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A Review: Role of Cyclooxygenase- 2 Inhibitors in Treatment of Colorectal Cancer.

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Abstract
Colorectal cancer, also called colon cancer or large bowel cancer or "CRC", includes cancerous growths in the colon, rectum and appendix. Experimental studies have shown that cyclooxygenase 2 **(**COX2) is involved in the colorectal tumour development and progression. Selective inhibitors of COX2 (coxibs) block tumour growth through many mechanisms, especially by antiangiogenic and proapoptotic effects[1]. In experimental models, coxibs potentiate the activity of cytotoxic agents, hormones, and radiotherapy. Large clinical studies have shown chemopreventive activity of coxibs in colorectal cancer. The findings of preclinical studies coupled with the overexpression of COX2 observed in advanced human tumours are the basis for new therapeutic anticancer strategies based on combinations of coxibs with other anticancer treatment modalities[3].

Key Words
Colorectal cancer, COX-2 inhibitors, Coxibs.

Introduction
The colon and rectum comprise the final portion of the human digestive tract, commencing at the ileocecal valve that marks the end of the small intestine, terminating at the anus, and measuring roughly one yard in length. Cancers of the colon and rectum are the second leading cause of cancer incidence and cancer death among adult Americans, with 135,000 new cases and 57,000 deaths in 2001, and with a 6% lifetime risk of developing the disease (Greenlee et al., 2001). Encouraging declines in the death rate from colorectal cancer in the last decade speak to the potential effectiveness of recent advances in prevention, screening, and therapy. Cancers of the colon arise from the colonic epithelial cells that line the lumen of the organ which renew themselves every five days from a stem cell population located at the base of colonic epithelial cell crypts[2]. Colon cancers are the end result of a multistep process of colon neoplasia that extends over several years First, neoplastic tubular colon adenomas arise as pedunculated polypoid structures growing into the colon lumen. With time, they acquire increasingly disordered villous histology and dysplastic cellular cytology, and are recognized as frank cancers only when invasive cells breach the underlying epithelial basement membrane. Various clinical stages of progression of colorectal cancer are illustrated in figure (Figure-1). Reproducible increases in incidence of the disease in populations that have migrated from low to high incidence regions of the world show the importance of environmental factors (Skibber et al., 2001). Cohort studies have rejected variations in intake of fiber, vegetables, and antioxidant vitamins as causative factors, but support that risk increases with red meat consumption, low folate intake, and sedentary lifestyle (Willett, 2001). The importance of genetic factors is shown by findings that germline mutations in key colon cancer genes give rise to familial hereditary colon cancer syndromes that account for 3%–7% of all cases annually [Kinzler and Vogelstein, 1996] and [Skibber et al., 2001].

Genetic susceptibility factors also likely play a role in typical "sporadic" colon cancers, as indicated by the 2- to 3-fold increased risk of colon cancers in first degree relatives of persons affected by either colon cancer or by colon adenomas developing before age 60[4].

Screening

In concept, most colon cancer could be prevented by detection and removal of premalignant colon adenomas. Likewise, considerable benefit would be predicted by detecting colon cancers at early stages when the disease is amenable to cure by surgical excision. These considerations have lead to recommendations for mass screening starting at age 50 for the average risk adult population, and earlier

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for individuals at higher risk due to family history or other predisposing factors ([Schoen, 2002] and [Smith et al., 2001]). Available screening modalities include chemical testing for the presence of occult blood in the stool, endoscopic visualization of the lower portion of the colon by sigmoidoscopy, or full endoscopic visualization of the colon by colonoscopy, with sensitivities for detecting cancer of 15%–30%, 60%, and 90%, respectively ([Schoen, 2002] and [Smith et al., 2001]). The adoption of mass colonoscopic screening has been impeded by the expense of the procedure and the 24 hr required to undergo both a pretest laxative preparation and a posttest recovery from sedation. The recent successful detection of colon cancer-specific mutations in DNA from the feces of colon cancer patients has spurred considerable hope for the development of molecularly based screening[5]. Invasive cancers that are confined within the wall of the colon (TNM stages I and II) are curable with surgery. If untreated, they spread to regional lymph nodes (stage III), where up to 73% are curable by surgery and chemotherapy. Cancer that metastasizes to distant sites (stage IV) is usually not curable, although chemotherapy can extend survival, and in rare cases, surgery and chemotherapy together have seen patients through to a cure. Radiation is used with rectal cancer[6].

