Expression of Cyclooxygenase-1 and Cyclooxygenase-2 in Mitotic Cells in Squamous Cell Carcinoma of the Head and Neck: a Preliminary Study

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Key Words: COX-1, COX-2, immunohistochemistry, prevention, treatment.

ABSTRACT

To clarify difference in terms of the expression of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) in squamous cell carcinoma in the head and neck (HNSCC), an immunohistochemical study was performed for specimens obtained from 4 patients with HNSCC, and 3 normal volunteers. COX-1 and COX-2 expressions were observed in small round cells, plasma cells and fibroblasts in interstitial tissue. An apparent difference in expression between COX-1 and COX-2 is that strong positive COX-2 expression was recognized in mitosis in 2 HNSCC patients, whereas COX-1 immunoreactivity was not seen in mitosis among 4. These preliminary findings suggest that COX-2 may have an important role in proliferation of HNSCC and COX-2 can be a target for the prevention or treatment of HNSCC.

Introduction

Cyclooxygenases (COX), which are prostaglandin endoperoxide synthase, are the rate-limiting enzymes, for the conversion of arachidonic acid to prostaglandins (VANE et al, 1998). There are two isoforms of COX: COX-1 is constitutively expressed and COX-2 is inducible (VANE et al, 1998; KUJUBU et al, 1991). COX-2 is expressed in inflammatory cells, such as a macrophage, fibroblast and vessel endothelial cell after being stimulated by antigens or cytokine (APPLETON et al, 1995; MAJIMA et al, 1997). COX-1, which is usually expressed in almost all cells and plays a role for maintaining blood circulation of gastric mucosa and kidneys (VANE et al, 1998), is not affected by such stimulation. In the past decade, epidemiological studies have shown that nonsteroidal anti-inflammatory drugs (NSAIDs), which suppress COX-1 and COX-2, have an obvious effect for decreasing the incidence of colon cancer statistically (THUN et al, 1991; ROSENBERG et al, 1991; GREENBERG et al, 1993; GIOVANNUCCI et al, 1995). According to basic research for colon carcinoma, COX-2 is a "constitutive" enzyme in colon carcinoma (Tsuji et al, 1997) and it can be seen in cancer cells. COX-2 mRNA is rapidly induced by proinflammatory or mitogenic stimuli including cytokine, endotoxins and interleukins (DUBOIS et al, 1994; HEMPEL et al, 1994; PRESCOTT et al,
In squamous cell carcinoma in the head and neck (HNSCC), mean levels of COX-2 mRNA are increased by nearly 150-fold compared with normal oral mucosa (CHAN et al., 1999). However, there are no reports that mention differences in expression between COX-1 and COX-2 in HNSCC. Additionally, there are few reports that describe where COX is observed immunohistochemically in HNSCC tissue (CHAN et al., 1999). In this study, we investigated differences in expression between COX-1 and COX-2 in HNSCC immunohistochemically, and especially observed COX-2 expression in cancer cells in terms of cell proliferation.

MATERIALS and METHODS

Materials

Reagents used for the experiment were as follows: Rabbit anti COX-1 antibody (CHEMICON International, Inc., Temecuma, CA), Rabbit anti COX-2 antibody (CHEMICON International, Inc., Temecuma, CA), Goat anti Rabbit IgG antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), avidin-biotin-peroxidase complex (ABC; Vector Laboratories Inc, Burlingame, CA), 3,3’-diaminobenzidine tetrahydrochloride (DAB; Sigma, St Louis, MO) and Triton X-100 (Sigma, St Louis, MO).

Patients sample

HNSCC was obtained from 3 male and 1 female patients who underwent operations of their cancer at Osaka Medical College: one patient each with oral cancer, mesopharyngeal cancer, hypopharyngeal cancer and laryngeal cancer. Two of them were well differentiated squamous cell carcinomas and the other two were moderately differentiated squamous cell carcinomas. Neither preoperative radiotherapy nor chemotherapy had been performed on these patients. Neither preoperative radiotherapy nor chemotherapy had been performed on these patients. No patient had oral intake of NSAIDs before operation. Specimens (5 × 5 × 5mm) were sharply cut from the middle part of HNSCC, fixed in 10% formaldehyde immediately after resection, embedded in paraffin, and sectioned at a thickness of 4 μm. Normal mucous membranes (2 oral and 1 laryngeal mucosae) were obtained for controls from 2 female and 1 male volunteers who underwent operations and specimens were processed in the same way as above. Sections of these specimens, which were mounted on silane-coated glass slides, were stained immunohistochemically.

