The development of new treatment strategies for oesophageal cancer
Buskens, C.J.

Citation for published version (APA):
Selective cyclooxygenase-2 inhibition as neo-adjuvant therapy for patients with oesophageal adenocarcinoma.

The results of the studies on COX-2 expression in oesophageal cancer led to the initiation of a clinical study to analyse the value of a selective COX-2 inhibitor (celecoxib) as neo-adjuvant therapy for patients with oesophageal adenocarcinoma. The effect of preoperative treatment with celecoxib on various tumour markers was assessed and although the trial is still continuing, the results of the first 26 patients are presented in this chapter.
INTRODUCTION

Adenocarcinoma of the oesophagus, developing via the Barrett's metaplasia-dysplasia sequence, is associated with a rapidly rising incidence and a poor prognosis.\(^1\) The best curative option is surgical resection, but even after extensive surgery, overall survival rates rarely exceed 25%.\(^3\) Advances in careful preoperative selection, radical surgery and (neo-) adjuvant chemo- and radiotherapy, have only shown limited improvement of prognosis.\(^4\) To improve the therapeutic options for patients with oesophageal cancer, current research focuses on novel molecular targets for specific chemotherapeutic treatment strategies.

Epidemiological studies have demonstrated approximately 50% reduction in the incidence of gastrointestinal adenocarcinomas in persons regularly taking aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs).\(^6\) One of the target enzymes of NSAIDs is cyclooxygenase-2 (COX-2), a rate-limiting enzyme in the formation of prostanoids, like prostaglandin E2 (PGE-2) from arachidonic acid.\(^7\) The importance of COX-2 in carcinogenesis and cancer progression has been implicated in cervical, breast, prostate and various gastrointestinal cancers, including oesophageal adenocarcinoma.\(^8\) During the multi-step tumour progression from Barrett’s epithelium towards oesophageal adenocarcinoma COX-2 is increasingly expressed, suggesting an important role in this carcinogenic process. In addition, COX-2 expression was recently identified as an independent prognostic variable for oesophageal adenocarcinoma, suggesting that this enzyme could be an attractive molecular target for specific chemotherapeutic treatment.\(^1\) Although the exact processes by which NSAIDs and in particular selective COX-2 inhibitors exert their anti-carcinogenic effects remain uncertain, several mechanisms have been suggested to be involved. The inhibition of COX-2 by NSAIDs results in a decreased synthesis of PGE-2, that mediates cellular processes involved in inflammation and growth. The decreased PGE-2 levels result in increased apoptosis, decreased proliferation and decreased angiogenesis in cancer cells.\(^12\) Consequently, NSAIDs and selective COX-2 inhibitors have been shown to decrease proliferation and induce apoptosis both in vitro and in vivo.\(^14\) Down-regulation of the hepatocyte growth factor (HGF) induced signal transduction has been suggested as one of the mechanisms.\(^16\) C-Met, the receptor for HGF, has been implicated in the progression and dissemination of several cancer types including oesophageal cancer.\(^18\) In malignancies of the colon, lung, stomach, breast, and nasopharynx, the expression of c-Met was reported as a prognostic factor for patient survival.\(^20\) In \textit{in vitro} models, the activation of c-Met causes increased cellular viability,
proliferation, angiogenesis and invasion of carcinoma cell lines. Therefore, inhibition of c-Met may constitute an important factor in the anti-carcinogenic NSAID effects. Although results from the various epidemiological studies and preclinical experiments all support the initiation of COX-2 inhibition as a novel chemopreventive or chemotherapeutic strategy for oesophageal adenocarcinoma, clinical implementation in daily practice is still debated. Most evidence of the anti-carcinogenic mechanisms of NSAIDs and selective COX-2 inhibitors is derived from in vitro studies with higher concentrations than those achievable in vivo and the biochemical anticancer effects in vivo remain to be elucidated. Therefore, the aim of this study was to characterize the potential clinical role of selective COX-2 inhibitors in the treatment of oesophageal adenocarcinoma. First, the anti-carcinogenic effects of a selective COX-2 inhibitor were assessed in vitro with concentrations achievable in vivo. Subsequently, to investigate the potential clinical significance of the biochemical results of neo-adjuvant selective COX-2 inhibition in vitro, the effects of celecoxib on COX-2 and c-Met expression with its influences on cell proliferation (assessed by Ki67 immunostaining), angiogenesis (assessed by immunohistochemical expression of the endothelial marker CD31), and apoptosis (assessed by cleaved caspase-3 staining as marker for cellular apoptosis) were analysed in vivo in a clinical phase II trial.

