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Antitumor effects of celecoxib in COX-2 expressing and non-expressing canine melanoma cell lines

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ABSTRACT

Cyclooxygenase-2 (COX-2) is a potential target for chemoprevention and cancer therapy. Celecoxib, a selective COX-2 inhibitor, inhibits cell growth of various types of human cancer including malignant melanoma. In dogs, oral malignant melanoma represents the most common oral tumor and is often a fatal disease. Therefore, there is a desperate need to develop additional therapeutic strategies. The purpose of this study was to investigate the anticancer effects of celecoxib on canine malignant melanoma cell lines that express varying levels of COX-2. Celecoxib induced a significant anti-proliferative effect in both LMeC and CMeC-1 cells. In the CMeC cells, treatment of 50 μM celecoxib caused an increase in cells in the G0/G1 and a decreased proportion of cells in G-2 phase. In the LMeC cells, 50 μM of celecoxib led to an increase in the percentage of cells in the sub-G1 phase and a significant activation of caspase-3 when compared to CMeC-1 cells. In conclusion, these results demonstrate that celecoxib exhibits antitumor effects on canine melanoma LMeC and CMeC-1 cells by induction of G_1-S cell cycle arrest and apoptosis. Our data suggest that celecoxib might be effective as a chemotherapeutic agent against canine malignant melanoma.

Melanomas are malignant tumors arising from melanocytes. Oral melanoma is the most common oral tumor in dogs (Goldschmidt, 1985). Unlike cutaneous melanomas of haired-skin, which are usually benign in the dog, oral melanoma is almost uniformly malignant and usually displays aggressive growth and metastasis to regional and distant sites (Ogilvie and Moore, 2006). Because of the lack of efficacious chemotherapeutic regimens for metastatic melanomas, several novel therapeutic strategies have been investigated (Rigel and Carucci, 2000; Withrow et al., 2012).

Cyclooxygenase-2 (COX-2) is expressed in more than half of spontaneous canine cancers and known as having a central role in the development and progression of some cancers (Pyrko et al., 2007). Increased COX-2 expression has been reported to mediate invasiveness of tumor cells (Kim et al., 2010), promotion of angiogenesis (Tegeder et al., 2001) and anti-apoptotic effects (Li et al., 2001). Altered COX-2 expression has been associated with the development and progression of human melanoma (Kuzbicki et al., 2006). In dogs, COX-2 was expressed in 21 of the 31 canine malignant melanomas (Pires et al., 2010), and oral malignant melanomas were specifically reported to have moderate to strong COX-2 expression.

Celecoxib (CELEBREX®, Onseral®, Pfizer, New York, USA), a selective COX-2 inhibitor, has been reported to inhibit the growth of human cancer cell lines (Bocca et al., 2011; Dhawan et al., 2010; Liu et al., 2009; North, 2001). There have been minimal reported data concerning the use of COX-2-specific inhibitors as potential antineoplastic drugs in canine malignant melanoma.

In this study, we investigated the anticancer effects of celecoxib either on COX-2 high expressing or null canine malignant melanoma cell lines.

Two different canine malignant melanoma cell lines (LMeC and CMeC-1) were used in our study (Inoue et al., 2004). Both cell lines were provided from Professor Nobuo Sasaki in the University of Tokyo. Description of detailed material and methods used in this investigation are provided as supplementary file.

COX-2 expression in CMeC-1 and LMeC cells treated with celecoxib was analyzed by Western blot analysis (Fig. 1a). COX-2 protein was abundantly expressed in LMeC cells but not expressed in CMeC-1 cells. After treatment with 20 or 50 μM celecoxib for

