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Cyclooxygenase-2 expression in epithelial neoplasms and its relevance as a targeted therapy in dogs

Expressão de ciclooxigenase-2 em neoplasias epiteliais e sua relevância como terapia alvo em cães

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— NOTE —

ABSTRACT

Targeted therapy of neoplasms is an emergent approach in human and veterinary medicine. Cyclooxygenase (COX) is a class of catalytic enzymes related to the formation of inflammatory mediators. COX-2 is expressed constitutively in a few body tissues, but it may be induced in specific pathophysiologic conditions, such as cancer. COX-2 expression in neoplams may be considered a potential predictive factor, due to the possible association of selective COX-2 inhibitors in adjuvant treatments. This scientific communication has the objective to report COX-2 expression in seven neoplasms of dogs and the usage of adjuvant treatment with COX-2 selective inhibitors as an effective and feasible option in cancer treatment.

Key words: *cancer, carcinoma, immunohistochemistry, antineoplastic drugs.*

RESUMO

O tratamento direcionado das neoplasias é uma abordagem emergente tanto na medicina humana, quanto na veterinária. A cicloxigenase (COX) é uma classe de enzimas catalíticas relacionada à formação de mediadores inflamatórios. A COX-2é expressa de forma constitutiva em poucos tecidos, mas pode ser induzida em condições patofisiológicas específicas, como os processos neoplásicos. A expressão da COX-2 em neoplasias pode ser considerada um fator preditivo em potencial, tendo em vista a possibilidade de associação de inibidores seletivos para COX-2 em tratamentos adjuvantes. Esta comunicação científica teve como objetivo relatar a expressão de COX-2 em neoplasias de sete cães e o tratamento adjuvante com inibidores seletivos para COX-2 como uma opção efetiva e viável no tratamento do câncer.

Palavras-chave: *câncer, carcinoma, imuno-histoquímica, antineoplásicos.*

An emergent tendency in cancer research is related to rational design and usage of drugs, in which it is necessary to identify a therapeutic target. Treatment randomness is no longer accepted within this tendency (PEEK, 2004).

Cyclooxygenase (COX) is a class of catalytic enzymes that promote aracdonic acid conversion into a variety of prostaglandins (BEAM et al., 2003). Prostaglandins have important functions in almost all systems and regulate physiologic processes related to immunity, reproduction, vascular tonus and integrity, nerve growth and development, and bone metabolism. These proteins are synthesized in a great variety of tissues acting as an autocrine or paracrine mediator in microenvironment signaling (GUPTA, 2001). Prostaglandins may pathologically contribute for the development of cancer through several mechanisms: increasing cellular proliferation, inhibiting apoptosis, modifying carcinogen metabolism or modulating the immune system (FISHER, 1997).

COX-2 expression may be related to its mechanism in tumoral promotion and progression phases during carcinogenesis. Therefore, it may be considered a poor prognostic factor in many neoplastic histologic types (MUTSAERS, 2013). COX-2 action in tumor progression is associated with inhibition of apoptosis, increase in cellular proliferation and angiogenesis, and enhanced tumoral invasiveness (MUTSAERS, 2013; CASSALI et al., 2014). COX-2

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expression may be considered a potential predictive factor because of the possible associations of selective COX-2 inhibitors in adjuvant treatments (targeted therapy). Although the duration of treatment is still empirical, it is known that it should be prolonged, since COX-2 selective inhibitors are capable of inhibiting receptor activation, but do not show any effect in receptor expression (LAVALLE et al., 2009).

This scientific communication has the goal to report COX-2 expression in seven canine epithelial neoplasms and adjuvant treatment with selective COX-2 inhibitors. Therefore, seven canine patients with early stage malignant epithelial neoplasms whose tumors showed high COX-2 expression were included in this study.

Age, breed, tumor location, stage, surgical margins, diagnosis, COX-2 score, and treatment duration with selective COX-2 inhibitor for each patient are listed in table 1. All patients underwent surgery for tumor resection. Tumors were fixed in buffered 10% formalin and sent to histopathology and COX-2 immunohistochemistry (IHC) analysis.

Histopathology diagnoses were squamous cell carcinoma (three cases), basal cell carcinoma, perianal mucinous adenocarcinoma, hepatoid carcinoma, and apocrine carcinoma with squamous differentiation.

