INTENSITY OF COX2 EXPRESSION IN CELLS OF SOFT TISSUE FIBROSACRCOMAS IN DOGS AS RELATED TO GRADE OF TUMOUR MALIGNANCY

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Abstract

Using immunohistochemical technique, localization and intensity of COX2 expression were estimated in canine soft tissue fibrosarcomas. The obtained results were related to tumour grade of malignancy. The material was sampled in the course of surgery from 23 dogs of various breed, 5 to 18 years of age. The presence of COX2 was demonstrated in cell cytoplasm in over 43% tumours. Augmented expression of COX2 was observed in samples of tumours manifesting higher grade of malignancy. Also strong positive correlation was detected (r=0.76) between expression of COX2 and grade of malignancy in the tumours, which might point to the involvement of the enzyme in neoplastic transformation of the cells from which fibrosarcomas derive in dogs.

Key words: dog, COX2, fibrosarcoma.

Cyclooxygenase (COX) or, in other words, prostaglandin H2 synthase, participates in the development of prostaglandins and thromboxanes. In the body, it is present in two isoforms coded by two distinct genes. Cyclooxygenase-1 (COX1) is a product of a cytohomeostatic gene and is involved in proper functioning of eicosanoids manifesting stable even if low expression in many different types of cells. The enzyme oxidises free arachidonate to prostaglandin G2, which in turn undergoes peroxidation in another active site of the enzyme to prostaglandin H2 (5). Cyclooxygenase-2 (COX2) is encoded by the so-called early response gene, which may be activated by various factors, such as carcinogens, growth factors, and products of activated oncogenes, oncoproteins (7). Due to such properties, expression of the enzyme is observed both in tumour cells and in cells of vascular endothelium and cells of inflammatory infiltrate (16). It should be added that proinflammatory cytokines activate synthesis of the induced isoenzyme, COX2, the activity of which provides grounds for pain reactions associated with inflammatory processes (e.g. in rheumatoid arthritis in humans) (5). Moreover, over expression of COX2 has been shown to accompany neoplastic transformation of epithelial cells in tumours such as cancers of mammary gland, lung, stomach, pancreas, urinary bladder, dermal spinocellular carcinoma, and colonic carcinoma (3, 8, 9, 14, 15, 18, 21-23). Augmented expression of COX2 was observed in around 50% of adenomatous polyps of the large intestine, and as many as 80% of adenocarcinomas, while levels of the enzyme in the epithelium of normal large intestine were almost undetectable (5). Elevated expression of COX2 was noted also in some tumours of mesenchymal origin in humans, i.e. in osteosarcomas, rhabdomyosarcomas, and Ewing’s sarcomas (5). An increase in COX2 expression in cells of various tumours can probably be linked to its oncogenic character, manifesting itself by its ability to inhibit apoptosis and to induce elevated levels of Bcl-2 protein expression (18-20).

Our studies were aimed at the determination of localisation and the extent of COX2 expression in cells of primary soft tissue fibrosarcomas of dogs using immunocytochemical techniques, and at comparing the obtained results with tumour malignancy grade.

Material and Methods

Material for the studies was sampled in the course of surgery from 23 dogs of various breed, aging 5 to 18 years. The tumours were verified histopathomorphologically as fibrosarcomas. Samples of the tumours were fixed in 7% buffered formalin and paraffin sections were prepared. The expression of COX2 was demonstrated using goat polyclonal antibodies (clone M 19; Santa Cruz Biotechnology; USA) at 1:100 dilution.
Reaction product was visualised using LSAB2° kit of reagents and diaminobenzidine (DAB). The sections were pre-exposed to procedure of boiling in a microwave oven, in Antigen Retrieval Solution, in order to unblock antigenic determinants. Each one of the reactions was accompanied by a negative control using Primary Negative Control. All the reagents originated from DakoCytomation (Denmark).