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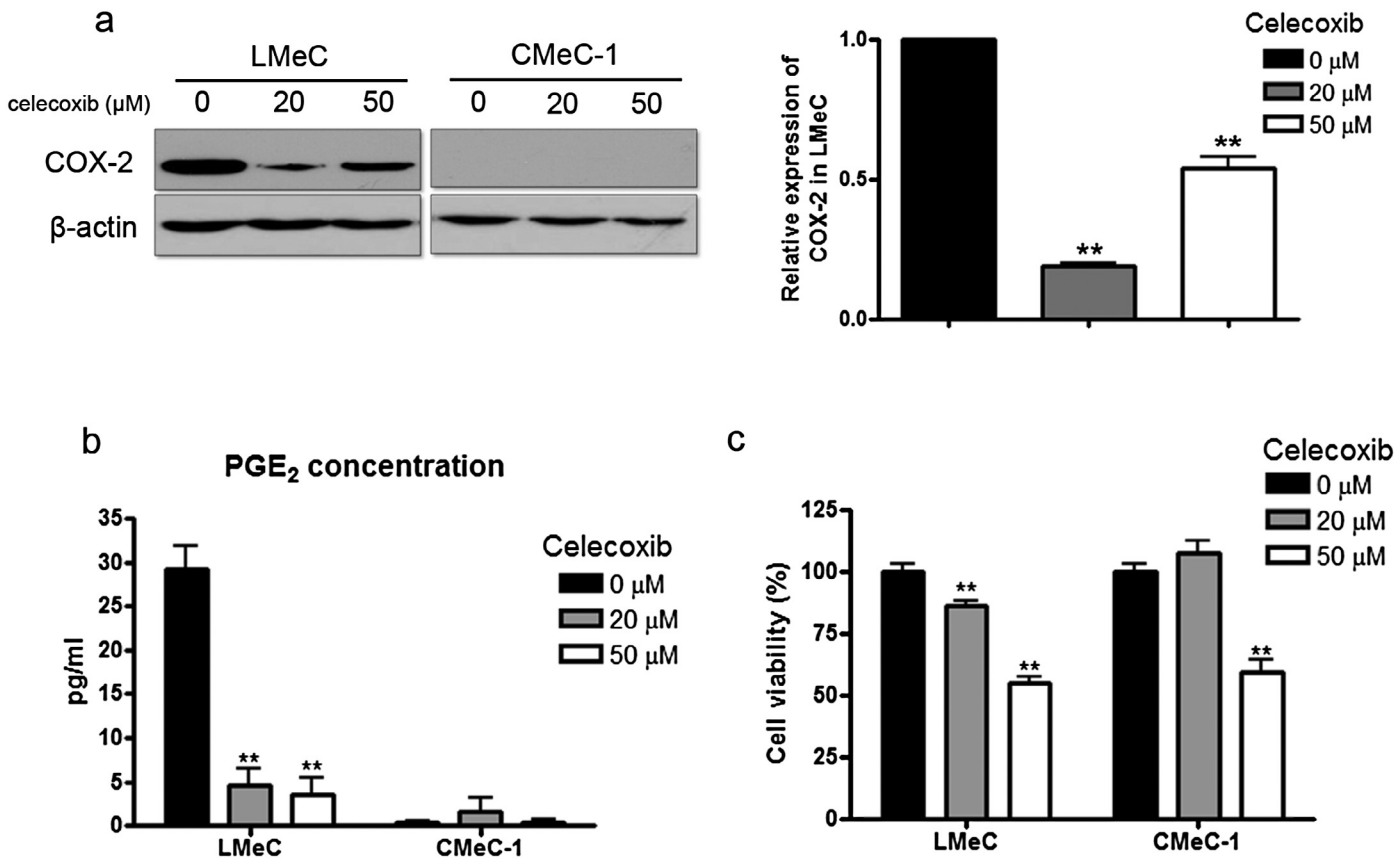

Fig. 1. The effects of celecoxib on COX-2 expression in canine maligmant melanoma and the inhibitory effects of celecoxib on cell proliferation. (a) Western blot analysis of COX-2 expression in LMeC and CMeC-1 canine melanoma cell lines. COX-2 expression was observed in LMeC cells and was absent in CMeC-1 cells. Celecoxib reduced COX-2 expression in LMeC cells. (b) Effects of celecoxib on production of COX-2's principal metabolic product, PGE_2. Cells were treated with celecoxib (20 and 50 μM) for 48 h. CMeC-1 cells did not produce any PGE_2. (c) The growth rates of LMeC and CMeC-1 cells measured by an MTT assay after treatment celecoxib. Proliferation was inhibited when LMeC were treated with 20, and 50 μM celecoxib. In CMeC-1 cells, the 50 μM celecoxib inhibited cell proliferation. Data are presented as the mean ± SD; $^{**}P < 0.01$.

