The development of new treatment strategies for oesophageal cancer

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COX-2 is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins.

It is usually not detectable in normal tissues, but can be induced in processes such as carcinogenesis.

This report reviews the mechanisms by which COX-2 can contribute to the development of cancer, its role in prognosis, and the possible place of selective COX-2 inhibitors in the prevention and treatment of gastrointestinal malignancies, focusing particularly on oesophageal adenocarcinoma.

The role of Cyclooxygenase-2 in the development and treatment of oesophageal adenocarcinoma

Chapter 4
INTRODUCTION

Oesophageal adenocarcinoma is one of the most aggressive human malignancies. Over the last decades, the incidence of this malignancy has increased in many Western countries, at a rate that exceeds that of any other tumour. It is now generally accepted that oesophageal adenocarcinomas develop from a premalignant lesion of the oesophagus, also referred to as Barrett’s oesophagus. Barrett’s oesophagus is a metaplastic change of the normal squamous cell epithelium of the oesophagus to a columnar type due to longstanding gastro-oesophageal reflux disease (GORD). Cancers in Barrett’s oesophagus evolve through a sequence of genetic alterations in which the metaplastic cells develop the ability of autonomous growth stimulation and acquire the ability to avoid triggering of the programmed cell death mechanism (apoptosis) which destroys cells that acquire genetic damage. The extending knowledge of mechanisms underlying carcinogenesis, provides insights that are necessary for the development of novel treatment strategies for both the prevention and treatment of (oesophageal) malignancies.

Over the past decade, a series of studies have suggested that the enzyme cyclooxygenase-2 (COX-2) represents a potential therapeutic target for cancer prevention and treatment. Numerous epidemiological studies showed that the long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclooxygenase enzymes, was associated with a reduced risk of developing malignant disease, especially gastrointestinal cancer. With the recent development of selective COX-2 inhibitors, which have antineoplastic activity in certain experimental models and in FAP patients but cause fewer serious adverse effects than traditional NSAIDs, the benefit-risk balance of this treatment strategy has improved. Since selective COX-2 inhibitors appear to be safe enough for long-term use (e.g. in rheumatoid arthritis patients), there are several ongoing chemoprevention and adjuvant therapy trials. This review considers the mechanisms by which COX-2 can contribute to carcinogenesis through effects on proliferation of cancer cells and to prognosis by increasing the metastatic potential of malignant cells. It focuses specifically on its role in the development of oesophageal adenocarcinomas and describes clinical and experimental data to provide a rationale for using selective COX-2 inhibitors as chemopreventive or (neo-)adjuvant agent in this malignancy.
CYCLOOXYGENASE-1 AND -2

COX is the rate-limiting enzyme in the conversion of arachidonic acid to prostaglandins (Figure 1). The first step in prostaglandin synthesis is hydrolysis of phospholipids to produce free arachidonate in a reaction catalysed by phospholipase A2. Next, COX catalyses a reaction in which molecular oxygen is inserted into arachidonic acid. This reaction produces an unstable intermediate, prostaglandin G2 (PGG2). PGG2 is converted rapidly to prostaglandin H2 (PGH2) by the peroxidase activity of COX. Specific isomers then catalyse the reactions of the specific common precursor PGH2 to different prostaglandins and thromboxane, which all have their own range of biological activities.

Recently, two COX isoforms have been cloned (COX-1 and COX-2) which share over 60% identity at amino acid level and have similar enzymatic activities. The most striking difference between the COX enzymes is in the regulation of expression. COX-1 is expressed constitutively in most tissues and seems to mediate production of prostaglandins that control normal physiological functions, such as protection of the stomach, regulation of renal blood flow, and platelet aggregation. COX-2 on the other hand, is usually not detectable in normal tissues, but can be readily induced in response to cell activation by hormones, proinflammatory cytokines, growth factors, and tumour promoters. In part, the inducibility of COX-2 is explained by the presence of the nuclear factor responsible for the interleukin-6 expression (NF-IL6) and the cyclic AMP response element (CRE) sites in the 5'-flanking promoter region of the COX-2 gene. On the other hand, enhanced stability of COX-2 mRNA may also contribute to an increased expression of COX-2.