Wnt signaling pathway

On the cellular and molecular level, colorectal cancer starts with a mutation to the Wnt signaling pathway (Figure 2). When Wnt binds to a receptor on the cell that sets in motion a chain of molecular events that ends with β-catenin moving into the nucleus and activating a gene on DNA. In colorectal cancer, genes along this chain are damaged. Usually, a gene called APC, which is a "brake" on the Wnt pathway, is damaged. Without a working APC brake, the Wnt pathway is stuck in the "on" position. Tumour progression in most colon cancers is characterized by cyclooxygenase-2 (COX-2) overexpression and aberrant activation of the WNT/β-catenin pathway. Along these lines, it has been suggested that the oncogenic potential of COX-2 may be mediated through the involvement of the recently discovered peroxisome proliferator-activated receptor δ (PPARδ) factor, which is again overexpressed in neoplastic tissue. β-Catenin is a binding partner for the product of the adenomatous polyposis coli (APC) tumour suppressor gene, which acts as a negative regulator of β-catenin and WNT signalling[7]. In normal cells β-catenin usually binds to APC and, following phosphorylation by glycogen synthase kinase-3β (GSK-3β), is then targeted to be degraded by the ubiquitin proteasome pathway. Following activation of the WNT signalling pathway, which operates through inhibition of GSK-3β, β-catenin remains unphosphorylated and proteasome degradation is prevented. Stabilized β-catenin is then released from the complex with APC and translocates into the nucleus acting as a transcriptional factor, leading to the increased expression of WNT target genes including c-myc and PPARβ/δ, which are known to play a relevant role in tumourigenesis[8]. Alternatively, β-catenin can form a complex with E-cadherin at adherens junctions located to promote cell–cell adhesion. Indeed, a decrease in cell–cell adhesion has been shown to be associated with changes in the structure or in the expression of the E-cadherin/β-catenin complex, potentially resulting in a loss of contact inhibition of cell proliferation and then favouring invasion and metastasis. E-cadherin loss and abnormal E-cadherin expression have been observed in tumour development, and correlate with colorectal carcinogenesis.

COX2 expression in human cancers:

Immunohistochemical studies have shown COX2 overexpression in premalignant lesions such as oral leucoplakia, actinic keratosis, prostatic intraepithelial neoplasia, and carcinoma-in-situ of the bladder and breast. COX2 is also upregulated in several invasive tumour types (table 1). In general, COX2 expression is higher in well to moderately differentiated tumours and in metastases. A significant relation between overexpression of COX2 and survival of patients with breast, colon, gastric, and lung cancers has been reported in retrospective studies[9]. Experimental studies have shown that cyclooxygenase 2 (COX2) is involved in tumour development and progression. Selective inhibitors of COX2 (coxibs) block tumour growth through many mechanisms, especially by antiangiogenic and proapoptotic effects. In experimental models, coxibs potentiate the activity of cytotoxic agents, hormones, and radiotherapy. Large clinical studies have shown chemopreventive activity of coxibs in colorectal cancer. The findings of preclinical studies coupled with the overexpression of COX2 observed in advanced human tumours are the basis for new

therapeutic anticancer strategies based on combinations of coxibs with other anticancer treatment modalities. Early clinical studies have documented the feasibility, good tolerability, and promising activity of coxibs combined with chemotherapy in patients with advanced colorectal and non-small-cell lung cancers[10].