PATIENTS, MATERIALS AND METHODS

Reagents
The selective COX-2 inhibitor celecoxib was kindly provided by Pharmacia (Pharmacia, St Louis, MO, USA). For in vitro analysis stock solutions were made in dimethyl sulfoxide (DMSO). Primary antibodies used for Western blot (WB) analysis and immunohistochemistry (IH) were: anti-COX-2 (Cayman Chemical Co., Ann Arbor, MI, USA), anti-c-Met (DL21 (WB) Do24 (IH); Upstate Biotechnology, Lake Placid, NY, USA), anti-Ki67 as marker for proliferation (clone MIB1; Ylem, Rome, Italy), the endothelial marker anti-CD31 (clone JC/70 A; DAKO, Glostrup, Denmark), anti-cleaved caspase-3 as marker for cellular apoptosis, (Cell Signalling Technology, Beverly, MA, USA), the housekeeping gene anti-β-actin for WB loading control (c19; Santa Cruz Biotechnology, CA, USA). PowerVision-horseradish peroxidase (HRP)-conjugated goat anti-rabbit/mouse Ig (PowerVision-GAR/AP) was purchased from ImmunoVision Technologies (Daly City, CA, USA). The substrate used in IH was Diaminobenzidine Peroxidase Substrate. (Sigma, St. Louis, Mo, USA)
Cell cultures
To evaluate the biochemical effects of COX-2 inhibition in vitro with concentrations achievable in vivo, two human oesophageal adenocarcinoma cell lines were used: OE19 and OE33. Both cell lines were originally derived from an oesophageal adenocarcinoma and obtained from the European Collection of Cell Cultures. The adherent cells were cultured in Dulbecco's modified Eagle medium (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% foetal calf serum (Integro, Leuvenheim, the Netherlands), 5 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen Corp., Carlsbad, CA, USA) at 37°C in a 5% CO2 atmosphere. Cells were passaged 20 times maximally. Cells were grown with or without the addition of celecoxib for 48 hours. The concentration range of celecoxib in the experiment was chosen to resemble the in vivo situation (10-80 μM). Equivalent concentrations of DMSO were used in the control samples (0.05%).

Cell viability in cell culture
The cell viability was assessed by the mitochondrial function, measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenil tetrazolium bromide (MTT) reduction activity as previously reported. Briefly, cells were seeded in a 14 well plate and stimulated with increasing concentrations of celecoxib (10-80 μM). After 72 hours, 0.5 mg/ml MTT (Sigma-Aldrich, St. Louis, MO, USA) was added for 30 min at 37°C. Subsequently, the media were aspirated and the cells were lysed in isopropanol/0.04 M HCl.

Western Blot analysis.
The OE19 and OE33 cell lines were seeded in 6-well plates to be at 50% confluence at the time of stimulation. After incubation, cells were kept on ice and washed with ice-cold phosphate-buffered saline. Cells were harvested by adding 300 μl of sample buffer (125 mM Tris/HCl, pH 6.8; 4% Sodium Dodecyl Sulfate (SDS); 2% -mercaptoethanol; 20% glycerol; 1 mg of bromphenol blue), and scraping after which lysates were stored at -20°C. After 5 sec of sonification and 5-min of incubation at 95°C, 20 μl was loaded onto SDS-PAGE and subsequently transferred to a polyvinylidene difluoride membrane. The membranes were blocked with Tris-buffered saline (TBS) supplemented with 0.05% Tween-20 (wash buffer) and 1% casein and incubated with the primary antibody overnight at 4°C, diluted in blocking buffer (COX-2 1:1000, c-Met 1:1500, cleaved caspase-3 1:1000, β-actin 1:4000). Subsequently, the membranes were washed and incubated with a HRP-conjugated secondary antibody in blocking solution, containing 2% low fat milk powder. Enhanced
chemiluminescence (ECL) was used for detection of the HRP-antibodies. Each sample was corrected for loading by comparison with the appropriate β-actin signal.