Immunohistochemistry was performed using a primary rabbit monoclonal anti-human COX-2 antibody (SP21, 1 : 20, Lab Vision, Freemont, CA), as described by LAVALLE et al. (2009). Sections from a human colon carcinoma known to express COX-2 were used as positive controls for COX-2, and negative controls were obtained with suppression of the primary antibody. According to LAVALLE et al. (2009), COX-2 IHC assessment was semi-quantitative; the distribution score was defined by the percentage estimate of positive cells in five high-power fields (400x magnification). Final score, from 0 to 12, was determined by the product of distribution and intensity values. For distribution, scores ranged from zero to four - zero for 0% of stained cells; one for 1% to 10% of stained cells; two for 11% to 30% of stained cells; three for 31% to 60% of stained cells; and four for more than 61% of stained cells. For intensity, scores ranged from zero to three - zero for no staining; one for weak staining; two for moderate staining; and three for strong staining.

Five patients were treated with selective COX-2 inhibitor; four of them received firocoxib (Previcox®), orally, in a daily dose of $5mgkg^{-1}$. The patient with basal cell carcinoma received mavacoxib (Trocoxil®), orally, $2mgkg^{-1}$ every 14 days and every 30 days after the second dose. Two patients were treated with electrochemotherapy only.

Follow-up included physical exams, complete blood counts, and biochemistry analyses every 30 days. No significant changes were seen in the exams. Two animals treated with firocoxib presented vomit, anorexia, diarrhea, and dark stool. Their treatments were discontinued, with resolution of clinical signs. Currently, treated patients are healthy, with disease free interval and overall survival time over one year.

Collective evidence in human, animal, and cell culture studies clearly indicates that targeted inhibition of COX-2 is a viable approach for cancer prevention and treatment (PEEK, 2004). Cyclooxygenase inhibitors efficacy was demonstrated

Table 1 - Histological type and COX-2 score for each patient (canine). T1 -tumor <2cm; T3 -tumor >5cm; N0 -no evidence of regional lymph node involvement; M0 -no evidence of distant metastasis.

Breed	Age (years)	Tumor location	Staging	Surgical margins	Diagnosis	COX-2 score (0-12)	Selective COX-2 inhibitor treatment duration (months)
Boxer	12	Perianal	T1N0M0	Compromised	Perianal mucinous adenocarcinoma	6	6
Poodle	9	Perianal	T1N0M0	Adequate	Hepatoid carcinoma	4	1
Golden Retriever	12	Left shoulder	T1N0M0	Adequate	Squamous cell carcinoma	6	2
Poodle	16	Left upper lip	T1N0M0	Adequate	Apocrine carcinoma with squamous diferentiation	4	3
Crossbreed	7	Prepuce	T3N0M0	Compromised	Squamous cell carcinoma	4	-
English Bulldog	11	Prepuce	T1N0M0	Compromised	Squamouscell carcinoma	6	-
Crossbreed	1	Face	T1N0M0	Compromised	Basal cell carcinoma	6	4

with palliative goal in dogs with transitional cell carcinomas (CHUN & THAMM, 2013) and inoperable squamous cell carcinomas (SCHMIDT et al., 2001), but with curative intent after chemotherapy in patients with inflammatory carcinomas (CAMPOS et al., 2011) and advanced stage mammary carcinomas (LAVALLE et al., 2009).

According to LAVALLE et al. (2012), selective COX-2 inhibitors may benefit patients with high (6-12) COX-2 immunohistochemistry scores. Early stage carcinoma and a high COX-2 score seen in most patients in this study qualified them as candidates for targeted therapy with selective COX-2 inhibitors, not for chemotherapy.

Non steroidal anti-inflammatory drugs (NSAIDs) side effects are based on their pharmacological mechanism of action. NSAIDs act mainly through the inhibition of COX activity in the biochemical cascade that leads to prostaglandin synthesis, and there are selective NSAIDs for COX-2 inhibition (KHWANJAI et al., 2011). COX-1 and COX-2 are involved in renal blood flow and tubular function regulation; therefore, selective COX-2 NSAIDs do not necessarily cause less renal side effects. In this study, renal function was monitored with monthly evaluation of blood urea nitrogen and creatinine, to detect early signs of renal impairment. COX selectivity is related only to the potential to reduce gastrointestinal (GI) side effects in healthy tissues and there is no association with renal or hepatic adverse effects, nor with effects in altered GI tract (KUKANICH et al., 2012). Side effects seen in two patients in this study are probably related to preexisting gastrointestinal disease, worsened with the usage of NSAID.

COX-2 immunohistochemistry score may be a prognostic and predictive factor in veterinary medicine, and COX-2 expression was demonstrated in different canine epithelial neoplasms, in this study. However, more studies are needed to determine the benefit of using selective COX-2 inhibitors for the adjuvant treatment of patients with advanced stage disease.

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