Expression of cyclooxygenase-2 in transitional cell carcinoma of the urinary bladder in dogs

K. Nasir M. Khan, DVM; Deborah W. Knapp, DVM; Dennis B. Denicola, DVM; R. Keith Harris, DVM

Objective—To evaluate expression of cyclooxygenase (COX)-1 and COX-2 in the urinary bladder epithelium of clinically normal dogs and in transitional cell carcinoma cells of dogs.

Animals—21 dogs with transitional cell carcinoma of the urinary bladder and 8 dogs with clinically normal urinary bladders.

Procedure—COX-1 and COX-2 were evaluated by use of isofrom-specific antibodies with standard immunohistochemical methods.

Results—COX-1, but not COX-2, was constitutively expressed in normal urinary bladder epithelium; however, COX-2 was expressed in neoplastic epithelium in primary tumors and in metastatic lesions of all 21 dogs and in new proliferating blood vessels in 3 dogs. Also, COX-1 was expressed in the neoplastic cells.

Conclusions and Clinical Relevance—Lack of expression of COX-2 in normal bladder epithelium and its substantial expression in transitional cell carcinoma cells suggest that this isoform may be involved in tumor cell growth. Inhibition of COX-2 is a likely mechanism of the antineoplastic effects of nonsteroidal anti-inflammatory drugs. (Am J Vet Res 2000;61:478–481)

Transitional cell carcinoma (TCC) is one of the most common neoplasms of the lower portion of the urinary tract in dogs and accounts for approximately 2% of all naturally occurring malignant tumors in this species. Transitional cell carcinoma in dogs bears many similarities to invasive bladder cancer in humans with regard to its morphologic pattern, biological behavior, and response to single-agent chemotherapy; thus, it may be useful as an animal model of human invasive bladder cancer.1 In a recent clinical trial of a nonsteroidal anti-inflammatory drug (NSAID), piroxicam, in 34 dogs with TCC, tumor responses included complete remission in 2 dogs, partial remission in 4 dogs, and stable disease in 18 dogs.1 The exact mechanisms of the anti-tumor effects of this drug are not known; however, an effect on arachidonic acid metabolism by inhibiting cyclooxygenase (COX) has been suggested.

In the arachidonic acid pathway, COX catalyzes the committed step in prostaglandin biosynthesis and exists in 2 related but unique isoforms, COX-1 and COX-2.2,3 Cyclooxygenase-1 is considered to be involved in the production of prostaglandins that modulate normal physiologic functions in several organ systems, including the kidneys, gastrointestinal tract, and platelets, whereas COX-2 is considered to be involved in the production of prostaglandins that modulate physiologic events in development, cell growth, and inflammation.

Because NSAID inhibit both COX isoforms, it is not clear whether their antineoplastic effects are attributable to inhibition of COX-1, COX-2, or both. This is an important issue because of the recent development of specific COX-2 inhibitors, especially considering their potential use in prevention of cancer.2,4 Thus, to attain further insight into the possible mechanism of antineoplastic effects of NSAID, the purpose of the study reported here was to evaluate expression of COX-1 and COX-2 in the urinary bladder epithelium of clinically normal dogs and in transitional cell carcinoma cells of dogs.

Materials and Methods

Tissue specimens—To evaluate the expression of COX-1 and COX-2 in the normal urinary bladder, tissue specimens were collected from 8- to 16-month-old Beagles (n = 8). To evaluate expression of these isoforms in TCC, primary tumor samples were collected by biopsy or at necropsy from 21 dogs referred to the Purdue Comparative Oncology Program at the School of Veterinary Medicine, Purdue University. Additionally, metastatic lesions in various organs were collected at necropsy from 7 of these dogs. All tissues were collected in neutral-buffered 10% formalin and processed with the National Institute of Health guidelines for the care and use of animals in research, and investigators received prior permission from the Purdue Animal Care and Use Committee.