CONTRIBUTION OF COX-2 TO CARCINOGENESIS

Beginning in the late 1970s, it was noted that neoplastic lesions had elevated concentrations of prostaglandins, and increased expression of COX-2 was found in many premalignant tissues and malignant tumours (e.g. colorectal, gastric, oesophageal, pancreatic, lung, breast, bladder, cervical and ovarian cancer). Various oncogenes, growth factors and tumour promoters were shown to be able to induce COX-2 expression in malignant cells. Moreover, wild-type but not mutant p53 suppresses COX-2 transcription, raising the possibility that p53 is also a determinant of COX-2 expression. There are also data which demonstrate a direct relation between induction of COX-2 expression and carcinogenesis. Knocking out the COX-2 gene in the
APC\textsuperscript{6716} mouse (a model for human familial adenomatous polyposis) caused a significant reduction in both the number and size of intestinal polyps\textsuperscript{19}, whereas overexpression of COX-2 was sufficient to induce tumorigenesis in the mammary gland in transgenic mice.\textsuperscript{20} Finally, pharmacological evidence also implicates a role for COX-2 in carcinogenesis. Selective COX-2 inhibitors such as celecoxib and rofecoxib reduced the formation of intestinal, breast, skin, lung and bladder tumours in animals\textsuperscript{10}, and the non-selective COX inhibitor sulindac causes the reduction of adenomas in familial adenomatous polyposis patients.\textsuperscript{21} These data suggest that COX-2 represents a potential molecular target for preventing and treating cancer.

**MECHANISMS BY WHICH COX-2 CONTRIBUTES TO CANCER DEVELOPMENT**

COX-2 affects many processes, which are important in carcinogenesis, and is therefore an attractive therapeutic target. These processes include apoptosis, proliferation, angiogenesis, invasiveness, immunosuppression and inflammation. Inhibition of these COX-2 processes by NSAIDs and selective COX-2 inhibitors may be the primary mechanisms of their antineoplastic action described in various studies (Figure 1). In addition, COX-2 independent mechanisms of action might also exist, possibly through inhibition of the wingless/wnt-pathway.\textsuperscript{22} Recent animal studies and in vitro experiments suggest that NSAIDs decrease transcriptional activity of the nuclear hormone receptor peroxisome proliferator-activated receptor δ (PPARS), a potential downstream target of the APC/β-catenin/T-cell factor 4 pathway.\textsuperscript{23} However, this review will only focus on the molecular mechanisms by which NSAIDs contribute to cancer-treatment by inhibition of its best-known target: the COX-2 enzyme.

**Apoptosis**

The size of a cell population depends on the balance between cell proliferation and programmed cell death (apoptosis). Decreased apoptosis may lead to a prolonged survival of abnormal cells, which favors the accumulation of sequential genetic changes that increase the risk of tumourigenesis, and inhibition of apoptosis will lead to clonal expansion. In vitro studies suggest that carcinoma cells expressing COX-2 have the tendency to become resistant to apoptosis.\textsuperscript{24,25} Epithelial cells overexpressing COX-2 have increased amounts of the anti-apoptotic protein Blc-2 and are
resistant to butyrate-stimulated apoptosis. Treatment with NSAIDs reversed this resistance to apoptosis. Recent studies also indicate that both selective and non-selective cyclooxygenase inhibitors induce the expression of the pro-apoptotic gene, prostate apoptosis response (Par-4), and that accumulation of arachidonic acid caused by the inhibition of cyclooxygenase enzymes activates the production of ceramide, a strong apoptosis inducer. Finally, NSAIDs have been shown to decrease the expression of nuclear factor κB (NF-κB), a transcriptional factor that prevents apoptosis.

**Proliferation**

COX-2 can induce activation of the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, which is one of the key growth-stimulating cascades that causes cellular proliferation, and NSAIDs can inhibit this process. It was demonstrated that selective COX-2 inhibitors have an anti-proliferative effect by preventing epithelial cells from progressing from the quiescent state (G0/G1) into the phase of DNA replication (S-phase). Sulindac decreases the
levels of mitotic cyclins, thereby reducing the phosphorylation of the retinoblastoma protein, which process normally allows the cell to enter the S-phase. This decrease in proliferation activity by NSAIDs was also demonstrated on colon carcinoma cells by a significant decrease in the Ki67 antigen, which is a proliferation marker, after treatment with sulindac.