RNA isolation and semi quantitative Reverse Transcription-PCR
Frozen resection specimens were selected after histological verification that they contained more than 70% tumour cells. Total RNA was isolated from the frozen resection specimens using Trizol reagent (Life Technologies Inc, Gaithersburg, MD, USA) according to the manufacturer’s protocol and quantified by spectrophotometry. Reverse transcription was performed with 5 µg of total RNA using superscript transcriptase III (Life Technologies Inc, Gaithersburg, MD, USA). Primers for c-Met mRNA were designed to amplify across an exon/exon boundary to prevent amplification of genomic DNA and were chosen to lie in regions of the sequence not showing significant homology with other known gene family members. Primers used for amplification of c-Met were 5’-CAGATGTTGTGGTCCTTTG-3’ (forward); 5’-ATTCGGGTTGTAGGAGTCT-3’ (reverse). The level of human TATA binding protein (TBP)-proteasome component, a housekeeping gene, was determined to serve as a control. The PCR was performed on the LightCycler instrument (Roche Diagnostics, Hamburg, Germany) using the LightCycler FastStart DNA Master SYBR green 1 reaction components (Roche Diagnostics). The final 15 µl reaction mixture contained PCR buffer (containing FastStart Taq polymerase and dNTP), MgCl2, 1.6 M primers and cDNA. The cycling conditions were as follows: initial denaturation/Taq activation at 95°C for 10 min, followed by 40 cycles of 95°C for 5s, 60°C for 10s and 72°C for 15s. Fluorescence acquisition was performed once per cycle at the 65°C annealing stage and all temperature transition rates were at 20°C/s. A melting curve was performed at the end of each run using a temperature range of 55°C to 85°C and 0.15°C/s temperature transition rate. All runs included a negative water control. The samples were quantified using Roche Lightcycler software, version 3.5 (Roche Diagnostics) and linregPCR software, version 7.4 as described previously.

Patients
To evaluate the biochemical effects of selective COX-2 inhibition in vivo, celecoxib (400 mg twice daily) was administered as neo-adjuvant treatment in patients with oesophageal adenocarcinoma. The duration of treatment was 4 weeks until the day of surgery. The protocol was approved by the institutional Medical Ethics Committee. All patients were informed and asked to sign a written informed consent before inclusion. Over a 12 months period, 12 consecutive patients were selected for celecoxib treatment and 15 patients
were included as a control group. Inclusion criteria were age >18 years, histologically proven adenocarcinoma of the distal oesophagus (Siewert type 1) and eligible for curative surgery. Patients were excluded in case of renal insufficiency, history of gastric/duodenal ulcer or inflammatory bowel disease, use of aspirin or other non-steroidal anti-inflammatory drugs, known hypersensitivity to these drugs, and history of heart failure or hypertension. All patients were preoperatively evaluated with CT-scan, PET-scan, endoscopic ultrasonography, external ultrasonography of the neck, indirect laryngoscopy and a chest X-ray to adequately determine cTNM stage. None of the patients underwent neo-adjuvant treatment other than celecoxib.

Tissue collection
At the time of diagnosis, endoscopic biopsies were taken from the primary tumour and adjacent normal oesophageal mucosa, snap-frozen in liquid Nitrogen at -180°C and stored at -80°C. In addition, biopsies taken from the same location were fixed in 10% neutral-buffered formalin and paraffin embedded according to standard procedures. After surgery, a small representative sample of the tumour and normal oesophageal tissue were taken from the resection specimen as assigned by a GI-pathologist (GJAO). The specimens were both snap-frozen at -180°C and stored in -80°C, and paraffin embedded. For the purpose of this study these tissue samples were immunohistochemically analysed for expression of COX-2, c-Met, ki-67, CD31 and cleaved caspase-3.

