasma.

### Material and Methods

Eighteen bitches of different breeds, aged 6-12 years, were used in the study. These animals were patients of the Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine in Lublin because of spontaneously occurring mammary gland tumours. These bitches (the experimental group) had their mammary gland tumours removed under atropine anaesthesia (*Atropinum sulphuricum* - Polfa, Poland) 0.05 mg/kg b.w., subcutaneously + xylazine (Rometar 2% - Spofa, Czech Republic) 2 mg/kg b.w., intramuscularly + ketamine (Narkamon 5% - Spofa, Czech Republic) 5-15 mg/kg b.w., intramuscularly. Six clinically healthy bitches, aged 3-8 years, were spayed in the clinic, and were considered as the control group. The experimental animals and the control group were fed commercial dog feed. All animals were clinically examined, and routine haematological and biochemical blood examinations, as well as urine examinations, were performed. In the experimental animals, there were no other diseases detected, apart from the tumours in the mammary glands.

Nine millilitres of blood from all bitches were collected from the saphenous vein into heparinised tubes immediately before anaesthesia. The plasma obtained after centrifugation was used for the determination of intensity of lipid peroxidation (LPI), concentration of reduced glutathione and β-carotene as well as TAC.

The intensity of lipid peroxidation process was determined by spectrophotometric method of Alberti *et al.* (3). The concentration of glutathione was determined by the use of commercial kit (Glutathione Assay Kit, Cayman Chemical Company, USA). The concentration of β-carotene was determined according to the procedure of Suzuki and Katoh (32). The determination of TAC was based on the method of Benzie and Strain (6). The concentration of protein was estimated by Lowry’s method (20).

Tumour tissue was fixed for 24 h in neutral 10% formalin. Paraaffin blocks were routinely prepared and cut into 4 µm slices. The slices were stained with haematoxylin and eosin. The classification of tumours and mammary gland dysplasia were performed in accordance with WHO requirements (25).

The results were expressed as arithmetic means and standard deviations. The results were analysed by the use of Student’s *t*-test. The differences were considered significant at *P*<0.05.

### Results

Results of the histopathological examination of the mammary gland tumours are presented in Table 1. Out of 18 bitches included in the study, 12 (66.7%) cases of malignant tumours were recognised. Tumours were benign in 6 (33.3%) bitches. The most often diagnosed malignant tumour was the complex carcinoma - 33.3% of cases. The majority of the benign tumours had mixed characteristics.

The results of biochemical determinations are presented in Table 2. The values of LPI did not differ significantly between the examined groups of animals. The concentration of glutathione in plasma either in cases with malignant or benign tumours was higher as compared to healthy bitches. However, the differences did not reach statistical significance. The concentration of β-carotene in plasma was similar in animals with malignant as well as benign tumours but was significantly lower (*P*<0.05) as compared to controls. The values of TAC were lower in bitches with tumours than in healthy animals. The difference between cases with malignant tumours and controls was significant (*P*<0.01).

### Discussion

It is well evidenced that oxidative stress plays a substantial role in aetiology of cancer (9, 15, 16). Lipid peroxidation is one of important consequences of oxidative stress (39). The determination of lipid peroxidation products allows for the estimation of the intensity of this process; moreover, it can be used for the evaluation of oxidative stress severity (14).
Table 1
Histological analysis of the mammary gland tumours

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Number of bitches (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant naoplasms</td>
<td>12 (66.67)</td>
</tr>
<tr>
<td>Complex carcinoma</td>
<td>6 (33.33)</td>
</tr>
<tr>
<td>Tubulopapillary carcinoma</td>
<td>3 (16.67)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>2 (11.11)</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>1 (5.55)</td>
</tr>
<tr>
<td>Benign neoplasms</td>
<td>6 (33.33)</td>
</tr>
<tr>
<td>Benign mixed tumours</td>
<td>4 (22.22)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>2 (11.11)</td>
</tr>
</tbody>
</table>