Microphotographs of the preparation were subjected to computer-assisted image analysis in a stand consisting of a computer coupled to Axiophot type light microscope (Carl Zeiss). The system had the potential to record images and to conduct their digital analysis. To the measurements, the MultiScaneBase V 14.02 software, working in the Windows environment, was applied.

Microscopic examination allowed for dividing the tumours into three groups of a variable malignancy grade. The technique of evaluating the malignancy grade, included three parameters scored in the scale from 0 to 3 points: histological differentiation of the tumour (extensive - 1 pt, moderate - 2 pts, low - 3 pts); number of mitoses per 10 large visual fields under 400x magnification (0-9 - 1 pt, 10-19 - 2 pts, ≥20 - 3 pts); region of necrosis (no necrosis - 0 pts, ≤50% tumour - 1 pt, >50% tumour - 2 pts). Sum of the points provided potential to distinguish 3 grades of malignancy (G) among the tumours: 2-3 pts - G1, 4-5 pts - G2, 6-8 pts - G3 (22).

For the evaluation of COX2 expression, a modified semiquantitative IRS scale of Remmele was applied (Table 1) (13).

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<th>A</th>
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<td>0 pts – no cells with positive reaction</td>
<td>0 pts – no colour reaction</td>
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<tr>
<td>1 pt – to 10% cells with positive reaction</td>
<td>1 pt – low intensity of colour reaction</td>
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<tr>
<td>2 pts – 11-50% cells with positive reaction</td>
<td>2 pts – moderate intensity of colour reaction</td>
</tr>
<tr>
<td>3 pts – 51-80% cells with positive reaction</td>
<td>3 pts – intense colour reaction</td>
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<td>4 pts – &gt; 80% cells with positive reaction</td>
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It should be mentioned that in none of the examined cases of soft tissue fibrosarcomas COX2 expression at the (++++) level has been disclosed.

Comparing the extent of COX2 expression with grades of malignancy, a pronounced positive correlation has been disclosed between the studied parameters. The correlation coefficient value amounted to $r = 0.76$ (Fig. 4).

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<td>Semi quantitative IRS scale taking into account both percentage of positive cells (A) and intensity of the reaction colour (B), with the final score representing product of the two variables (A x B)</td>
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Fig. 1. Moderate intensity of COX2 expression (++) in the cytoplasm of cells in canine soft tissue fibrosarcomas. 400x

Fig. 2. Weak intensity of COX2 expression (+) in cytoplasm of cells in canine soft tissue fibrosarcomas. 400x

Fig. 3. Intensity of COX2 expression in canine soft tissue fibrosarcomas.
This illustrates augmented COX2 expression level in tumours of a more malignant character. Among aggressive tumours included to the G2 category, 75% cases have demonstrated COX2 expression at the level of (+++) and 25% cases at the level of (+). In the case of G1 tumours not a single case of COX2 expression at the level of (+++) or higher has been demonstrated. It should be added that among tumours classified as G1 over 68% tumours have demonstrated no COX2 expression. The obtained results have justified the assumption that in canine soft tissue sarcomas increase in tumour aggressiveness is accompanied by elevated levels of COX2 synthesis. A similar tendency has been observed in mammery tumours of women and bitches and in many other tumours of epithelial origin in both humans and animals (10, 11). Ristimaki et al. (15) noted in human mammery gland carcinoma increased expression of COX2, positively correlating with tumour size and with intensity of Ki-67 proliferation associated antigen. The authors found also an unfavourable effect of COX2 over expression on duration of patient survival in the presence of metastases to the lymph nodes.

Summing up, it should be concluded that the activation of COX2 gene with subsequent overproduction of the enzyme might play a significant role in cell neoplastic transformation, which in dogs results in soft tissue fibrosarcomas. Detailed recognition of the mechanisms, which control the processes, might, on one hand, broaden the knowledge on biology of canine tumour cells and, on the other, permit to develop more effective ways of the prevention and treatment of the tumours, based, e.g., on selective COX2 inhibitors.

References