Immunohistochemical methods—Standard immunohistochemical procedures and commercially available reagents were used for detection of COX-1 and COX-2 as described,5 with minor modifications. In general, tissues were paraffin-embedded, sectioned at 4 to 5 µm, and mounted on positively charged slides.6 Sections were dewaxed, rehydrated in xylene and decreasing concentrations of alcohol, and blocked for endogenous avidin-biotin. All tissues were peroxidized (0.3% Triton, 0.2% saponin, 1% bovine serum albumin in phosphate-buffered saline [0.9% NaCl] solution [PBSS]), preblocked in PBSS containing 10% normal goat serum,5 and incubated in primary antibody overnight at 4 C. The COX-1 and COX-2 primary antibodies were prostaglandin H synthase-1 polyclonal rabbit anti-human.
and prostaglandin H synthase-2 polyclonal rabbit anti-human antibodies, respectively. These antibodies were diluted 1:100 in 1% bovine serum albumin-PBSS. Immunoreactive complexes were detected via an enhanced streptavidin-biotin affinity system and stained with diaminobenzidine that reacts with peroxidase to give a brown reaction product. Slides were counterstained briefly with Mayer hematoxylin. All control slides were processed without primary antibody and incubated with biotinylated goat anti-rabbit IgG or biotinylated horse anti-rabbit IgG at the appropriate dilution to accompany the test slides.

Percentage of neoplastic cells with COX immunoreactivity and staining intensity (mild, moderate, and intense) were estimated by evaluation of 5 to 10 fields under high magnification (50×).

Results

Expression of COX-1 and COX-2 in normal urinary bladder epithelium—Immunoreactivity for COX-1 was detected in the mucosal epithelium of the urinary bladder collected from control dogs (n = 8) and in the normal urinary bladder adjacent to neoplasms (7) collected from dogs with TCC (Fig 1); however, COX-2 was not detected in the normal urinary bladder epithelium.

Expression of COX-1 and COX-2 in TCC—Histologically, TCC was classified as papillary infiltrative in 9 cases and nonpapillary infiltrative in 7 tumors. Histologic classification was not possible in 5 tumors because of sample size or orientation. Moderate to intense COX-2 immunoreactivity was detected in neoplastic epithelial cells within primary tumors in all 21 dogs and in the metastatic lesions in lungs, liver, stomach, spleen, regional lymph nodes, adrenal glands, kidneys, and blood vessels of 7 dogs (Table 1; Fig 2 and 3). The endothelial cells in small proliferating blood vessels within neoplasms had COX-2 immunoreactivity in 3 instances (Fig 4). The stroma and blood vessels in all other instances did not have COX-2 immunoreactivity. Staining for COX-2 was diffuse throughout the cytoplasm. The percentage of neoplastic epithelial cells staining for COX-2 was slightly higher in primary tumors (56.7 ± 24.9%) than metastatic lesions (44.3 ± 23.7%).

Similar to the normal urinary bladder epithelium, COX-1 immunoreactivity was also detected in neoplastic epithelial cells in all 21 primary tumors and 7 metastatic lesions. In addition, endothelial cells of blood vessels within 1 tumor had COX-1 immunoreactivity. Staining for COX-1 was coarse and granular and detected throughout the cytoplasm. The percentage of neoplastic epithelial cells staining for COX-1 was slightly higher in primary tumors (59.0 ± 22.1%) than metastatic tumors (46.7 ± 25.9%).

Discussion

Nonsteroidal anti-inflammatory drugs are nonselective inhibitors of both COX isoforms and are commonly used for the clinical management of rheumatologic disorders. Recently, results of several epidemiologic studies in humans,6,7 experimental studies in laboratory animals,8,9 and clinical studies in dogs10 and humans11 have suggested preventative or anti-tumor effects of NSAID (eg, aspirin, piroxicam, sulindac, and ibuprofen) against naturally occurring or chemically induced neoplasia. For example, dogs with naturally occurring TCC of the urinary bladder, when treated with piroxicam, had partial or complete remissions and had improved median survival times.12

The exact mechanism of anti-tumor activity of NSAID is not known; however, it is hypothesized that specific or nonspecific inhibition of arachidonic acid metabolism via inhibition of COX enzymes by these compounds may modulate eicosanoid production and affect tumor cell proliferation, neovascularization within the tumor, and immune responsiveness. This effect could be induced by NSAID inhibition of one or both isoforms of COX. In the study reported here, COX-2 was expressed in neoplastic transitional epithelial cells and was not detected in normal urinary bladder epithelium suggesting that COX-2 may be involved in the progression of TCC of the canine urinary bladder. The role of COX-1 in tumorigenesis is less clear, because this isoform was expressed in normal and neoplastic epithelial cells. In the urinary bladder of mice, both COX isoforms are reported to express constitutively.13

