Expression of cyclooxygenase-2 in canine uveal melanocytic neoplasms

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Objective—To determine whether cyclooxygenase-2 (COX-2) is expressed in benign or malignant canine uveal melanocytic neoplasms and whether expression correlates with malignancy.

Sample Population—Tissue sections from 71 globes; 57 with benign (n = 15), malignant (34), or mixed (8) uveal melanocytic neoplasms; 10 with nonneoplastic disease; and 4 with no abnormalities.

Procedures—Bleached sections from all globes and canine kidney were incubated with mouse monoclonal antibody directed against rat COX-2 protein or mouse antibody isotype control. Location, intensity, and percentage of immunolabeled cells were scored.

Results—Expression of COX-2 was detected in all but 5 globes, all of which contained neoplasms. Expression of COX-2 was detected in regions infiltrated by neoplasia in 21 globes; however, definitive labeling of tumor cells was detected in only 2 of those. In the remaining 19 globes, COX-2 expression was detected in areas also labeled in globes without disease and in globes with nonneoplastic disease, especially the aqueous outflow tract and ciliary body. However, only globes with uveal malignant melanomas had detectable COX-2 expression in the iris. Expression of COX-2 was detected in the ciliary body of more globes with uveal malignant melanoma (20/34) than in those without disease (13/41), with nonneoplastic disease (4/10), or with melanocytoma (3/15) or mixed neoplasms (3/8).

Conclusions and Clinical Relevance—Canine globes with uveal melanocytic neoplasia appeared to express COX-2 in similar sites and with similar intensity as globes without neoplasia. Differentiation of benign from malignant canine uveal melanocytic neoplasms was not possible. (Am J Vet Res 2009;70:1284–1290)
plasms and whether the degree of COX-2 expression correlates with malignancy.

**Materials and Methods**

**Sample population**—Sixty-seven paraffin-embedded canine globes were selected from the Comparative Ocular Pathology Laboratory of Wisconsin collection. All globes underwent histologic review by a board-certified veterinary pathologist (RRD) and were categorized as diseased but without evidence of neoplasia or containing uveal melanocytic neoplasms (melanocytomas, malignant melanomas, or mixed neoplasms). Criteria used to diagnose each neoplasm type were based on morphology of neoplastic cells, presence of heavily pigmented cells (often characterized as benign), and presence or absence of mitotic figures. Histologic diagnoses were based on review of H&E-stained sections. Occasionally, sections bleached with potassium permanganate were also used. Four nondiseased globes obtained from the University of California Veterinary Pathology Service following postmortem removal from dogs euthanatized for nonocular disease served as additional controls in this study. These nondiseased globes lacked any histologic lesions. For all globes used in this study, data regarding clinical diagnoses were obtained from the submitting clinician and complete histologic diagnoses were obtained from the pathology report. In particular, clinical or histologic evidence of the presence or absence of glaucoma was noted.

**Immunohistochemical evaluation**—Tissue sections (5 μm) from all globes were mounted onto positively charged slides and underwent immunohistochemical detection of COX-2. Formalin-fixed, paraffin-embedded tissue sections of healthy canine (puppy) kidney served as a positive control for COX-2 expression and were processed in an identical manner to that used for globes. All tissue sections were deparaffinized in xylene and rehydrated in an alcohol series of decreasing concentration. Melanin was bleached, and endogenous peroxidases were depleted by incubation in a 10% solution of hydrogen peroxide in PBS solution at 22°C for approximately 3 hours. Antigen retrieval was performed in citrate buffer heated in a microwave at 180 W for 10 minutes and allowed to cool on the benchtop for 20 minutes. Nonspecific binding was blocked by use of 10% heat-inactivated horse serum in PBS solution for 20 minutes at 22°C.

The primary antibody was a mouse monoclonal antibody directed against the rat COX-2 protein. A purified mouse IgG, isotype control was used for negative controls. Both antibodies were used at a dilution of 1:30 in PBS solution. Sections were incubated with primary antibody for 90 minutes and incubated for 30 minutes with goat anti-mouse antibody linked to horseradish peroxidase. All antibody incubations were performed in a humidified chamber at 22°C. Immunocomplexes were visualized by use of aminoethyl carbazole substrate and counterstained with Mayer hematoxylin. Slides were washed with PBS solution after bleaching, antigen retrieval, primary antibody incubation, secondary antibody incubation, aminoethyl carbazole, and counterstaining steps. Slides were preserved in mounting media and protected with a coverslip.