**Angiogenesis**

The growth of a tumour partly depends on an increase in blood supply. Tumour cells ensure their own growth by secreting growth factors, especially vascular endothelial growth factor (VEGF), that stimulate angiogenesis. COX-2 has been implicated in this aspect of carcinogenesis as well. Overexpression of COX-2 in cancer cells increases the production of vascular growth factors, and the formation of capillary-like networks in vitro. These effects can be blocked by selective COX-2 inhibitors. It was demonstrated that selective COX-2 inhibitors also diminish angiogenesis through inhibition of the MAPK pathway in endothelial cells.

**Invasiveness**

COX-2 is important in modulating the invasive properties of human cancer cells. When COX-2 is overexpressed in cancer cell lines, the production of prostaglandins increased, and the cells became more invasive. This increased invasiveness was associated with the activation of the matrixmetalloproteinases (MMPs) 1 and 2. These enzymes digest the collagen matrix of the basement membrane, thus stimulating the invasive and motile phenotype of tumour cells. Additionally, overexpression of COX-2 was associated with increased amounts of CD44, the cell surface receptor for hyaluronate, and specific blockade of CD44 significantly decreased tumour cell invasion. Consistent with these in vitro findings, selective COX-2 inhibitors have been observed to inhibit dissemination in animals.

**Immunosuppression**

The growth of tumours is associated with suppression of the immune system. Colony-stimulating factors released by tumour cells activate monocytes and macrophages to synthesize PGE₂, which suppresses T-cell and B-cell proliferation, lymphokine production, macrophage activation, and the cytotoxic activity of natural killer cells. PGE₂ also inhibits the production of tumour necrosis factor-α while inducing the production of interleukin 10 (IL-10), which has immunosuppressive effects. These actions may allow the tumour to escape normal immune surveillance. By inhibiting prostaglandin synthesis, NSAIDs can indirectly enhance immune responses. Additionally, they might
upregulate expression of major histocompatibility complex antigens, as was demonstrated in azoxymethane-induced colonic tumours in the rat.\textsuperscript{43} Mechanistically this can depend on cytokine microenvironment, since COX-2 dependent synthesis of prostanoids by lung cancer cells altered release of IL-10 and IL-12 from lymphocytes and macrophages resulting in repression of host immunity.\textsuperscript{46}

**Inflammation**

Chronic inflammation, which is particularly associated with the development of a Barrett’s oesophagus, is a recognized risk factor for carcinogenesis.\textsuperscript{45} Inflammation induces the synthesis of prostaglandins via a cytokine-mediated induction of COX-2. With the data reviewed above, a mechanism can be suggested in which chronic inflammation and an increased expression of COX-2 contribute to the malignant degeneration of a Barrett’s oesophagus.

**COX-2 EXPRESSION IN BARRETT’S METAPLASIA-DYSPLASIA-CARCINOMA**

English-language reports on COX-2 expression in the Barrett’s metaplasia-dysplasia-adenocarcinoma sequence are summarized in Table 1. In all studies, the normal squamous epithelium of the oesophagus was consistently negative or only weakly positive. Wilson et al. reported for the first time an elevated COX-2 mRNA expression in 81% of the Barrett’s metaplasias and a consistently increased COX-2 protein expression in associated oesophageal adenocarcinoma when compared to normal tissue.\textsuperscript{46}

<table>
<thead>
<tr>
<th></th>
<th>Normal epithelium</th>
<th>Metaplasia</th>
<th>Low-grade dysplasia</th>
<th>High-grade dysplasia</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson et al. 1998 (44)</td>
<td>0%</td>
<td>81%</td>
<td>nd</td>
<td>nd</td>
<td>100%</td>
</tr>
<tr>
<td>Zimmerman et al. 1999 (45)</td>
<td>0%</td>
<td>0%</td>
<td>nd</td>
<td>nd</td>
<td>77.8%</td>
</tr>
<tr>
<td>Shirvani et al. 2000 (46)</td>
<td>Significant increase in expression of COX-2 (p &lt; 0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morris et al. 2001 (47)</td>
<td>0%</td>
<td>75%</td>
<td>83%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