Tissue microarray
For each of the patients a representative tumour sample was selected as donor block for the microarray. Using H&E-stained slides, three morphologically representative regions were defined for each of the tumour samples. From these regions, cylindrical core tissue specimens (diameter = 0.6 mm) were acquired and arrayed precisely into a new recipient paraffin block (20 x 35 mm) using a custom-built precision instrument (Beecher Instruments, Silver Spring, MD, USA).

Immunohistochemical analysis.
The clinical study was set up to immunohistochemically investigate the biochemical effects of celecoxib treatment in vivo. To assess direct effects of selective COX-2 inhibition, it would be ideal to compare tumour tissue before and after treatment. However, due to the heterogeneous expression pattern of various tumour markers, it was acknowledged that preoperative biopsies could reflect sampling errors. Therefore, the study was designed to analyse
patient material in two ways. First, the results of the patient group preoperatively treated with celecoxib were compared to the non-treated control group. In addition, biopsies at the time of diagnosis of the patients included for preoperative celecoxib treatment were compared to oesophageal resection specimen samples after treatment, for internal patient control.

Four-micrometer-thick sections of representative blocks from each patient were deparaffinized in xylene, rehydrated, and treated with 3% H2O2 in methanol for 10 min to block endogenous peroxidase activity. All specimens were subjected to heat-induced epitope retrieval for ten minutes at 95°C. Sections were incubated with anti-COX-2 (1:100), anti-c-Met (1:100), anti-Ki67 (1:100), anti-CD31 (1:50), anti-cleaved caspase-3 (1:200) diluted in TBS with 1% bovine serum albumin (BSA) overnight at 4°C. Specificity of the antibody was confirmed by negative controls using irrelevant mouse or rabbit IgG instead of primary antibodies. Colon cancer tissue was included as a positive control. The analysis of all tissue sections was independently performed by two different investigators (CJB and JBT) without patient identification parameters. For COX-2 immunohistochemical staining, the following scoring criteria of tumour cells were agreed upon before the analysis: 0, no staining; 1+, weak diffuse cytoplasmic staining (may contain stronger intensity in less than 10% of cancer cells); 2+, moderate to strong granular cytoplasmic staining in 10-90% of cancer cells; 3+, over 90% of tumour cells stained with strong intensity. These scoring criteria have previously been described and are identical to those used in our previous report on COX-2 expression in oesophageal adenocarcinoma. For evaluation of c-Met the same scoring criteria were used, since the staining pattern is similar except for the membranous localization of the c-Met receptor. The evaluation of microvessel density by CD31 was performed as described by West et al. with minor modifications. Briefly, tumour sections were analysed at low-power magnification to assess areas of neo-angiogenesis. Within these areas, individual microvessels were counted in two or three separate random fields within a superimposed grid (area, 0.0625 mm²). A single countable microvessel was defined as any endothelial CD31-positive cell or group of cells that was clearly separated from other vessels, stroma, or tumour cells without the necessity of a vessel lumen or red blood cells within the lumen. Areas of diffuse haemorrhage or necrosis were neglected. Ki67 and cleaved caspase-3 immunopositivity was determined as the percentage of tumour cells with positive staining counted in three separate x40 microscopic fields (at least 200 cells per field were counted). Subsequently samples were scored as 1, when less than 1% of the cancer cells were positive. A score of 2 was considered if 1-5% immunostained
cancer cells were positive, a score of 3 when 5-10%, a score of 4 when more than 10% of the tumour cells were positive.

**Statistical analysis**
Data obtained from Western Blot analysis or immunohistochemistry were analysed using the Student's t-test (two sided) with a level of significance at p-value < 0.05. Graphically, data are shown as bar graphs depicting the mean values and the standard errors of mean (SEM). All statistical analyses were performed using the Statistical Software Package version 11.5 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Effects of celecoxib on cell viability and apoptosis in vitro**
Before the effects of COX-2 inhibition in patients with oesophageal cancer were investigated, it was essential to show that oesophageal adenocarcinoma cells react to COX-2 inhibition by decreasing viability and by inducing apoptosis. For this purpose, two human oesophageal adenocarcinoma cell lines OE19 and OE33 were cultured and exposed to celecoxib at various concentrations. Celecoxib reduced viability by inducing apoptosis in both cell lines at 20 μM and more as detected by the reduced incorporation of trypan blue by the MTT assay after 72h (Figure 1a). Accordingly, after 72h incubation with celecoxib, an increase in cleavage of caspase-3, an unequivocal marker for cellular apoptosis was detected on Western blot analysis (Figure 1b).

