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Lipid peroxidation in neoplastic tissue of dogs with mammary cancer fed with different kinds of diet

Maria KARAYANNOPOULOU[1,*], Anna FYTIANOU[1], Nikolaos ASSALOUMIDIS[1], Dimitra PSALLA[2], Ioannis SAVVAS[1], Eleni KALDRYMIDOU[2]

[1]Department of Clinical Studies - Companion Animal Clinic, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2]Laboratory of Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, University Campus, Thessaloniki, Greece

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Abstract: Increased lipid peroxidation due to reactive oxygen species (ROS)-induced oxidative stress has been demonstrated in the neoplastic mammary tissue of dogs with malignant mammary tumors. Dietary factors implicated in ROS production may influence mammary cancer incidence or prognosis. Balanced diets (as commercial dog food is considered) may be more protective against oxidative damage than homemade diets. The purpose of this preliminary study was to compare the extent of lipid peroxidation, as evidenced by thiobarbituric acid-reactive substances (TBARS) and α-tocopherol concentration in the neoplastic mammary tissue, among dogs with mammary cancer fed with different kinds of diet (commercial or homemade). Eighteen dogs with mammary cancer, assigned into 2 groups according to diet, were used. Neoplastic and normal mammary tissue , 1 cm^3 each, was used for TBARS and α-tocopherol measurements. Serum TBARS, α-tocopherol, total cholesterol, and triglycerides were also measured. No significant differences in serum parameters evaluated were found between the 2 diet groups. The increased lipid peroxidation due to oxidative stress induction (increased TBARS and decreased α-tocopherol concentration) found in neoplastic compared to normal mammary tissue of dogs with mammary cancer was similar in both groups of dogs fed with a different kind of diet, commercial or homemade.

Key words: α-Tocopherol, antioxidants, dog, lipid peroxidation, mammary tumors, oxidative stress

1. Introduction

Oxidative damage to cellular macromolecules (lipids, proteins, or nucleic acids) by an excess of reactive oxygen species (oxidative stress) is believed to be involved in the pathogenesis of mammary cancer. Reactive oxygen species (ROS) may be produced during carcinogenesis (i.e. after a longstanding exposure to estrogens), by neutrophils and macrophages that infiltrate the tumor (immune system activation and/or tumor necrosis), or during neoplastic tissue hypoxia and reperfusion (1,2). The ROS target primarily the polyunsaturated fatty acids in cell membranes and cause lipid peroxidation. The products of lipid peroxidation may induce DNA damage and consequently mutagenesis and carcinogenesis (2,3). A biomarker of lipid peroxidation that can be measured in both blood and tissues is thiobarbituric acid-reactive substances (TBARS) (3,4). Antioxidative defense mechanisms using endogenous enzymes and diet-derived antioxidants moderate the effects of ROS. Vitamin E (α-tocopherol) is a lipid-soluble dietary antioxidant and is protective against oxidative damage due to its ability to inhibit lipid peroxidation (3,5). In human studies (2,6,7) and in the few similar studies performed in dogs (4,8), increased or decreased oxidative/antioxidative parameters have been found in the blood or tumor tissue of patients with mammary tumors.

The role of nutritional factors, on the other hand, in the pathogenesis of breast cancer in women is unclear. Dietary fat, alcohol, red meat, and carcinogens produced during cooking are some of the factors that may increase the risk of breast cancer, whereas fruits and vegetables, fiber-rich food, or vitamin supplementation may have a protective effect (9–11). In dogs, the role of nutrition on the incidence or prognosis of mammary tumors has also been investigated (12), but to a lesser extent. In a case-control study (13) in dogs bearing mammary gland dysplasia or tumors, homemade diet (with high beef and pork content and low chicken content) was associated with a higher incidence of mammary tumors as compared to commercial food. The opposite was found in another

* Correspondence: marikara@vet.auth.gr

case-control study (14) performed in dogs with malignant mammary tumors, where the intake of dietary fat and table food was found inversely related to the risk of mammary cancer.