\textit{nd = not determined}
In contrast, Zimmerman et al. found no immunoreactivity for COX-2 in metaplastic columnar epithelial cells, whereas COX-2 immunostaining was observed in smooth muscle cells, fibroblasts and endothelial cells. In two studies (Shirvani et al., and Morris et al.), progressively enhanced expression of COX-2 was shown with each stage of oesophageal carcinogenesis from Barrett's metaplasia via low-grade dysplasia, high-grade dysplasia and adenocarcinoma on both mRNA and protein level. So far, the clinical significance of upregulated COX-2 expression in Barrett's carcinomas is still debated. In one study, an association between COX-2 expression and decreased overall survival has been found, but this relation to prognosis could not be confirmed by other studies. In addition, no association between COX-2 expression and prognosis was found in oesophageal squamous cell carcinomas and gastric adenocarcinomas. The discrepant results found in the different studies, may partially depend on specificity and sensitivity issues related to different COX-2 antibodies and staining and scoring protocols or on tissue collection and handling procedures.

SELECTIVE COX-2 INHIBITORS AS CHEMOPREVENTION

The evidence to use NSAIDs as a chemopreventive agent in carcinogenesis first came from epidemiological studies. Of the five published observational studies on oesophageal cancer, four demonstrated a protective effects of NSAIDs (Table 2). Thun et al. found that subjects who used aspirin 16 times per month or more often for at least one year, had an approximately 40% lower risk of oesophageal cancer (p=0.054). Data from the National Health and Nutrition Examination Survey and the National Epidemiological Follow-up Studies showed a 90% (95% CI = 0.01-0.76) decreased risk of developing oesophageal cancer in subjects who reported occasional aspirin use. Data from a large population-based case-control study showed a reduced risk of oesophageal adenocarcinoma (OR=0.37, 95% CI=0.24-0.58), and squamous cell carcinoma (OR=0.49, 95% CI=0.28-0.58) in patients with aspirin use relative to nonusers. Langman et al. also demonstrated the protective effects of NSAIDs against oesophageal cancer (OR = 0.64, CI = 0.41-0.98). Although the last study (Coogan et al.) did not find a significant reduced risk of oesophageal cancer with NSAIDs, the odds ratio was < 1.0 with regular NSAIDs use relative to never use. In addition, in vitro studies and animal studies have been published which support the possible chemopreventive effect of selective COX-2 inhibitors in Barrett's epithelium. Buttar et al demonstrated in primary cultured endoscopic
TABLE 2
Summary of retrospective studies on the protective effect of NSAIDs for oesophageal adenocarcinoma.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Sample</th>
<th>Outcome</th>
<th>NSAIDs results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thun et al. (49)</td>
<td>Epidemiologic</td>
<td>n = 635,031</td>
<td>Death rates</td>
<td>RR = 0.59, 95% CI = 0.34-1.03</td>
</tr>
<tr>
<td>Funkhouser et al.</td>
<td>Epidemiologic</td>
<td>n = 14,407</td>
<td>Oesophageal cancer</td>
<td>RR = 0.10, 95% CI = 0.01-0.76</td>
</tr>
<tr>
<td>Farrow et al. (51)</td>
<td>Case control</td>
<td>Cases: 650</td>
<td>Oesophageal cancer</td>
<td>OR = 0.37, 95% CI = 0.24-0.58</td>
</tr>
<tr>
<td>Langman et al. (52)</td>
<td>Population-based</td>
<td>Controls: 695</td>
<td>incidence</td>
<td>95% CI = 0.41-0.98</td>
</tr>
<tr>
<td>Coogan et al. (53)</td>
<td>Hospital based</td>
<td>Cases: 1,149</td>
<td>Oesophageal cancer</td>
<td>OR = 0.8, 95% CI = 0.5-1.4</td>
</tr>
</tbody>
</table>