For each globe, an immunolabeled slide and a negative control were assessed for immunolabeling by 1 author (DP) by use of light microscopy. All regions of the globe were assessed, and any ocular tissue that expressed COX-2 was reported. Each globe was scored for positive immunolabeling of the sclera, each corneal layer, all sections of the uvea, aqueous outflow tract, all parts of the lens, and retina. For the purpose of COX-2 expression, the aqueous outflow tract was defined as the trabecular meshwork and ciliary cleft. Uveal areas containing melanocytic neoplastic tissue were closely assessed in an attempt to define whether neoplastic cells were specifically labeled. Scoring was based on labeling intensity and percentage of positively labeled neoplastic cells. Briefly, labeling intensity was graded semiquantitatively as negative (−), weak (+), moderate (++), or strong (+++) labeling. Percentage of positively labeled neoplastic cells was graded as 0 (negative), 1 (rare positive neoplastic cells), 2 (< 5% of cells), 3 (3% to 10% of cells), or 4 (> 10% of cells).

**Results**

**Sample population**—Histologic diagnoses for the 57 globes with uveal melanocytic neoplasia included anterior uveal melanocytoma (n = 15), anterior uveal melanoma (n = 19), uveal melanoma with choroidal extension (n = 16), choroidal melanoma (n = 2), anterior uveal melanoma with conjunctival extension (n = 1), and choroidal melanoma with ciliary body extension (n = 1).

- **Figure 1**—Photomicrographs of collecting duct epithelial cells in the renal medulla of nondiseased canine kidney that has (A) or has not (B) undergone bleaching with hydrogen peroxide prior to labeling for COX-2. Immunohistochemical stain.
malignant melanoma (34), and mixed melanocytic neoplasm (malignant melanoma arising within uveal melanocytoma; 8). Histologic diagnoses in diseased globes without neoplasia (n = 10) included chronic superficial keratitis (10), corneal endothelial attenuation (4), lens capsule rupture (2), preiridal fibrovascular membrane (2), peripheral anterior synchiae (2), phthisis bulb (2), cataract (1), lens luxation (1), intraocular hemorrhage (1), asteroid hyalosis (1), and goniodysgenesis (1). Thirty-nine (68.4%) globes with neoplasia and 7 of 10 diseased globes without neoplasia had clinical or histologic evidence of glaucoma or both.

Immunohistochemical findings—Expression of COX-2 was detected in collecting duct epithelial cells in the renal medulla of all positive control canine kidney samples irrespective of whether they were bleached or not (Figure 1). Expression of COX-2 was also detected in all 4 nondiseased canine globes. Various sites in nondiseased globes were labeled, including the ciliary body musculature, the trabecular meshwork of the aqueous outflow tract (Figure 2), lens epithelium, choroidal stroma, and scleral lamina cribrosa (Table 1). The COX-2 staining was variably granular in the cytoplasm. No nondiseased globes had detectable COX-2 expression in the cornea, corneoscleral limbus, anterior sclera, iris, or retina.

Labeling for COX-2 was noted in 52 (91.2%) globes with uveal neoplasia. Globes in which COX-2 expression was not identified included 3 with malignant melanocytic neoplasms, and 1 with choroidal melanocytomas, and 1 with uveal melanomas.

Table 1—Summary of COX-2 expression based on anatomic location in nondiseased canine globes, canine globes with melanocytic neoplasms, and canine globes with nonneoplastic disease. Values indicate number of globes.

<table>
<thead>
<tr>
<th>Location</th>
<th>Tissue</th>
<th>Nondiseased globes (n = 4)</th>
<th>Uveal melanocytomas (n = 15)</th>
<th>Uveal malignant melanomas (n = 34)</th>
<th>Mixed melanocytic neoplasms (n = 8)</th>
<th>Diseased globes without neoplasia (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>Epithelium</td>
<td>NA</td>
<td>0</td>
<td>1</td>
<td>11</td>
<td>2</td>
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<tr>
<td></td>
<td>Stroma</td>
<td>NA</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Limbus</td>
<td>Collagen</td>
<td>2</td>
<td>5</td>
<td>18</td>
<td>5</td>
<td>3</td>
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<tr>
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<td>Blood vessels</td>
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<td>2</td>
<td>9</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adjacent to neoplasm</td>
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<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Iris</td>
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<td>0</td>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Lamina cribrosa</td>
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<td>NA</td>
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<td>Dilator muscle</td>
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<td>NA</td>
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<tr>
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<td>3</td>
<td>20</td>
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<td>4</td>
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<tr>
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<td>11</td>
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<td></td>
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<td>8</td>
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<tr>
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<td>20</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Stromma</td>
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<tr>
<td></td>
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<tr>
<td>Retina</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Lens epithelium</td>
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</tbody>
</table>