Cyclo-oxygenase (COX), the enzyme that catalyses the conversion of arachidonic acid to prostaglandins, is involved in several physiological and pathogenetic pathways (Figure 3). Two isoforms are known. COX1 is constitutively expressed in most tissues and produces prostaglandins involved in maintenance of the gastric mucosa, regulation of renal blood flow, and platelet aggregation. The inducible form, COX2, is expressed in inflamed and neoplastic tissues and is induced by proinflammatory and mitogenic stimuli, such as growth factors (epidermal growth factor [EGF], vascular endothelial growth factor [VEGF], fibroblast growth factor [FGF]) and cytokines (tumour necrosis factor α [TNFα], interleukins 1α and 1β). Certain mutations also upregulate COX2 (eg, *v-src*, *v-Ha-ras*, HER-2/*neu*, and *Wnt*). The presence of *cis*-acting elements in the 5–flanking region of the COX2 gene, including nuclear factor kB (NFkB), nuclear factor–interleukin 6 (NF-IL6), and cAMP response element (CRE) sites, and increased stability of COX2 mRNA may upregulate COX2 overexpression. COX2 is expressed in macrophages, synoviocytes, fibroblasts, osteoblasts, tumour cells, and "activated" endothelial cells. Recent research suggests a role of COX2 in neoplasia, including hyperproliferation, transformation, tumour growth, invasion, and metastasis.

Mechanisms of COX2 regulation

Overexpression of COX2 in human cancers is likely to occur via several pathways (figure 4). Associations of COX2 with mutated *RAS* and *c-MYB* have been found. In human colon and liver carcinogenesis, COX2 is transcriptionally downregulated by *APC* and upregulated by nuclear accumulation of β-catenin, through the *Wnt*-signalling pathway, whereas *K-RAS* induces stabilisation of COX2 mRNA. However, whether the mechanisms of modulation of COX2 expression are the same in different cell lines is not known at present. Increased expression of COX2 in human cancers is likely to occur via several pathways: mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), c-Jun N-terminal kinase (JNK), p38, and protein kinase A (PKA), that induce cAMP synthesis and activation of NFkB and NF-IL6, as well as the CRE promoter site. COX2 gene transcription is induced through NFkB by an extracellular-signal-related kinase (ERK2), p38, and JNK, through NF-IL6 via p38, and through CRE via ERK2 and JNK pathways. PKC seems to mediate COX2 transcription through all the three promoter sites. COX-2 is transcriptionally downregulated by APC and upregulated by c-Myb, and nuclear accumulation of β-catenin, through the Wnt-signalling pathway, in human colon and liver carcinogenesis, whereas K-ras induces COX2 mRNA stabilisation. DR, death receptor; FADD, Fas-associated death domain protein.

COX2 is overexpressed in several cell types, such as macrophages, synoviocytes, fibroblasts, osteoblasts, tumour endothelial cells, and "activated" endothelial cells, and may contribute to tumour growth through several mechanisms: COX2-dependent - prostaglandins can stimulate intracellular receptors (intracrine mechanism), self-prostaglandin membrane receptors (autocrine mechanism), and prostaglandin membrane receptors of different cells, such as endothelial cells, with proangiogenic effects (paracrine or landscaping effect)[11].(Figure 5).