**Effects of celecoxib on COX-2 protein and c-Met expression in vitro**
Subsequently, the expression of COX-2 and c-Met were determined in vitro by Western blot analysis after 48 hrs of celecoxib treatment to avoid cell death. Expression levels were corrected for protein levels to ensure equal loading. Celecoxib administration resulted in decreased COX-2 protein levels. Moreover, the levels of c-Met protein were decreased when compared to the levels of β-actin (Figure 2).

**Clinical data**
To investigate the potential clinical significance of the biochemical results obtained in vitro, 12 patients with an adenocarcinoma of the distal oesophagus were preoperatively treated with celecoxib (Celebrex® 200mg tablets) 400 mg orally twice a day, from the day of diagnosis until the day
FIGURE 1
A: The viability of oesophageal adenocarcinoma cells (OE19 and OE33) was measured by MTT assay. After 72 hours of exposure to celecoxib, cell viability was decreased at low concentrations of celecoxib while higher concentrations resulted in almost 75% percent decrease in viability.

B: Western blot analysis of OE33 cell lysate after 72h incubation with celecoxib. The cleaved caspase-3 products of 17 and 19 KDa are shown as a marker for apoptosis. Celecoxib progressively induces apoptosis at increasing concentrations. The level of β-actin is shown as a loading control.
FIGURE 2
Western blot analysis of OE33 cell lysate after 48h incubation with celecoxib. COX-2 protein levels decreased after 48h incubation with low concentrations (20 μM, 40 μM and 80 μM celecoxib), whereas c-Met protein is downregulated at higher concentrations (80 μM celecoxib). The level of β-actin is shown as a loading control.

before surgery. One adverse event of celecoxib treatment was registered in a patient experiencing a mild cutaneous rash which spontaneously resolved after discontinuation of celecoxib treatment. This patient was excluded for further analysis.

The median treatment duration of the remaining 11 patients was 4 weeks with a compliance rate of 100%. Expression data were compared to those in the control group consisting of 15 prospectively included patients, who did not receive celecoxib or any other NSAIDs medication. The clinicopathological parameters of both groups are described in Table 1.
FIGURE 3
Examples of immunohistochemical analysis of human resection specimens plotted in the tissue microarray.
A: high COX-2 expression; B: low COX-2 expression; C: high c-Met expression; D: low c-Met expression; E: high CD-31 expression; F: low CD-31 expression; G: high Ki67 expression; H: low Ki67 expression; I: high cleaved caspase-3 staining; J: low cleaved caspase-3 staining. Magnification = 10x. The squares at the lower right corner represent the same tissue at magnification = 50X.
TABLE 1
Clinicopathological parameters of patients preoperatively treated with celecoxib in comparison to the non-treated control group.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Untreated</th>
<th>Treated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>65±9</td>
<td>64±8</td>
<td>0.6</td>
</tr>
<tr>
<td>Gender</td>
<td>Male (23)</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Female (3)</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumour characteristics</th>
<th>Untreated</th>
<th>Treated</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of invasion†</td>
<td>T1 (1)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>T2 (3)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>T3 (22)</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Lymph node involvement‡</td>
<td>N0 (5)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>N1 (21)</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Differentiation grade</td>
<td>Well (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Moderate (16)</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Poor (10)</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

*T1: tumor limited to the submucosa, T2: tumor infiltrates muscularis propria, but not adventitia, T3: tumor infiltrates adventitia; NO: no regional lymph node metastasis. N1: regional lymph node metastasis present.