AOT = Aqueous outflow tract. NA = Not applicable. NCBE = Nonpigmented ciliary body epithelium. NFL = Nerve fiber layer. RPE = Retinal pigment epithelium.
melanoma, 1 with melanocytoma, and 1 with a mixed neoplasm. A wide variety of cells and tissues in globes with neoplasia expressed COX-2 (Table 1). Of the 57 canine globes with neoplasia, 21 (36.8%) expressed COX-2 in regions infiltrated by the uveal melanocytic neoplasm. However, because these neoplasms invaded and were admixed with nondiseased tissue in many globes, it was possible to definitively identify labeling of neoplastic cells in only 2 of these 21 globes, 1 with a mixed neoplasm and 1 with malignant melanoma (Figure 3). In the remaining 19 globes, COX-2 expression was evident in those areas in which it was also seen in nondiseased globes and diseased globes without neoplasia; especially the trabecular meshwork of the aqueous outflow tract (Figure 4) and the nonpigmented epithelium of the ciliary body (Figure 5). All diseased globes without neoplasia expressed COX-2 in at least 1 ocular tissue (Figure 3). However, only globes with uveal malignant melanoma had detectable COX-2 expression in the iris. The percentage of globes in which COX-2 expression was detected in the ciliary body was notably greater in globes with uveal malignant melanoma (58.8%) than in nondiseased globes (1/4), diseased globes without neoplasia (4/10), or globes with melanocytoma (3/15) or mixed neoplasms (3/8). Among the globes with neoplasia, labeling score (percentage of cells labeled for COX-2) and labeling intensity were highly variable; however, moderate labeling intensity and scoring grades (+ and 2, respectively) were noted most commonly. Moderate labeling intensity was noted in 16 (47%) malignant melanomas, 2 melanocytomas, and 5 mixed neoplasms. A moderate scoring grade was assigned to 19 (55.9%) malignant melanomas, 3 melanocytomas, and 5 mixed neoplasms.

In all, 46 of 67 (68.7%) diseased globes had clinical or histologic evidence of glaucoma; 7 were diseased globes without neoplasia and 39 were globes with neoplasia. There was COX-2 expression in the aqueous outflow tract of 25 and in the ciliary body of 18 of these 46 globes. In 5 of the 18 globes with ciliary body labeling, COX-2 expression was localized to the nonpigmented ciliary body epithelium. Of the remaining 21 globes that did not have evidence of glaucoma, 16 and 12 had COX-2 expression in the aqueous outflow tract and ciliary body, respectively. Uveitis was diagnosed in 2 diseased globes without neoplasia and 8 globes with neoplasia. All 10 globes with evidence of uveitis expressed COX-2, and all but 1 expressed COX-2 in the aqueous outflow tract.

Discussion

In this study, definitive intraneoplastic expression of COX-2 was detected in only 3.5% of canine uveal melanocytic neoplasms. However, it is highly likely that this represented an underestimate of the expression of this enzyme in these neoplasms because ocular tissues that frequently were abutted or invaded by the uveal neoplasm (such as the iris, ciliary body, aqueous outflow tract, and anterior sclera) expressed COX-2 but were not included in this count unless expression was categorically identified in a neoplastic cell. The find-
ing that COX-2 expression was infrequently (ciliary body muscle and aqueous outflow tract) or never (iris, ciliary body stroma, nonpigmented ciliary body epithelium, and anterior sclera) detected in these adjacent tissues in nondiseased globes strongly supports the theory that COX-2 expression in these surrounding tissues may actually have originated from invasive and expansile neoplastic cells. However, the proportion of globes with neoplasia in which definitive COX-2 expression was detected was lower than that reported for human uveal malignant melanomas. In 3 studies of a total of 87 human uveal malignant melanomas, the percentage of tumors in which COX-2 expression was detected was 13%, 30% and 91%. The different rates reported among those studies may have resulted from the methods used or interpretation of labeling. For example, none of the studies used bleached sections as in the present study. However, bleaching with hydrogen peroxide is effective when used in conjunction with immunohistochemical analysis, and this method has been used effectively in a study examining COX-2 expression. Additionally, in the present study, COX-2 expression was consistent between positive control samples that underwent hydrogen peroxide bleaching and those that did not. Therefore, it appears unlikely that bleaching affected COX-2 detection in the present study. It is also possible that the relatively infrequent COX-2 expression detected in canine neoplasms represents a true species difference between dogs and humans or may be because only uveal malignant melanomas were examined in the reports of human disease, whereas benign and mixed tumors, in addition to malignant melanomas, were examined in the present study.