Immunohistochemistry

Sections on slides from 4 patients with HNSCC and one normal volunteer were dewaxed in xylene, rehydrated in descending ethyl alcohol from 100% to 50% and rinsed twice in distilled water. Endogenous peroxidase was inactivated by incubation with 0.3% H2O2 in methanol. After rinsing in phosphate-buffered saline (PBS), microwave antigen retrieval incubation was performed with 10 mM sodium citrate for 10 minutes. Nonspecific antigen binding was inhibited by incubation with blocking solution containing 2% nonfat dry milk, 2% normal goat serum and 0.1% Triton X-100 for 1 hour at room temperature. The sections were incubated with the anti-COX-1 or COX-2 antibody at 4°C overnight. These primary antibodies were used at a 1:625 dilution in PBS with 0.5% Triton X-100. After rinsing in PBS, sections were incubated with anti-rabbit IgG antibody as a secondary antibody, which was diluted at a 1:200 in PBS. After rinsing in PBS again, sections were incubated with avidin-biotin-peroxidase complex for 1 hour at room temperature. The color was developed with 0.02% DAB in 4% sodium acetate, plus 0.003% hydrogen peroxide for 3 minutes. Sections were dehydrated in a graded series of ethanol (70%, 80%, 95% and 100%), then immersed in xylene for 15 minutes twice. Finally, these sections were coverslipped and examined under a light microscope.

Results

Expression of COX-1 in Normal Pharyngeal Squamous Epithelium and in HNSCCs

Immunohistochemically, normal squamous epithelium showed weak positive staining of COX-1 in the cytoplasm (Fig. 1A). Cells in the subepithelial layer were not stained. In HNSCC, COX-1 immunoreactivity was found weakly in cancer cells, but seen strongly in the cytoplasm of small round cells, plasma cells and fibroblasts in interstitial tissue (Fig. 1B).

Expression of COX-2 in Normal Pharyngeal Squamous Epithelium and in HNSCCs

Normal squamous epithelium showed negative staining of COX-2 in epithelial cells or cells in the subepithelial layer. In 3 of the 4 patients with HNSCC, faint COX-2 immunoreactivity was found in cytoplasm of cancer cells. Among 3 COX-2 positive carcinomas, 2 cases had strong COX-2 immunoreactivity observed in mitosis in cancer cells (Fig. 2A, B). In interstitial tissue of HNSCC,
small round cells, plasma cells and fibroblasts had positive immunoreactivity in the cytoplasma in 3 of the 4 cases.

DISCUSSION

This immunohistological study shows the difference in expression between COX-1 and COX-2 in normal pharyngeal squamous epithelium and HNSCC. The most remarkable observation is that strong positive COX-2 expression was recognized in mitosis in HNSCC, whereas COX-1 immunoreactivity was not seen in mitosis. Positive COX-2 expressions in mitosis of cancer cells have not been reported in HNSCC or other carcinomas, e.g., colon cancer or breast cancer. This result indicates the possibility that COX-2 might be induced at the phase of mitosis in HNSCC cells. If this possibility is shown to be true, and we can reveal how COX-2 plays a role in the phase of mitosis in cancer cells and cell proliferation, then we can consider the application of NSAIDs, which suppress COX-2, to the prevention or treatment of HNSCC in combination with cell cycle phase-specific anticancer agents, e.g., cisplatin, daunorubicin, dacarbazine, busulfan and cyclophosphamide. Paik et al demonstrated that NSAIDs can inhibit mitogen-induced COX-2 expression.

Fig. 1  Expression of COX-1. A, normal oral squamous epithelium shows weak positive staining of COX-1 in cytoplasma. Cells in subepithelial layer are not stained. The calibration bar represents 100 μm. B, in HNSCC, immunoreactivity is weak in cancer cells, but strong in cytoplasma of small round cells, plasma cells and fibroblasts in interstitial tissue. The calibration bar represents 100 μm.

Fig. 2  Expression of COX-2 in HNSCC. A, strong COX-2 immunoreactivity is observed in mitosis in cancer cells of HNSCC of oral mucosa (arrowheads). Faint COX-2 expression is seen in cytoplasma of cancer cells. In interstitial tissue of HNSCC, small round cells, plasma cells and fibroblasts had positive immunoreactivity in cytoplasma. The calibration bar represents 100 μm. B, similarly, strong COX-2 expression is observed in mitosis in cancer cells of HNSCC in larynx (arrowheads). The calibration bar represents 100 μm.
and downstream markers of inflammation in addition to their inhibitory effect on cyclooxygenase enzyme activity (PAIK et al, 2000). Investigation into COX-2 expression in HNSCC cell lines is needed, not only immunohistochemically but also using molecular biological approach to reveal the role of COX-2 in the phase of mitosis.

In HNSCC, diffuse faint COX-2 expression was also observed in cancer cells without mitosis. This result was similar to a previous report that investigated esophageal squamous cell carcinoma (ZIMMERMANN et al, 1999). In normal mucosa, COX-2 expression was not seen. This observation includes suggestion that COX-2 might be a "constitutive" enzyme in HNSCC as well as colon adenocarcinoma (TSUJI et al, 1997).

In normal squamous epithelium, weak COX-1 expression was seen in the epithelium but could not be observed in the subepithelial layer. In HNSCC, weak expression of COX-1 was observed in cancer cells, but obvious COX-1 staining was seen in cells in interstitial tissue. These findings suggest that head and neck cancer tissues have close relationship to inflammation. In terms of COX-1 expression, the difference between normal head and neck mucosa and HNSCC might be helpful when we consider how to use NSAIDs for the prevention or treatment of HNSCC, as well as COX-2 expression.

References


Received 12 May, 2003
Accepted 20 June, 2003