Comparison between tumoural biochemical parameters in patients after celecoxib treatment and control patients
A tissue microarray of paraffin embedded resection tissue with histologically confirmed adenocarcinoma of patients treated with celecoxib and control patients was used to immunohistochemically evaluate COX-2 and c-Met expression, and proliferation (Ki67), angiogenesis (CD31) and apoptosis (cleaved caspase-3) in the adenocarcinoma tissue (Figure 3).
In line with the results obtained in vitro, the level of COX-2 protein was significantly lower in patients treated with celecoxib when compared to the untreated control group (p=0.002). Also, a significant decrease in c-Met expression was observed (p=0.003). No significant discrepancy in the immunohistochemically determined level of proliferation, angiogenesis, and apoptosis was detected, although a trend was observed towards a decrease in proliferation (p=0.1, Figure 4).
FIGURE 4
Quantification of immunohistochemical analysis in resection specimens. After preoperative celecoxib treatment, tumoural COX-2 and c-MET protein levels were significantly decreased (p=0.002 and p=0.003 resp.) in patients treated with celecoxib when compared to non-treated control patients. Proliferation, as quantified by Ki67 staining, was decreased in treated patients although this did not reach statistical significance (p=0.1). A decrease in angiogenesis (CD-31 expression) or increase in apoptosis (cleaved caspase-3 expression) was not observed.

FIGURE 5
Quantification of c-Met RNA from frozen resection specimens. Total RNA was extracted from surgical resection specimens containing at least 70% tumour tissue. After correction for TATA binding protein-proteasome component, a housekeeping gene, c-Met RNA levels were decreased in patients preoperatively treated with celecoxib when compared to the control group, although this did not reach statistical significance (p=0.2).
FIGURE 6
Quantification of immunohistochemical analysis in biopsies before treatment compared to resection specimens after neo-adjuvant celecoxib treatment in the same patient.
A: In 2 out of 3 patients COX-2 expression was decreased. B: In 3 out of 4 patients a decline of c-MET was demonstrated. C: The amount of apoptotic cancer cells, detected by immunohistochemical analysis of cleaved caspase-3, is decreased in 2 out of 3 patients.

**A**
COX-2 protein before and after treatment

**B**
c-Met expression before and after treatment

**C**
apoptosis before and after treatment
To validate the results obtained by the immunohistochemical analysis, RNA levels of c-Met in the resection specimens of patients treated with celecoxib versus control patients were determined. The relative amount of c-Met RNA was decreased in patients treated with celecoxib as compared to untreated control patients although this did not reach statistical significance ($p=0.2$, Figure 5)

Comparison between tumoural biochemical parameters in preoperative biopsies and surgical resection specimens after celecoxib treatment
To confirm the effects of celecoxib on biochemical parameters observed both in vitro and in vivo, the expression patterns of COX-2, c-Met, Ki67 (proliferation), CD31 (angiogenesis), and cleaved caspase-3 (apoptosis) were also measured in tissue samples from the same patient taken before and after treatment. So far, we have only been able to analyse the tissue specimens of three patients. Soon the data of the eight remaining patients will become available, after which a more definite statistical analysis will be performed. Again, a decline in COX-2 and c-Met protein expression was detected by immunohistochemistry. In addition, apoptosis was substantially increased during the four weeks of treatment which is consistent with the in vitro data described above (Figure 6).
DISCUSSION

This study demonstrates that low concentrations of the selective COX-2 inhibitor celecoxib significantly decrease cell viability in oesophageal adenocarcinoma cell lines and that they induce cell apoptosis at higher concentrations in vitro after 72 hours. This decrease in viability was associated with diminished COX-2 protein expression levels after 48 hours, in combination with decreased expression of c-Met protein. These findings led to the initiation of a clinical phase II study with selective COX-2 inhibition as neo-adjuvant treatment in patients with an adenocarcinoma of the oesophagus. The preliminary results of this study show that 4 weeks celecoxib treatment significantly inhibits the tumoural expression of COX-2 and c-Met in vivo. Although the decrease in proliferation and angiogenesis or increase in apoptosis in vivo was not statistically significant, the current results suggest that the role of selective COX-2 inhibition in the treatment of oesophageal adenocarcinoma is promising.