The potential roles COX-2 plays in tumor cell growth include direct effects on cell proliferation or indirect effects secondary to the release and function of other cytokines, as suggested by the role of COX-2 in mitogenesis, ovulation, and kidney and bladder development.13-15 Some investigators have suggested that COX-2 may be involved in new capillary growth in tumors; however, with the exception of 3 tumors, COX-2 was not detected in tumor vasculature in the study reported here. It is possible, however, that COX-2-mediated prostaglandins play a vasodilatory role in blood vessels within tumors. Thus, inhibition of these prostaglandins by NSAID may deplete the local blood supply, resulting in shrinkage of the tumor mass. The NSAID seem to have great potential as anti-neoplastic and preventive agents against cancer in human and veterinary medicine; however, in many patients these nonselective COX inhibitors cannot be consistently used over a long period at therapeutic doses because of their gastrointestinal and renal toxicity.12,16 Protection of gastric mucosal cells, renal blood flow, and renal handling of electrolytes are believed to be regulated by COX-1, and gastric toxicity seen with NSAID is attributable to inhibition of COX-1.4,7 We have reported that COX-1 is the most abundant isoform in dog, rat, monkey, and human kidneys, whereas COX-2 is expressed at low concentrations under basal conditions, suggesting that COX-1 plays the major role in maintaining renal functions under normal conditions.7 This data is further supported by observations that nonselective COX inhibitors (eg, meloxicam and relafen) at near-therapeutic dosages induce renal papillary necrosis in dogs, whereas selective COX-2 inhibitors (eg, celecoxib) spared dogs from this toxicity even when administered at dosages several fold above the therapeutic requirements.5 Thus, selective COX-2 inhibitors presently being developed aggressively within the pharmaceutical industry are expected to be highly efficacious for inhibiting inflammatory prostaglandins with little or no toxicity.4,6

Cyclooxygenase-2 was substantially expressed in the neoplastic epithelium of urinary bladder TCC and not in normal urinary bladder epithelium of dogs in the study reported here. Cyclooxygenase-1 was expressed in normal bladder epithelium and in neoplastic cells. Constitutive expression of COX-1 in the normal urinary bladder epithelium suggests that this isoform may be involved in basal physiologic functions, whereas lack of expression of COX-2 in the nor-

Table 1—Results (No. of positive results/No. of tissues tested) of immunohistochemical staining for cyclooxygenase (COX)-1 and COX-2 in dogs with normal urinary bladder epithelium and dogs with transitional cell carcinoma of the urinary bladder

<table>
<thead>
<tr>
<th>Tissue</th>
<th>COX-1</th>
<th>COX-2</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal bladder epithelium</td>
<td>8/8</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Adjacent normal tissue*</td>
<td>2/21</td>
<td>1/21</td>
<td>3/21</td>
</tr>
<tr>
<td>Neoplastic cells</td>
<td>2/21</td>
<td>21/21</td>
<td>21/21</td>
</tr>
<tr>
<td>Metastatic lesions</td>
<td>7/7</td>
<td>7/7</td>
<td>7/7</td>
</tr>
<tr>
<td>Neovascular tissue†</td>
<td>1/21</td>
<td>3/21</td>
<td>0/21</td>
</tr>
</tbody>
</table>

*Adjacent normal tissue was obtained from bladder epithelium adjacent to a transitional cell carcinoma. †Neovascular tissue was obtained from within tumors.
mal bladder and its substantial expression in TCC sug-
gest that this isomform may be involved in tumor cell
growth, and inhibition of this isomform may be a mech-
anism of the anti-neoplastic effects of NSAID.

activity between cisplatin and piroxicam in canine transitional cell
carcinoma of the bladder, a model of human invasive bladder can-
2. Paraplast Xtra, Oxford Labware, St Louis, Mo.
3. Superfrost Plus, Erite Scientific Co, Portsmouth, NH.
4. Sigma Chemical Co, St Louis, Mo.
5. Vector Laboratories Inc, Burlingame, Calif.
8. Dako Corp, Carpenteria, Calif.
    tyn of a specific COX-2 inhibitor versus NSAIDs in dogs (abstr). Vet
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