Summary. Cyclooxygenases catalyze the initial, rate-limiting steps of prostaglandin synthesis from arachidonic acid. Two isoforms of this enzyme exist in mammalian and avian species: COX-1 and COX-2. COX-1 is constitutively expressed and is the major isoform of gastrointestinal tissue. COX-2 is induced in response to inflammatory stimuli. COX-2 has been implicated in carcinogenesis of several neoplasms. Furthermore, COX-2 over-expression has been noted in many solid tumours and has been correlated with a worse prognosis in colorectal cancer, non-small-cell lung cancer, mesothelioma and gastric cancer. In this review, the most recent findings on the mechanisms by which COX-2 promote tumorigenesis are discussed, with particular emphasis on the studies involving spontaneous canine neoplasms.

Key words: COX-2, Canine tumors, Chemotherapy

The Cox enzyme and cancer

The enzyme prostaglandin G/H synthase, also named cyclooxygenase (COX), is responsible for the first rate limiting step of the synthesis of all the prostaglandins from arachidonic acid (Smith, 1992). The enzyme is a homodimer composed of two subunits of 70000 Daltons and heme group (van der Ouderaa et al., 1979) and has two catalytic activities that mediate the conversion of arachidonic acid into several different prostanoids. Two isoforms of Cox have been identified and have been named Cox-1 and Cox-2 (Williams and DuBois, 1996), they share many similarities at a protein level but are encoded by different genes and are transmitted by different RNA messengers. The expression of the two isoforms is remarkably different in that Cox-1 is present in a broad spectrum of tissues and is referred to as the constitutive form, while Cox-2 is generally undetectable in most tissues and is synthesized under the influence of a variety of agonists, thus being referred to as the inducible form, with the exceptions of the adult kidney and the central nervous system (Nasir et al., 1998; Ostrom et al., 2001; Teather et al., 2003) It is generally believed that Cox-1 is involved in maintaining tissue homeostasis, whereas Cox-2 is involved in inflammatory and immune responses (Vane et al., 1998). Cox-2 is strongly induced by proinflammatory cytokines, growth factors, carcinogens and oncogenes. (Subbaramaiah et al., 1996; Mestre et al., 1997). Of interest, Cox-2 is upregulated in the early phases of oncogenesis (Nathan et al., 2001) and is strongly expressed by several tumors in humans. In particular, it seems that epithelial neoplasms are particularly prone to express high levels of the inducible form; high levels of the enzyme have been evidenced in colorectal cancer, gastric carcinoma, squamous cell carcinoma of the neck, bladder carcinoma, carcinoma of the cervix and endometrium, thyroid carcinoma, and mammary carcinoma (Ristimaki et al., 1997; Chan et al., 1999; Hao et al., 1999; Reddy et al., 2002; Mohammed et al., 1999; Kulkarni et al., 2001; Half et al., 2002; Specht et al., 2002). Few non epithelial neoplasms have been found to express Cox-2 so far, in particular chondrosarcoma and malignant mesothelioma (Baldi et al., 2004; Sutton et al., 2004). Several studies pointed out the multiple actions played by Cox-2 overexpression in cancer such as increased production of PGE2 that contributes to the malignant phenotype (Parhar and Lala, 1988; Brock et al., 1999) and causes impairment of the immune system (Sharma et al., 2003), as well as regulation of cell cycle molecules in some tumors (Baldi et al., 2004). Cox-1 and Cox-2 are targeted by a broad variety of non steroidal anti-inflammatory inhibitors (NSAIDs). Cox-2 inhibitors, and among others, piroxicam, are currently used as anti-inflammatory drugs, primarily to treat arthritis in men (Ando and Lombardino, 1983). Of interest, anti-Cox-2 molecules have shown anti-tumor activity in chemically induced murine neoplasm,
Cox-2 in spontaneous canine tumors

Spontaneous neoplasms of companion animals are a formidable and still underexploited tool to make rapid advances in cancer therapy by testing new compounds and delivery systems that have shown promise in vitro (Knapp et al., 1997, 2000; Spugnini and Porrello, 2003; Porrello et al., 2004). The advantages of this model system have been reviewed (MacEwen, 1990; Hahn et al., 1994; Vail and MacEwen, 2000) and include: 1) similarities between specific types of canine and human cancer with regards to histopathological appearance, biological behavior and response to therapy, 2) significant similarity in drug metabolism between dogs and humans, 3) pet owners’ willingness to enter their tumor-bearing dogs in humanely conducted clinical trials due to the absence of a well defined “standard” therapy for many canine cancers, 4) the compressed lifespan of dogs, which allows the completion of clinical trials in a timely manner, 5) the fact that dogs share the same environment as their owners but lack their unhealthy habits such as alcohol consumption and cigarette smoking which act as “confounding” factors in many clinical and epidemiological studies, 6) the larger size of dogs compared to rodents which makes it feasible to perform many medical procedures, 7) novel interventional strategies developed in vitro or in laboratory animal studies can be tested in dogs affected by cancer, whereas similar studies might be unacceptable or less feasible in human patients, especially when a standard, yet only partially effective treatment exists.