Role of COX 2 inhibitors

An interaction of nitric oxide and COX has been shown. Nitric oxide activates COX by heme oxidation or indirectly through the production of peroxynitrile and hydroxyl radical, leading to increased lipid peroxidation. Also, increased production of superoxide anion by nitric oxide synthase could potentially activate COX. In human colon and breast cancers, increased expression of inducible nitric oxide synthase and prostanoids is associated with COX2 overexpression, in both cancer cells and stroma; these findings suggest a close association between these two factors. Eight types of prostanoid receptors have been identified. All are G-proteincoupled cell-surface receptors, encoded by different genes. The IP, DP, EP_2, and EP_4 receptors mediate a rise in cAMP—"relaxant" receptors. The TP, FP, and EP_1 receptors induce calcium mobilisation—"contractile" receptors. The EP_3 receptor causes a decline in cAMP concentrations—"inhibitory" receptor. The intracellular effects of prostaglandin receptors are a function of concentration and subtype of

prostanoids. EP_4 activates the phosphatidylinositol-3-kinase (PI3K)/ AKT pathway. Prostaglandins also modulate cellular pathways by acting directly in the nucleus. COX2 has been localised in the perinuclear envelope, and both PGI2 and metabolites of PGD2 transactivate members of the peroxisome-proliferator-activated receptor (PPAR) family of nuclear hormone receptors. However, another study did not confirm these findings. The physiological role of prostaglandins as PPAR activators has not been critically examined, partly because coxibs are themselves PPAR ligands[12].

Coxibs: pharmacology, drug interactions, and safety profile

Three classes of COX inhibitors have been developed: aspirin; indomethacin and other NSAIDs; and coxibs (celecoxib, rofecoxib, valdecoxib, etoricoxib, and COX-189 or luminaroxib). Selectivity for COX2 can be assessed by whole-blood assays in vitro or in vivo, based on the production of thromboxane B2 during blood clotting (an index of platelet COX1 activity) and the production of PGE2 by bacterial lipopolysaccharide in whole blood (an index of monocyte COX2 activity). Genetic variability in the target or metabolising enzymes, drug interactions, and the clinical characteristics of the patient can influence both efficacy and toxicity of coxibs. The differences between celecoxib and rofecoxib are related to their pharmacokinetic characteristics, such as oral bioavailability, half-life, and hepatic metabolism. The selectivity depends on plasma drug concentrations. Rofecoxib seems to have ten times greater major COX2 selectivity than celecoxib, particularly at the high doses used in people with cancer. This point is relevant because most of the experimental studies have shown dose-dependent antitumour activity of coxibs. Celecoxib is oxidised by cytochrome P450 2C9, 3A4 and interacts with inhibitors of this enzyme and warfarin, resulting in higher drug plasma concentrations and possible haemorrhagic events. Also, rofecoxib interacts with warfarin, but it is less influenced by renal insufficiency. Both the drugs increase blood pressure and are influenced by hepatic impairment. On the basis of results of phase III studies, the FDA approved celecoxib for the treatment of osteoarthritis and rheumatoid arthritis and rofecoxib for osteoarthritis and acute musculoskeletal pain. However, whether the two coxibs are equally effective in the same disease is unknown. Coxibs can exacerbate the late phase of inflammation characterised by the production of antiinflammatory prostaglandins mediated by COX2[13]. Two large trials assessed the safety and efficacy of coxibs: the Vioxx Gastrointestinal Outcomes Research (VIGOR) and the Celecoxib Long-term Arthritis Safety Study (CLASS). Only the VIGOR study showed absolute and relative reduction of the risk of peptic ulcers or gastrointestinal bleeding (2·4% and 50%, respectively) in the rofecoxib group, probably because of a cardioprotective effect of naproxen. However, other mechanisms cannot be discounted, such as a prothrombotic effect of coxibs. That trial documented a higher rate of cardiovascular events in patients treated with rofecoxib; such a toxic effect of coxibs needs further investigation. Prostaglandins preserve renal blood flow in the presence of volume depletion, and inhibition of this homoeostatic response accounts for the renal side-effects of NSAIDs. COX2 has been localised in renal vasculature, cortical macula densa, and medullary interstitial cells of the kidney, and its content increases with age. By contrast, COX1 is expressed in vasculature, collecting ducts, and thin loops of Henle. An analysis of the postmarketing data for celecoxib indicates a rate of 2·1 % for peripheral oedema, 0·8 % for hypertension, and 0·6 % for exacerbation of pre-existing hypertension. Also, rofecoxib showed a rate of 3·8 % for peripheral oedema. The risk of nephrotoxicity is higher in patients with impaired renal perfusion.