Table 2
Lipid peroxidation intensity (LPI), glutathione, and β-carotene concentrations, and total antioxidant capacity (TAC) in plasma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bitches with malignant tumour</th>
<th>Bitches with benign tumour</th>
<th>Healthy bitches</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPI (µmol/g protein)</td>
<td>0.14±0.03^a</td>
<td>0.15±0.01^a</td>
<td>0.17±0.03^a</td>
</tr>
<tr>
<td>Glutathione (mmol/g protein)</td>
<td>0.19±0.17^a</td>
<td>0.17±0.11^a</td>
<td>0.10±0.03^a</td>
</tr>
<tr>
<td>β-carotene (µmol/g protein)</td>
<td>1.43±0.59^A</td>
<td>1.38±0.19^A</td>
<td>3.05±1.62^B</td>
</tr>
<tr>
<td>TAC (µmol/g protein)</td>
<td>5.83±1.69^a</td>
<td>7.10±2.46^ab</td>
<td>11.72±5.75^b</td>
</tr>
</tbody>
</table>

a, b – different superscripts denote significant differences at P<0.05;
A, B – different superscripts denote significant differences at P<0.01

An increase in the content of lipid peroxidation products in blood plasma was detected in women suffering from mammary gland cancer (19, 27), as well as from other kinds of human cancer (15, 17).

The higher intensity of lipid peroxidation was described in patients with malignant than with benign tumours (19). There was also evidence that the concentration of lipid peroxidation products was significantly higher in tumour than in surrounding healthy tissues (19). After surgical removal of the tumour, the intensity of lipid peroxidation decreased (15). It may indicate that cancer cells itself can be the source of higher amount of ROS in patients suffering from cancer. Chronic activation of the immune system leading to proinflammatory cytokine excess and in consequence to increase in ROS production may be another reason for ROS increase in cancer (22). In our experiment, the intensity of lipid peroxidation did not differ among the examined groups of bitches. It confirms the results of earlier studies where no differences in the concentration of lipid peroxidation products (thiobarbituric acid reactive substances) as well as proteins (-SH groups content) between bitches suffering from mammary gland tumours and healthy animals were detected (33). It is worth mentioning however, that in both studies, the markers of lipid peroxidation were determined once. The collection of samples more frequently would indicate the dynamics of possible changes within lipid peroxidation process.

A decrease in the capacity of antioxidative defence mechanisms is one of reasons leading to oxidative stress. That is why antioxidative status of a biological sample can be used as the marker of oxidative stress. In many cases, low antioxidative capacity of tissues or body fluids is the consequence of oxidative stress. The increase of ROS may be responsible for the consumption of non-enzymatic antioxidants and decrease in their concentration in tissues and body fluids (11, 14). Moreover, an increase in antioxidative enzyme activities as well as an increase in low molecular
antioxidant concentration may occur as the response to current challenges connected with ROS overproduction (14). Significantly, lower concentration of β-carotene and TAC in plasma of bitches with mammary gland tumours could be the result of the consumption of plasma antioxidants during neutralisation of ROS excess and may indicate general oxidative stress. It corresponds with earlier studies, which showed an increase in erythrocyte antioxidative enzymes in bitches suffering from mammary gland tumours. It can be considered as the result of compensative mechanisms accompanying oxidative stress (33). An increase in antioxidative enzyme activities in patients with malignant tumours was observed in humans (15, 27). A decrease in the concentration of glutathione and other antioxidants in patients suffering from cancer as well as a decrease in TAC was described by Saygili et al. (29), Kumaragaruparan et al. (19), and Afrasyap et al. (2), respectively. Moreover, it was suggested that an increase in β-carotene concentration and TAC in serum was connected with a decrease in risk of breast cancer (9, 16).

In conclusion, the alterations in antioxidative status suggesting the presence of general antioxidative stress in bitches with mammary gland tumours occur. These alterations are not accompanied by lipid peroxidation increase. The necessity of more frequent sample collection for the detection of dynamic changes in peroxidation process revealing the intensity of oxidative stress in bitches suffering from mammary gland tumours should be considered.

References