Several studies have investigated the expression of Cox-2 in canine tumors. In Table 1 data available from the scientific literature are summarized.

A phase I study on piroxicam in 62 dogs affected by a broad spectrum of neoplasms documented 8 partial remissions (3/10 transitional cell carcinoma (TCC), 3/5 squamous cell carcinoma (SCC), 1/3 mammary carcinoma, 1 transmissible venereal tumor), suggesting a Cox-2 inhibition mechanism at the base of the antitumor response (Knapp et al., 1992). A second study was performed to better assess the mechanisms of Cox-2 inhibition in a canine model of TCC. In that investigation, the levels of PGE2 were inversely related to the tumor response to therapy while a correlation with the activity of the natural killer cells was not observed (Knapp et al., 1994). Further studies confirmed the effectiveness of piroxicam at inhibiting TCC in dogs (Knapp et al., 2000; Henry et al., 2003). Prostaglandin E2 concentrations have been measured in tumor tissue samples and in the culture medium of several canine cancer cell lines; the results showed that high levels were present in TCC, prostate carcinoma and osteosarcoma derived cell lines (Mohammed et al., 2001). Histopathology studies have identified expression of Cox-2 in sections of canine primary tumors, in particular prostatic adenocarcinoma, TCC, SCC, mammary carcinoma, renal cell carcinoma, intestinal epithelial neoplasms, and epithelial nasal tumors (Tremblay et al., 1999; Khan et al., 2000, 2001; Pestilli de Almeida et al., 2001; McEntee et al., 2002; Dorè et al., 2003; Kleiter et al., 2004). These studies helped to identify potential targets for anti-Cox-2 therapy, leading to hypothesize the possible use of such molecules as radiosensitizers or in synergy with chemotherapy and immunotherapy. A paper recently published suggests a possible correlation between Cox-2 levels in appendicular osteosarcomas and overall survival time (Mullins et al., 2004). Of interest, one of the authors (EPS) observed a complete remission in a mammary inflammatory carcinoma and two complete and two partial remissions (lasting 6 and 14 months, respectively) in four dogs with osteosarcoma. In all the patients piroxicam has been administered as anti-inflammatory and pain killer to palliate the symptoms related to the neoplasms; these results are very interesting, especially the response of the inflammatory carcinoma that is an incurable cancer in women. It would be worth investigating the expression of such metabolic pathways in these cancers, because it could lead to better targeted therapies. The only prospective study on the effects of piroxicam in a canine TCC model, available in literature, suggested that tumor response to anti-Cox-2 drugs was related to induction of apoptosis and reduction of angiogenic growth factors as indicated by the reduction of basic growth factors concentration in the urine (Mohammed et al., 2002). Finally, only one recent paper

### Table 1. Pooling of canine neoplasms expressing positivity for Cox-2.

<table>
<thead>
<tr>
<th>TUMOR TYPE</th>
<th>TUMOR EXPRESSING COX-2</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCC</td>
<td>54/61</td>
<td>Mohammed 1999, 2001; Khan, 2000</td>
</tr>
<tr>
<td>SCC oral</td>
<td>47/50</td>
<td>Pestilli de Almeida, 2001</td>
</tr>
<tr>
<td>Intestinal adenoma</td>
<td>13/20</td>
<td>McEntee, 2002</td>
</tr>
<tr>
<td>Intestinal carcinoma</td>
<td>7/15</td>
<td>McEntee, 2002</td>
</tr>
<tr>
<td>Nasal carcinoma</td>
<td>17/21</td>
<td>Kleiter, 2004</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>3/3</td>
<td>Khan, 2001</td>
</tr>
<tr>
<td>Prostatic carcinoma</td>
<td>23/30</td>
<td>Tremblay, 1999</td>
</tr>
<tr>
<td>Mammary adenoma</td>
<td>15/63</td>
<td>Dore, 2003</td>
</tr>
<tr>
<td>Mammary carcinoma</td>
<td>55/92</td>
<td>Mohammed, 2001; Dore, 2001</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>38/89</td>
<td>Mohammed, 2001; Mullins, 2004</td>
</tr>
<tr>
<td>Melanoma</td>
<td>5/10</td>
<td>Mohammed, 2001</td>
</tr>
</tbody>
</table>

TCC: Transitional cell carcinoma; SCC: squamous cell carcinoma.
investigated the expression of Cox-2 in feline tumors, of interest, very few tumor types were positive for the enzyme. positivity was restricted to 7 out of 19 TCC and 2 out of 21 SCC. Pulmonary, mammary, and intestinal carcinomas were all negative as well as sarcomas, this might reflect interspecies differences or could be ascribed to the smallness of the sample tested (Beam et al., 2003)

In conclusion, studies on the expression of Cox-2 in tumors of companion animals and its inhibition should warrant further investigation in order to gain insights into the mechanisms involved at a gene level (e.g. p53 expression) and to have better therapeutic protocols.

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COX-2 in canine tumors