IMMUNOHISTOCHEMICAL LOCALIZATION OF COX2 IN CELLS OF MAMMARY ADENOCARCINOMAS IN BITCHES AS RELATED TO TUMOUR MALIGNANCY GRADE

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Received for publication October 21, 2005.

Abstract

Using immunohistochemistry the localization and expression intensity of COX2 were evaluated in mammary adenocarcinomas in bitches. The obtained results were related to the tumour malignancy grades. Material for the studies was sampled in the course of surgery from bitches of various strains which developed the primary mammary tumour. In cytoplasm of mammary adenocarcinoma cells the presence of COX2 was disclosed in 74% of the examined tumours. Augmented expression of COX2 was noted in samples of tumours demonstrating high grades of malignancy. A moderate positive correlation was disclosed between expression of COX2 and the grade of histological malignancy (r = 0.41), which might point to the involvement of the enzyme in neoplastic transformation of cells in the mammary glands.

Key words: bitch, COX2, adenocarcinomas, mammary gland.

Cyclooxygenase (COX) is the enzyme responsible for the transformation of arachidonic acid to prostaglandins. In the body it exists in two isomers coded by two distinct genes. Cyclooxygenase-1 (COX1) (the constitutive isoform) represents a product of a housekeeping gene and participates in normal functioning of eicosanoids, demonstrating stable although a low activity in several mammary types of cells. Cyclooxygenase-2 (COX2), in turn, is coded by the so-called gene of early reaction which may be activated by various agents, including growth factors, carcinogens, and oncogenes, the products of activated oncogenes (7). Therefore, its expression is noted mainly in tumour cells as well as in cells of vascular endothelium and cells of inflammatory infiltrates (19). Overexpression of COX2 was noted to accompany neoplastic transformation of epithelial cells in tumours such as mammary carcinoma (18), and cancers of lungs (8), colon (20), stomach (17), pancreas (25), and urinary bladder (26) as well as in spinocellular cancer of the skin (3). Augmented expression of COX2 in cells of various epithelial tumours is probably related to its oncogenic character, manifested, i.a., by its ability to inhibit apoptosis and by its induction of augmented expression of Bcl-2 protein, which disturbs normal cell cycle (20, 23, 24). COX2 exerts also a strong immunomodulatory function and exhibits pro-inflammatory effects (13). Independently of the above, in tumours of epithelial origin augmented production of prostaglandins was demonstrated, as compared to their normal tissues of origin (1). In mammary carcinoma of females overexpression of COX2 might promote invasiveness of tumour cells, their metastatic potential (21) and angiogenesis (27). Majima et al. (23) have found that COX2 influences expression of several factors linked to angiogenesis (e.g. vascular endothelial growth factor and basic fibroblast growth factor). In turn, a decreased expression of the above listed factors may follow the administration of selective inhibitors of the enzyme.

Present studies aimed at determination of localization and expression of COX2 in cells of primary adenocarcinomas of the mammary gland in bitches using an immunohistochemical technique and at relating the obtained results to grades of tumour malignancy.

Material and Methods

Material for the studies was sampled in the course of surgery from 23 bitches of various strains, aged 5 to 14 years, which developed mammary tumour. The neoplastic tumours were verified by histopathology. The tumours sampled were fixed in 10% buffered formalin, dehydrated and embedded in paraffin. Determination of COX2 expression in the paraffin sections took advantage of goat polyclonal antibodies...
(clone M 19; Santa Cruz Biotechnology; USA). The antigen presence was visualized employing LSAB2® and diaminobenzidine (DAB) reagent kits. Every staining was accompanied by an appropriate negative control using the primary negative control kit. All the reagents originated from DakoCytomation, Denmark.

The obtained preparations served to prepare microphotographs, which were subjected to computer analysis in a stand consisting of a computer coupled to Carl Zeiss microscope, model Axiphot. The equipment had the potential to register the pattern and to conduct its digital analysis. The measurements took advantage of the MultiScaneBase V 8.08 software, working in the Windows environment.

The microscope studies allowed to classify the tumours into three groups of different malignancy grades (G). The grade was established using the scale of Bloom-Richardson in modification of Elstonia and Ellis (5). The technique takes into account three variables, evaluated in the scale of 1 to 3 pts.: formation of tubules (evident, moderate, slight), polymorphism of cell nuclei (slight, moderate, marked), and number of mitotic figures per 10 microscope fields at the magnification of 400x (0-7, 8-16, ≥ 17). Sum of the points allows to distinguish three grades of differentiation of cancer cells, manifesting, 0-5 pts. – I grade, 6-7 pts. – II grade, 8-9 pts. – III grade respectively. Expression of COX2 was appraised employing a modified semiquantitative IRS scale according to Remmele and Stegner (16) (Table 1).