Recently, in dogs with mammary cancer fed both commercial and homemade diets, we found increased lipid peroxidation (increased TBARS and decreased α-tocopherol concentration) in neoplastic tissue compared to adjacent normal mammary gland tissue (15). As dietary factors such as lipids or vitamin E are implicated in lipid peroxidation occurring in mammary cancer cases, we hypothesized that balanced diets (as commercial dog food is considered) may be more protective against oxidative damage than homemade meals. Therefore, the purpose of this preliminary study of 18 dogs with mammary cancer was to explore any significant differences in the extent of lipid peroxidation (evidenced by TBARS and α-tocopherol concentration in neoplastic tissue) between dogs fed exclusively with commercial dog food and dogs fed exclusively with a homemade diet.

2. Materials and methods

2.1. Animals and study design

A total of 18 intact female dogs with malignant mammary tumors were included in this study according to the following criteria: 1) they had malignant mammary tumors confirmed on postsurgical histological examination, 2) no obvious distant metastases were observed on thoracic and abdominal radiographs or on physical examination, 3) the dogs were otherwise apparently healthy, 4) the dogs had a nonobese body conformation, and 5) they were fed exclusively with commercial dog food or with homemade diet at least 6 months before admission. All dogs were admitted for care to the Companion Animal Clinic of the Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece. Their owners gave their consent for the study. They were also asked to fill out a questionnaire about their dogs' diets (i.e. brand names of the commercial food or the most usual constituents of the homemade diets, and feeding frequency).

These 18 dogs were assigned into 2 groups according to their diet: group A (commercial dog food) and group B (homemade diet). Group A consisted of 10 dogs aged 7 to 10 years (mean: 8.05 years), whereas group B included 8 dogs with an age that ranged from 6 to 10 years (mean: 7.69 years).

Every dog underwent a complete physical examination and had a complete blood cell count, serum biochemical analysis, and urinalysis. Thoracic and abdominal radiographs were also taken of all dogs. Single mammary tumors were excised by regional mastectomy and multiple tumors by chain mastectomy.

2.2. Sampling

Blood samples were collected from all dogs on the day of mastectomy, immediately before the administration of proanesthetic medication. The dogs had been fasted for 12 h prior. A sample of 10 mL of blood taken via jugular venipuncture was placed into a serum tube and centrifuged to obtain serum. An aliquot of the serum was stored in the freezer (–20 °C) for α-tocopherol determination at a later date. The rest of the serum was used for immediate determination of TBARS, total cholesterol, and triglycerides.

One cubic centimeter of excised mammary tumor and 1 cm^3 of an adjacent, ipsilateral normal mammary gland were harvested, washed with normal saline, and immediately frozen (–70 °C) for later determination of α-tocopherol and TBARS content. A second sample of the excised mammary tumor was fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for histological examination. The classification of mammary tumors was performed following the last WHO classification scheme (16).

2.3. Biochemical estimations

The tissue TBARS concentration was assayed by a spectrophotometric method (Hitachi U-2000 spectrophotometer, Tokyo, Japan) as the assay results in a pink-colored chromogen formed by the reaction of thiobarbituric acid with the products of lipid peroxidation (17). The serum TBARS concentration was measured using a modification (18) of the above method. Both tissue and serum samples were read at 532 nm and were reported as nmol/g tissue or nmol/mL, respectively. The tissue and serum α-tocopherol concentrations were determined by fluorometric methods (Hitachi F-2000 fluorometer) with the samples read at excitation λ 290 nm and emission λ 329 nm (19,20), and they were expressed in μg/g tissue or μg/mL, respectively. Serum, total cholesterol, and triglycerides concentrations were measured enzymatically (Flexor E, Vital Scientific, the Netherlands) and reported as mmol/L.