Because COX plays a role in the release of prostaglandins during inflammation, the COX-2 expression detected in globes with uveal melanocytic tumors may have been expressed by nonneoplastic or neoplastic cells as part of the associated inflammatory response rather than as a direct result of the neoplasm. Certainly, some of the globes with neoplasia that expressed COX-2 in the present study had histologic evidence of uveitis. For this reason, a control group of inflamed globes without neoplasia was included for comparison. Although statistical comparisons among groups were not possible in this study, some subjective observations were possible. Expression of COX-2 was largely similar between the globes with neoplasia and diseased globes without neoplasia; however, 2 major differences were noted. All 10 diseased globes without neoplasia expressed COX-2 in the cornea, compared with only 7% to 32% of globes with neoplasia and none of the nondiseased globes. By contrast, COX-2 expression was never detected in lens epithelium of diseased globes. COX-2 expression was detected in 12% of globes with melanocytic tumors and 1 in 4 of nondiseased globes. No expression was detected in lenses of globes with mixed tumors. Finally,

Figure 5—Photomicrograph of a section of a canine globe with anterior uveal melanocytoma. Notice the ciliary body, ciliary processes, and adjacent neoplasm. There is finely granular intracytoplasmic COX-2 immunoreactivity in the nonpigmented ciliary body epithelium (inset). Immunohistochemical stain.

Figure 6—Photomicrograph of sections of 2 diseased globes without neoplasia from dogs with chronic keratitis. Notice the granular intracytoplasmic COX-2 immunoreactivity predominantly in the corneal epithelium and occasionally in stromal keratocytes of 1 globe (A) and in the nonpigmented ciliary body epithelium of the other globe (B). Immunohistochemical stain.
diseased globes (those with neoplasia and those with nonneoplastic diseases) expressed COX-2 in a range of tissues such as the cornea, corneoscleral limbus, and retina that did not express COX-2 in any of the nondiseased globes. Because the most common histologic diagnosis in diseased globes without neoplasia of the present study was chronic superficial keratitis, and because corneal COX-2 expression has been reported, especially following corneal injury, corneal COX-2 expression in this group was expected. Taken together, these data suggest that COX-2 expression is likely upregulated by canine ocular disease in general and specifically in some globes with uveal neoplasia.

It is also possible that the coexistence of glaucoma in some of the globes with neoplasia may have altered COX-2 expression. In a study of 17 glaucomatous and 5 nondiseased canine globes, COX-2 expression was greater in glaucomatous globes, particularly in the cornea, sclera, trabecular meshwork, angular aqueous plexus, and ciliary body. The authors suggested that the expression of COX-2 might correlate with prostaglandin involvement in the pathogenesis of glaucoma; however, results from inflamed but nonglaucomatous globes were not reported. By contrast, less COX-2 expression has been reported in the nonpigmented ciliary body epithelium of human globes with end-stage primary open-angle glaucoma, compared with nondiseased human globes. In addition to species differences, it is likely that the pathogenesis of primary open-angle glaucoma, which may in part be caused by impaired COX-2 expression, varies substantially from the primary or secondary angle-closure glaucoma that occurs in canine globes. In the present study, it appeared that COX-2 expression in the aqueous outflow tract and ciliary body occurred in inflamed globes irrespective of the presence of glaucoma.

An additional purpose of the present study was to assess the value of COX-2 expression as a predictor of malignancy. When COX-2 expression of uveal malignant melanomas and mixed tumors was compared with that of benign uveal melanocytomas, 3 observations were possible. First, definitive intraneoplastic labeling was detected only in the globes infiltrated by malignant melanoma or a mixed tumor. Second, most globes with uveal malignant melanoma had COX-2 expression in the ciliary body whereas this was a less common finding in globes with mixed or benign tumors. Third, only globes with uveal malignant melanoma had detectable COX-2 expression in iridal tissue. Although this was not a consistent finding in globes with uveal malignant melanoma, COX-2 expression in the iris was not observed in globes from any other category. Therefore, although detection of COX-2 expression might be used to support a diagnosis of malignancy, its absence should not be used as confirmation that a uveal melanocytic tumor is benign. By contrast, in human uveal malignant melanomas, a combination of COX-2 expression score and intensity was found to be predictive of death caused by metastasis in 1 study. This difference may represent differences in the way these tumors behave among species or may reflect the reported difficulty in correlating histologic and behavioral criteria of malignancy for this tumor type in dogs. A study examining COX-1 and COX-2 expression in various canine neoplasms found that COX-2 expression was apparent in 9 of 15 oral malignant melanomas. However, like us, the authors of that study also detected variable intensity of COX-2 expression in those neoplasms. Although limited COX-2 expression in the uveal melanocytic neoplasms in the present study was detected, therapeutic inhibition of COX-2 by use of NSAIDs may still be effective. For example, response to COX inhibitors was detected in COX-2-negative human colorectal cancer cells, and COX inhibitors may exert non-COX–related antitumor mechanisms. In particular, it is possible that COX inhibition in the vasculature and not the neoplasm itself is important for antitumor activity.

References