The technique took into account both the content of positive cells and intensity of the reaction (colour intensity) and the final score represented the product of the evaluated variables and ranged from 0 to 12 pts (no reaction: 0 pts; faint reaction: 1-2 pts (+); moderate reaction: 3-4 pts (++), strong reaction: 6-12 pts (+++)).

**Results and Discussion**

Our results demonstrated the presence of COX2 in cells of mammary adenocarcinoma of all three grades (G) of histological malignancy (Figs 1-3).

Expression of COX2 was noted in 74% of the studied tumours. The results exceeded those described by Dore et al. (4). The authors examined 84 adenocarcinomas of the mammary gland in bitches and demonstrated the expression of COX2 in 56% of the tumours. In turn, Half et al. (6) examined mammary cancers in women and detected COX2 expression in 63% of cases, Soslow et al. (22) noted it in 56%, while Ristimaki et al. (18) in 37% of cases. The results have demonstrated the relatively extensive diversity of the results noted either in tumours of women or in those of bitches. It should be added that all the authors noted higher expression of COX2 in cancers than in adenomas as well as higher expression in adenomas than in normal tissue. In our studies 43% of adenocarcinomas manifested the presence of COX2 at the (+) level, 22% on the (++) level and 9% at the (+++) level. In 26% of malignant mammary tumours in bitches no COX2 expression could be disclosed (Fig. 4).

Comparing the extent of COX2 expression with grade of tumour malignancy (G), we detected a positive although moderately expressed correlation between the examined variables. The correlation coefficient value amounted to $r = 0.41$ (Fig. 5).

This pointed to augmented levels of COX2 in tumours of a more malignant character. Among the aggressive tumours included to the G3 category, 50% of the cases showed COX2 expression at the (++) level, in cases of G2 tumours such expression was noted in only 9% tumours and no G1 tumour manifested COX2 expression at such a high level. In addition, among tumours included to the G1 category 50% of them manifested no COX2 expression. The obtained results permit to assume that growing aggressiveness of mammary adenocarcinomas in bitches is accompanied by rising levels of COX2 synthesis. A similar tendency has been noted in mammary carcinomas in women and in several other human tumours of epithelial origin. In female mammary cancer, Ristimaki et al. (24) have noted growing expression of COX2, which has been positively correlated with tumour size, intensities of Ki67 antigen and p53 protein expression and with amplification of HER2 (Human Epidermal Growth Factor Receptor) gene. The authors have detected also an unfavourable effect of COX2 overexpression on duration of survival of patients with metastases to lymph nodes. Expression of cyclooxygenase 2 protein is thought to be affected by autocrine polypeptide growth factors, heregulins (HRG), which participate, i.a. in formation of HER2/HER3 heterodimers, the strongest mitogens formed by receptors of HER family (10, 28).

**Table 1**

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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 pts – to 10% cells with positive reaction</td>
<td>1 pts – low intensity of colour reaction</td>
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<tr>
<td>2 pts – 11-50% cells with positive reaction</td>
<td>2 pts – average intensity of colour reaction</td>
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<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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Fig. 1. Intense reaction for COX2 (+++) in cytoplasm of mammary gland adenocarcinoma cells in a bitch. 400x

Fig. 2. Moderate intensity reaction for COX2 (++) in cytoplasm of mammary gland adenocarcinoma cells in a bitch. 400x

Fig. 3. Faint reaction for COX2 (+) in cytoplasm of mammary gland adenocarcinoma cells in a bitch. 400x
Apart from involvement in mammary gland neoplastic transformation, overexpression of COX2 plays a significant role in carcinogenesis of human colon. It has been demonstrated in 80% of cancers of this fragment of the alimentary tract (2, 15). This has been confirmed also by studies of Oshima et al. (14), conducted on mice with mutation of the suppressor APC (adenomatous polyposis coli) gene, responsible in humans for the development of familial adenomatous polyposis. Such mice have been hybridized with the mice carrying mutations of both APC gene and the gene coding for COX2. In the homozygotic progeny (with damaged both alleles of COX2 gene) approximately sevenfold lower number of polyps has been disclosed as compared to individuals with the unchanged COX2 gene. The authors have also found that administration of selective COX2 inhibitors results in a decreased number of the polyps. In turn, studies of Kakiuchi et al. (9) have documented a significant effect of COX2 overexpression in colon cancer on increased metastazing potential of the tumour cells. Moreover, Li et al. (11) have induced in Syrian hamsters spinocellular carcinoma by the administration of dimethylbenzanthracene. Parallel administration of the selective COX2 inhibitor, celecoxib, has reduced incidence of the tumours from 76% to 50% of the treated animals.

Summing up, it should be assumed that activation of COX2 gene with the subsequent overproduction of the enzyme plays a significant role in neoplastic transformation of epithelial cells in the mammary gland of bitches. Detailed recognition of the involved pathomechanisms in the processes might allow to work out more effective approaches to prevention and treatment, based on selective inhibitors of COX2.
References