The decrease in COX-2 expression after selective COX-2 inhibition in vivo is in agreement with the study of Kaur et al. They convincingly showed a decrease of COX-2 expression and cell proliferation in patients with metaplastic Barrett's epithelium after 10 days of rofecoxib, a selective COX-2 inhibitor. In addition, celecoxib administration in patients with cervical cancer has also been shown to decrease COX-2 expression. In in vitro and in animal studies, this decrease in COX-2 expression is associated with a decrease of tumour proliferation, angiogenesis and increase in apoptosis. A possible explanation for this observation is that selective COX-2 inhibition reduces oncogenic signal transduction mediated by growth factors and their receptors, such as c-Met, via downregulation of PGE-2. Pai et al. demonstrated that PGE-2 transactivates epidermal growth factor receptor and c-Met, which latter was demonstrated to increase tyrosine phosphorylation and nuclear accumulation of b-catenin. Tyrosine phosphorylation of b-catenin by HGF is suggested to promote metastatic potential and tumour invasiveness by stabilizing b-catenin and binding of T cell factor/lymphoid enhanced binding factor-1 (TCF/LEF-1) DNA transcription factors, which in turn stimulate cell proliferation. This increase in c-Met by PGE-2 stimulation can explain our in vivo finding that celecoxib treatment was associated with a significantly lower tumoural COX-2 and c-Met expression when compared to COX-2 and c-Met expression in the non-treated patient group, and that a similar decrease in expression levels was observed before and after celecoxib therapy in the same patient. Although no definite conclusions can be drawn about the exact anti-carcinogenic pathway, the significant concomitant
decrease in COX-2 and c-Met expression suggests that c-Met is indeed an important target of selective COX-2 inhibition. This is in agreement with a previous report demonstrating that celecoxib delayed wound healing by down-regulation of the HGF/c-Met signalling pathway in a rat model for oesophageal ulcers.\(^{34}\)

C-Met is expressed in various cancers and is associated with an advanced stage of disease. Moreover the activation of the HGF receptor is one of the main mechanisms involved in the development of metastases.\(^{26, 27}\) In this study it was demonstrated that the patients with oesophageal cancer have a high expression of the c-Met protein. Compared to other cancers of the gastrointestinal tract, lymphatic dissemination occurs relatively early during the development of oesophageal cancer.\(^5\) It has been suggested that this relatively early lymphatic dissemination is due to the extensive lymphatic network in the submucosa of the oesophagus, which is absent elsewhere in the digestive tract. However, specific molecular mechanisms like the expression of adhesion molecules and growth receptors can also account for this oncogenic behaviour. The observed decrease of C-Met expression by short term celecoxib administration in vivo, provides a rational to analyse its significance in prevention of cancer progression and dissemination.

It should be realized that this is a phase II trial comparing treated and non-treated patients, set up to create insight in the biochemical mechanisms underlying selective COX-2 inhibition in vivo. Whether selective COX-2 inhibition truly improves patient survival has to be demonstrated in a randomized controlled trial with long term survival data which is currently being performed. It is acknowledged that the results of this study may be hampered by sampling errors since oesophageal biopsies might be affected by the tissue handling. However, this is less likely since the observed changes in the described parameters were also present when non-treated patients were compared to celecoxib treated patients in resection tissue analysis.

In conclusion, this study demonstrates for the first time that neo-adjuvant celecoxib administration can downregulate COX-2 and the HGF receptor c-Met in vivo, in oesophageal adenocarcinoma. Therefore, not only COX-2 but also c-Met is interesting as a promising molecular target for selective COX-2 inhibition. So far, most studies have predominantly focused on the potential chemopreventive value of NSAIDs and selective COX-2 inhibitors. Since COX-2 and c-Met are both important oncogenes involved in cancer progression and the development of distant metastases, the role of selective COX-2 inhibition in the treatment of oesophageal adenocarcinoma seems also promising. However, the results of a randomized controlled trial with long-term follow-up data will have to be awaited before this new treatment strategy can be implemented in daily clinical practice.
REFERENCES