48 h, expression of COX-2 protein was decreased in LMeC cells (Fig. 1a).

Expression of prostaglandin E2 (PGE_2) protein from the supernatant of cell lines was assessed by enzyme-linked immunosorbent assay (Fig. 1b). In the presence of 20 or 50 μM celecoxib, LMeC cells showed a significant decrease of PGE_2 protein expression compared with those of control. The values of PGE_2 protein level were as follows: untreated (29.2 ± 4.6 pg/ml), 20 μM celecoxib treated (4.63 ± 3.11 pg/ml; $P < 0.001$) and 50 μM celecoxib treated (3.61 ± 3.23 pg/ml; $P < 0.001$). Additionally, the level of PGE_2 protein was much lower in CMeC-1 cells than LMeC cells (untreated: 0.34 ± 0.33 pg/ml). With 20 and 50 μM of celecoxib, PGE_2 production in CMeC-1 cells did not significantly change ($P = 0.582$ treated with 20 μM of celecoxib, $P = 0.998$ at 50 μM; Fig. 1b). To investigate whether celecoxib affects the proliferation of CMeC-1 and LMeC cells, each cell line was incubated for 48 h with celecoxib. Cell viability and cell surviving fraction were analyzed (Fig. 1c and supplementary Fig. S1 in the online version at doi:10.1016/j.rvsc.2014.03.003). Treatment with celecoxib significantly reduced LMeC cell viability in a dose-dependent manner (Fig. 1c; $P = 0.002$ treated with 20 μM of celecoxib, $P < 0.001$ at 50 μM). In CMeC-1 cells that lack of COX-2 expression, no significant change of cell proliferation was observed at the presence of 20 μM celecoxib ($P = 0.458$) while cell proliferation was markedly reduced at the 50 μM concentration of celecoxib (Fig. 1c; $P < 0.001$ at 50 μM). Similar results were observed using a clonogenic assay (Supplementary Fig. S1 in the online version at doi:10.1016/j.rvsc.2014.03.003).

To assess celecoxib-induced anti-proliferative ability, cell cycle analysis was performed (Fig. 2a). The results indicated that celecoxib arrested the cell cycle at the G_0/G_1 phase after 48 h compared to the untreated control. In the CMeC-1, 50 μM celecoxib caused an increase in cells in the G_0/G_1 phase accompanied by a decrease in the G-2 phase (Fig. 2a). In LMeC cells, there was a numerical increase in cells in the G_0/G_1 phase induced by 50 μM of celecoxib although this difference did not show statistical significance (Fig. 2a). To elucidate whether celecoxib may influence cyclin D1 expression associated with a G0-G1 arrest, expression of cyclin D1 was evaluated (Fig. 2b). Celecoxib treatment decreased the levels of cyclin D1 in both cell lines in a dose-dependent manner. In the presence of 50 μM celecoxib, cyclin D1 expression was remarkably lower in CMeC-1 cells than LMeC cells (Fig. 2b).

To determine whether the reduced viability of celecoxib-treated CMeC-1 and LMeC cells was mediated by apoptosis, we analyzed the sub-G_1 phase cell cycle, active caspase-3 expression and inter-nucleosomal DNA fragmentation (Fig. 2c, d). An increased proportion of cells in the sub-G_1 phase of CMeC-1 and LMeC cells were observed (Fig. 2a). The percentage of LMeC cells in the sub-G_1 phase after treatment with 50 μM of celecoxib (15.26 ± 4.75%) increased compared to the control cells (Fig. 2a, $P = 0.049$). In CMeC-1 cells, the percentage of sub-G1 phase was increased from 5.65 ± 0.66% to 10.79 ± 0.62%, after treatment of 50 μM celecoxib ($P = 0.021$).

Consistent with the inhibition of cell growth, LMeC cells underwent apoptosis after treatment with 50 μM of celecoxib. The results of Western blot demonstrated that celecoxib treatment