2.4. Statistical analysis

The normality of the data distribution of the parameters evaluated was tested using the Shapiro–Wilk test. For the assessment of the extent of lipid peroxidation in each diet group (tissue measurements of TBARS and α-tocopherol), a general linear model for repeated measurements was used, with 1 between-subjects factor (diet: commercial versus homemade) and 1 within-subjects factor (site: neoplastic versus normal mammary tissue). For the assessment of any significant differences in the parameters evaluated between the 2 diet groups, Student's t-test for independent samples (for serum and tissue measurements of TBARS and α-tocopherol) or the Mann–Whitney test (for serum cholesterol and triglycerides measurements)

was used. Kendall correlation coefficients were computed to detect any correlation between serum TBARS, total cholesterol, or triglycerides concentration in the total of 18 dogs. For each of the comparisons, differences were considered significant at $P \leq 0.05$. All statistical analyses were performed using SPSS (version 19.0, IBM-SPSS Science, Chicago, IL, USA).

3. Results

3.1. Histopathology

The diagnosis of mammary tumors for dogs fed commercial food included simple carcinoma in 5/10 dogs, complex carcinoma in 2/10 dogs, and carcinoma in benign mixed tumor in 3/10 dogs. The diagnosis for dogs fed homemade diet included simple carcinoma in 4/8 dogs, complex carcinomas in 3/8 dogs, and carcinoma in benign mixed tumor in 1/8 dogs.

3.2. Diet content

According to the questionnaire filled out by the dogs' owners, all dogs were usually fed once daily. Commercial diet included canned or dry dog food or both, whereas a rough estimation of the composition and analysis of the commercial diets used was approximately as follows: beef or poultry meat or derivatives (~25%), fats and oils (~15%), derivatives of vegetables and cereals (~2%–4%), and vitamins, minerals, and trace elements (in minimal quantities). Homemade diets often included chicken or pasta and, less often bread, red meat and vegetables, cooked by traditional Mediterranean methods of boiling and use of olive oil.

3.3. Serum concentrations

No statistically significant differences were found in serum TBARS ($P = 0.308$), α-tocopherol ($P = 0.551$), total cholesterol ($P = 0.689$), and triglycerides ($P = 0.965$) concentration between the 2 groups of dogs fed with different kinds of diet (Table 1). Furthermore, no correlation was found between serum TBARS and total cholesterol or triglycerides concentration ($P = 0.171$ and 0.748, respectively) in the total of 18 dogs.

3.4. Tissue concentrations

The TBARS concentration was significantly higher whereas α-tocopherol concentration was significantly lower in neoplastic tissue compared to normal mammary tissue, either in dogs fed with commercial dog food ($P = 0.004$ and $P = 0.04$, respectively) or in dogs fed a homemade diet ($P = 0.003$) (Table 2). In comparisons made between the 2 diet groups, no statistically significant differences were found in TBARS ($P = 0.484$) or α-tocopherol ($P = 0.163$) concentration in the mammary neoplastic tissue.

4. Discussion

In this study, the significantly higher TBARS and lower α-tocopherol concentration found in the neoplastic tissue of dogs with mammary cancer, when compared to the normal tissue of an adjacent mammary gland (Table 2), is indicative of increased lipid peroxidation due to oxidative stress induction. Altered oxidative/antioxidative parameters in mammary tumor tissues have also been reported by others in both dog (8) and human (6,7) studies, although the results were not always similar to ours. The tissue concentration of α-tocopherol reflects long-term dietary intake of vitamin E and is considered a more reliable antioxidative marker than its blood concentration (6,21). Moreover, increased blood levels of lipid peroxidation products are usually metabolized and detoxified rapidly after the oxidative challenge, and thus their tissue measurement has been advisable (22).

The extent of increased lipid peroxidation found in the neoplastic mammary tissue in our study was similar in both groups of dogs fed with different kinds of diet; no significant differences in the neoplastic tissue TBARS or α-tocopherol concentration (Table 2) were found between dogs fed with commercial food and those fed a homemade diet. Additionally, no statistically significant differences were found in serum TBARS and α-tocopherol concentration (Table 1) between the 2 diet groups. Much work has been done on the implication of different kinds of dietary fats in the pathogenesis of breast cancer (9,23). Dietary fats, as well as obesity, are considered risk factors for mammary cancer (23), as estrogens can be produced after aromatization of androgens, which occur primarily in fat tissues (9,24). Estrogens can produce ROS and consequently higher amounts of lipid peroxidation products, mutagenesis, and carcinogenesis (2,25). Hormonal status might also be perturbed by phytoestrogens, which appear in diet components such as olive oil (used in homemade diets in this study), and

Table 1. Mean ± standard deviation of serum concentrations of thiobarbituric acid-reactive substances (TBARS), α-tocopherol, total cholesterol (TC), and triglycerides (TG) in 18 dogs with mammary cancer fed either commercial dog food or homemade diet.