RR = relative risk; CI = 95% Confidence Interval; OR = Odds Ratio.

biopsy specimens from patients with Barrett's oesophagus that selective COX-2 inhibitors significantly decreased COX-2 activity and decreased proliferation of epithelial cells by 55% (95% CI 47.1-63.8). The same investigators also found in a rat model that two different selective COX-2 inhibitors reduced the relative risk of developing oesophageal cancer by 55% (95% CI 43-66, p<0.008) and by 79% (95% CI 68-87, p<0.001) after oesophagojejunostomy, when compared with controls. However, the prevalence of a Barrett's oesophagus was not significantly different between the groups (p=0.98). Finally, Kaur et al. indirectly demonstrated the possible chemopreventive effect of selective COX-2 inhibitors in vivo. Biopsy specimens of human Barrett's epithelium were compared with biopsy specimens obtained after 10 days of therapy with rofecoxib 25 mg orally daily. After rofecoxib therapy, the COX-2 expression decreased by 77% (p<0.005), the PGE2 content decreased by 59% (p<0.005), and the proliferating cell nuclear antigen (PCNA) expression decreased by 62.5% (p<0.005).

These findings led to the initiation of one phase II and one phase III randomised clinical trial, which are currently being performed. In a multicenter study, Forastiere et al. (Baltimore, Maryland, USA) randomise patients with
low- or high-grade columnar dysplasia between daily selective COX-2 inhibitors and no additional treatment with dysplasia regression as primary endpoint. Attwood et al. (Manchester, UK) recently started a large European trial with a two-by-two factorial design, in which patients with a Barrett's oesophagus are randomised for selective COX-2 inhibition plus proton pomp inhibitors, versus only selective COX-2 inhibition, only acid inhibition or no additional treatment, to analyse their value in the prevention of oesophageal adenocarcinoma.

SELECTIVE COX-2 INHIBITORS AS (NEO-) ADJUVANT THERAPY

Therapeutic trials are easier to conduct and more cost-effective than prevention trials because of their smaller size and shorter follow-up. There are strong experimental data supporting the initiation of therapeutic trials with selective COX-2 inhibitors as (neo-)adjuvant therapy for oesophageal adenocarcinoma. In vitro studies have shown that NSAIDs inhibit cellular growth and induce apoptosis in carcinoma cell lines. So far, the emphasis has predominantly been on the chemopreventive effects of NSAIDs and solid evidence from randomized trials suggesting that selective COX-2 inhibitors inhibit recurrences or distant metastases of gastrointestinal malignancies, and prolong survival is not yet available. However, various trials (mainly for colorectal cancer) have started with NSAIDs as adjuvant therapy. In Sweden, a randomized trial has been started in patients with adenocarcinoma of the oesophagus or the gastro-oesophageal junction, with celecoxib after potentially curative oesophageal resection, to evaluate the influence of adjuvant selective COX-2 inhibition on survival.

CONCLUSIONS AND FUTURE DIRECTIONS

Numerous epidemiological and experimental studies indicate that NSAIDs show promise as anticancer drugs in gastrointestinal malignancies. So far, the clinical application of these drugs is still limited. Several randomized clinical trials have shown the chemopreventive effect in FAP patients, but only indirect evidence is available for the value of these drugs as adjuvant therapy. In addition, unresolved questions about the mechanism(s) by which these drugs act, the optimal drug, drug dose and the balance of risks and benefits of the longterm use in different populations need to be answered.
Recently, other novel therapeutic agents (e.g. drugs activating apoptotic pathways and inhibiting angiogenesis) have been developed and combination therapies including COX-2 inhibitors, should be tested for a chemopreventive or adjuvant effect. In this respect, Torrance et al. showed that the combination of sulindac with a novel inhibitor of epidermal growth factor receptor kinase was more efficient than either agent alone in reducing intestinal tumour formation in APC^{min} knockout mice. In conclusion, the role of COX-2 and selective COX-2 inhibitors in the prevention and treatment of gastrointestinal tumours and more specifically of oesophageal cancer is promising, but further investigations are needed before they can be incorporated in daily clinical practice.
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