Abstract The expression of Cox-2 protein was studied by immunohistochemistry in normal oral mucosa and in mucosa with various lesions of oral leukoplakia, including hyperplasia and dysplasia of squamous epithelium and frank invasive squamous carcinoma. A gradient of Cox-2 staining was found: the expression of Cox-2 was lowest in normal epithelium, somewhat increased in hyperplastic epithelium, further increased in dysplastic epithelium, and highest in invasive squamous cell carcinomas. The presence of Cox-2 in squamous cell carcinomas of the oral mucosa and its precursor lesions indicate that Cox-2 could participate in the carcinogenic process of these oral malignancies.

Keywords Cyclo-oxygenase-2 · Oral dysplasia · Oral carcinoma · Carcinogenesis · Immunohistochemistry · Leukoplakia

Introduction

Idiopathic leukoplakia is a clinical term which includes hyperplastic, dysplastic, and carcinomatous changes, usually related to the use of tobacco [24, 27]. Some leukoplakias are known to develop into squamous carcinoma. Rates of transformation vary considerably from study to study [14, 16]. Oral cavity cancer account for 4% at all malignancies in men and 2% in women. Practically all cancers of oral cavity are squamous cell (epidermoid) carcinomas and 16% of the tumors in oral cavity are tongue carcinomas. Oral cavity cancers have been associated with tobacco and alcohol. Most cases occur in men over the age of 50 years [16]. Intraoral squamous cell carcinomas are usually moderately or poorly differentiated. Those located at the base of the tongue tend to be particularly undifferentiated and solid. The epithelium adjacent to the invasive tumor often shows carcinoma in situ or dysplastic changes. The main prognostic factors in carcinomas of the oral cavity are the stage of the disease, location, and microscopic grading. The overall 5-year survival rates are about 60% for tumors of the anterior tongue and 40% of the posterior tongue.

Several epidemiological studies have shown that prolonged use of aspirin is associated with reduced risk of colorectal cancer [5, 22]. In addition, a large prospective mortality study found the use of aspirin to be associated with reduced risk of esophageal, gastric, and colorectal cancers but not of those outside the gastrointestinal tract [22]. However, more recent studies suggest that aspirin also reduces the incidence of lung and breast cancers [7, 18]. The best known target of nonsteroid anti-inflammatory drugs, including aspirin, is cyclo-oxygenase (Cox), the rate-limiting enzyme in the conversion of arachidonic acid to prostanoids (Cox is also called prostaglandin endoperoxide synthase). Two Cox genes have been cloned (Cox-1 and Cox-2) that share over 60% identity at the amino acid level and have similar enzymatic activities [8, 20, 25]. Cox-1 is considered a housekeeping gene, and prostanoids synthesized via the Cox-1 pathway are thought to be responsible for cytoprotection of the stomach, for vasodilation in the kidney, and for production of a proaggregatory prostanoid, thromboxane, by platelets. In contrast, Cox-2 is an inducible immediate-early gene, and its pathophysiological role has been connected to inflam-
mation, ovulation, and carcinogenesis, i.e., cellular growth and differentiation. Cox-2 expression seems to be especially prominent in gastrointestinal tumors [4, 10, 15, 17, 21], and it plays an important role in the development of intestinal adenomas and carcinomas in experimental animal models [6]. However, expression of Cox-2 has not been extensively studied outside the gastrointestinal tract, although recent studies suggest that it is present in skin, breast, and lung carcinomas [1, 9, 11, 26]. Cox-2 expression is also increased in squamous carcinoma of the esophagus, nasopharynx, and skin [2, 13, 19, 28]. We have now studied Cox-2 expression in human tongue carcinomas and in precursor lesions leading to this malignancy.

**Materials and methods**

**Patient samples**

Human tongue tissue samples, removed surgically because of cancer, were collected from the archives of Department of Pathology in Helsinki University Central Hospital from the years 1987–1988 and 1990–1992, and they were fixed in 10% neutral-buffered formalin and then embedded in paraffin. We had 38 normal tongue tissue samples, 41 tongue tissue samples with hyperplastic epithelium, 23 dysplastic tongue tissue samples, and 73 tongue carcinoma samples. All cancers were primary tongue tumors, and they were classified by the same pathologist according to WHO typing (T.P.) [12].

**Immunohistochemistry**

Tissue samples were embedded in paraffin, sectioned (4–5 µm), deparaffinized, and microwaved for 4×5 min in 0.01 M Na-citrate buffer (pH 6.0). The slides were first immersed in 1.6% hydrogen peroxide in methanol for 30 min and then in blocking solution buffer (pH 6.0). The slides were first immersed in 1.6% hydrogen peroxide in methanol for 30 min and then in blocking solution buffer (pH 6.0). The slides were first immersed in 1.6% hydrogen peroxide in methanol for 30 min and then in blocking solution buffer (pH 6.0).

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**Evaluation**

The tongue samples were divided into four groups, namely normal, hyperplasia, dysplasia, and cancer by J.R. and T.P. The epithelial Cox-2 staining was quantitated on a scale from 0 (none) to 4 (very high staining) in consensus of two investigators (J.R. and T.P.). The final staining score took into account both the intensity of the staining and the number of positive cells according to the following scheme: both were quantitated from 1 to 4 and the sum of 1 or 2 received the final score 1 and the sum of 3 or 4 the final score of 2, etc.

**Results**

To investigate the expression of Cox-2 protein in benign and malignant tongue epithelium we used immunohistochemistry on a large number of patient samples. Our material was formalin-fixed and paraffin-embedded tissues, normal tongue epithelium (n=38), hyperplastic epithelium (n=41), dysplastic epithelium (n=23), and the squamous cell carcinoma epithelium (n=73). The degree of staining was semiquantitated with a scale from negative (0) to strong positivity (4).

Figure 1 presents the distribution of epithelial Cox-2 expression in different types of epithelial changes as determined by degree of staining with a score from negative (0) to strong positivity (4). The normal lesions do not express substantial quantities of Cox-2 and over 25% of the samples are totally negative. The level of Cox-2 expression increased when leukoplakias were investigated. The distribution of Cox-2 expression levels in hyperplastic samples was greater than normal. When the lesions studied were of dysplastic epithelium the expression of Cox-2 was further increased. No significant differences were between low and severe dysplasia, although the score in severe dysplasia was higher (data not shown). The highest levels of expression were seen in invasive carcinomas. Representative immunoperoxidase stainings are shown in Fig. 2.

The mean values of the intensity of Cox-2 expression in tongue epithelium are shown in Fig. 3. The mean value of the Cox-2 staining intensity increased gradually from normal epithelium (0.97) to hyperplastic lesions (1.58) and more to dysplastic lesions (2.04). The highest mean expression was in squamous cell cancer (2.42). The difference between Cox-2 expression in the normal epithelium and that in the hyperplastic epithelium is statistically significant (P=0.0041, paired t test) and the difference between normal and dysplastic epithelium highly significant (P=0.0011), as is that between normal and cancerous epithelium (P<0.0001).

Cancer material was further divided into histological grade I (n=22), grade II (n=30), and grade III (n=21) carcinomas. The mean ±SE Cox-2 staining scores in the different grades (I, II, III) were 2.14±0.23, and 2.24±0.27, and 3.05±0.22, respectively. The differences were significant between grades I and III (P<0.01) and between grades II and III (P<0.05).