Diet	TBARS (nmol/mL)	α-Tocopherol (μg/mL)	TC (mmol/L)	TG (mmol/L)
Commercial n = 10	2.7 ± 1.2	19.1 ± 5.3	6.3 ± 2.3	0.7 ± 0.3
Homemade n = 8	2.1 ± 0.8	20.9 ± 7.1	6.5 ± 1.4	0.7 ± 0.2

Table 2. Mean ± standard deviation of thiobarbituric acid-reactive substances (TBARS) and α-tocopherol concentrations in neoplastic and normal mammary tissue of 18 dogs with mammary cancer. P-value indicates significant difference between neoplastic and normal tissue concentrations.

Diet	Mammary tissue	TBARS (nmol/g)		α-Tocopherol (μg/g)	
Commercial n = 10	Neoplastic	17.7 ± 6.0	P = 0.004	34.8 ± 18.5	P = 0.04
	Normal	13.1 ± 5.3		62.7 ± 22.3	
Homemade n = 8	Neoplastic	16.0 ± 3.2	P = 0.003	51.9 ± 30.7	P = 0.003
	Normal	10.6 ± 3.4		100.5 ± 54.1	

can exert estrogen-like or antiestrogenic activity (9,23). The blood concentration of lipids (total cholesterol and triglycerides) or lipoproteins merely reflects the fat intake by diet (26). Increased concentration of serum lipids has been associated with breast cancer. It has been suggested that cholesterol is susceptible to oxidation, resulting in higher amounts of lipid peroxidation products during oxidative stress (7,27). Nevertheless, the blood concentration of TBARS has not been found to correlate with total cholesterol or triglycerides levels (18,28), which was also the case in our study. Furthermore, the blood concentration of vitamin E may be affected by lipid parameters (21). However, in this study, the concentration of serum lipids (total cholesterol and triglycerides) did not differ significantly between the 2 diet groups (Table 1), suggesting a rather similar fat content in the food of both groups, and also suggesting that our results were rather not affected by diet. This fact might explain the similar extent of lipid peroxidation found in both diet groups.

On the other hand, dietary fat contains nonsaponifiable components such as carotenoids and fat-soluble vitamins (A, D, E), which have shown antioxidative activity (23,29). This may be the reason why Sonnenschein et al. (14) found, in dogs with mammary tumors, that dietary fat and table food were inversely associated with breast cancer risk. Nevertheless, in human studies, neither a diet low in fat and rich in antioxidative vitamins of vegetables and fruits nor vitamin E supplementation have shown a significant overall protective effect against breast cancer incidence or mortality (9,11,30).

In this preliminary study of 18 dogs, a gross and indirect evaluation of the influence of the kind of diet on lipid peroxidation evidenced in the neoplastic tissue of dogs with mammary cancer was performed. However, it is well known that commercial diets, although considered "balanced", might not always be, due to reasons such as, for example, preservation conditions. On the other hand, since there is a wide range of ingredients and recipes for homemade diets, it is difficult to evaluate whether such diets are protective or not against oxidative damage. Many dietary variables (polyunsaturated fatty acids, carotenoids, α-tocopherol content, etc.) have to be assayed in commercial or homemade diets in order to evaluate whether they offer any protection against oxidative damage.

In conclusion, in this preliminary study, the increased lipid peroxidation due to oxidative stress induction in dogs with mammary cancer, as evidenced by increased TBARS and decreased α-tocopherol concentration in neoplastic tissue, was found to be similar in both groups of dogs fed with different kinds of diet, commercial or homemade.

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