Apoptosis

Treatment of colorectal-carcinoma cells with NSAIDs or coxibs increased the concentration of arachidonic acid by stimulation of sphingomyelinase (which converts sphingomyelin to ceramide, a potent inducer of apoptosis). Unesterified arachidonic acid modulates mitochondrial permeability and causes release of cytochrome C, leading to apoptosis. Inhibition of lipo-oxygenases leads also to cell death through increased cytoplasmic concentrations of arachidonic acid. However, COX-independent mechanisms of NSAID-mediated apoptosis have been described. NSAIDs inhibit the activity of IkB-kinase β, which catalyses the NFkB pathway by phosphorylation of the inhibitory subunit of NFkB (IkBα), targeting it for proteosome destruction. Another mechanism involves PPARδ, a growth-promoting protein suppressed by *APC* gene,

inhibited by sulindac. In colorectal cancers, in which the *APC* gene is commonly mutated, the PPARδ concentration is higher than in normal cells and NSAIDs block this activity. In other studies, NSAIDs decreased expressionof the antiapoptotic gene *BCLXL*, thereby increasing the cellular ratio of BAX to BCLXL. $BAX^{-/-}$ cells are resistant to NSAID-induced apoptosis. Finally, Grosch and colleaguesshowed that the G_0/G_1 cell-cycle block caused by celecoxib in colon-cancer cell lines and in-vivo models is related to decreased expression of cyclins A and B1, and to expression of the cell-cycle inhibitory proteins p21WAF1 and p27KIP1. Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) have long been studied as agents that may influence cancer development and progression. In particular, data from observational studies and intervention trials consistently demonstrate that usage of these agents reduces the risk of colorectal adenomas and/or cancer. Hypotheses for the mechanism of action of these agents include inhibition of the cyclooxygenase (COX) family of enzymes (increasing arachidonic acid which stimulates the conversion of sphingomyelin to ceramide that mediates apoptosis as well as altering prostaglandin production which will decrease angiogenic factors), inhibition of the activation of nuclear factor-κ-B, interference of the binding of peroxisome-proliferator-activated receptor *δ* (PPAR*δ*) to DNA and other potential non-COX mediated pathways. Regardless of the precise mechanism, the data is so consistent that causality is generally accepted. In the Nurses' Health Study, an over 30-year ongoing prospective observational study of 121,000 women, a dose- and duration-dependent protective effect of aspirin on colorectal cancer incidence was demonstrated. Amongst women who regularly used aspirin (≥2 standard [325-mg] tablets per week), the multivariate relative risk (RR) for colorectal cancer was 0.77 (95% confidence interval [CI], 0.67–0.88) compared with nonregular users. A statistically significant risk reduction required more than 10 years of use. The strongest benefit was seen in subjects using more than 14 aspirins per week (RR 0.68 [95% CI, 0.49–0.95]). In a randomized, placebo-controlled study of aspirin in patients with prior colorectal adenomatous polyps, aspirin reduced the risk of advanced adenomas at 3 years by 30%. Similarly, in a phase III trial of aspirin versus placebo in patients with a prior history of colorectal cancer, treatment with aspirin decreased subsequent adenomatous polyp formation by 35% in the 3-year follow-up period.

The discovery of the second isoform of cyclooxygenase, COX-2, resulted in extensive research on the different roles of COX-1 and COX-2 in normal and abnormal cell function. Studies emerged that suggested that COX-2 was induced by inflammation and COX-1 was more constitutive, particularly in the gastrointestinal tract. The subsequent conclusion was that inhibitors specific to COX-2 would have more therapeutic specificity with less gastrointestinal toxicity (as well as minimizing inhibition of platelet aggregation by having a more modest effect on thromboxane A_2 synthase). Thus, there was great enthusiasm to develop COX-2-specific inhibitors. In less than 10 years since the initial discovery of COX-2, celecoxib and rofecoxib were approved by the Food and Drug Administration (for arthritis)[14].

Conclusions

There is compelling experimental evidence that inhibition of COX2 causes tumour regression. Pharmacodynamic studies have shown several mechanisms for the anticancer effects of coxibs: blocking of angiogenesis, promotion of apoptosis, modulation of inflammation and immunoresponse, and others. However, we do not yet know which of these the major pathway of control of tumour growth is. Another unresolved issue concerns systematic comparative studies with the available coxibs to establish which the compound of choice for anticancer therapy is. Preclinical studies suggest possible additive or synergistic activity of combinations of coxibs with conventional anticancer treatments and with novel molecular targeting compounds. Since coxibs are expected to have therapeutic indications as a component of multitargeted treatments rather than as single agents, this issue is a topical and expanding subject of research. The search for surrogate biomarkers able to select the patients who are most likely to benefit from coxibs and to monitor the pharmacological effects in individual patients is another important issue. As anticancer therapy, coxibs present important theoretical advantages—they are orally active, have moderate side-effects, and have few medical contraindications. Indeed, coxibs combined with taxane or inhibitors of topoisomerase I may potentiate the antitumour effects and keep to a

minimum certain typical side-effects of chemotherapy such as mucositis, diarrhoea, and other inflammatory toxic effects. Coxibs are also useful for control of both chronic pain and fever. Coxib-based combinations should find rational indications for therapy of advanced cancer, especially in chemoprevention and adjuvant settings, since these drugs have a good toxicological profile and can be given for long durations.

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Figure 1: Clinical staging of colon cancer.

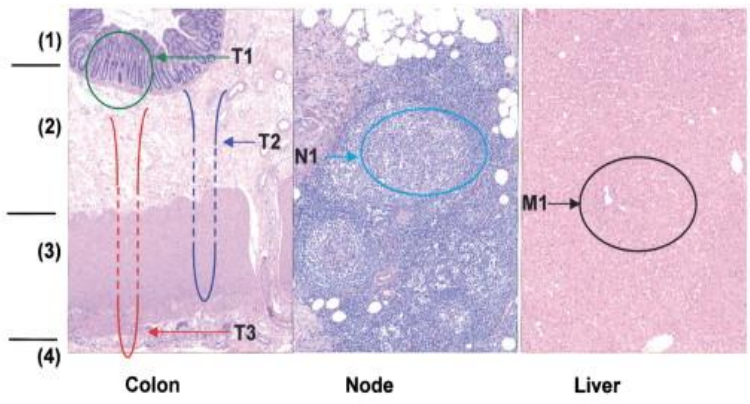

Figure 2: Wnt signaling pathway.

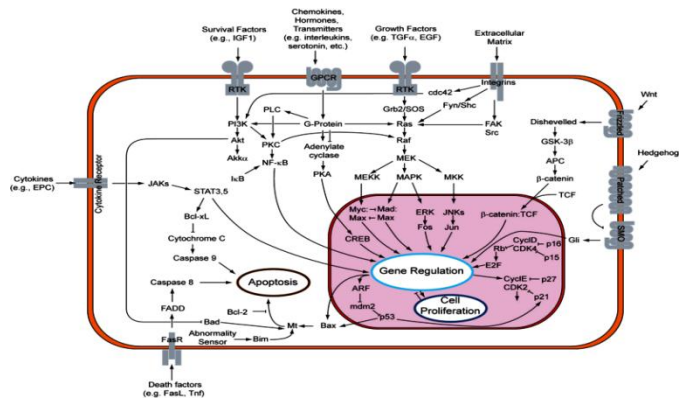

Figure 3: The pathways which stimulate tumour growth through COX2.

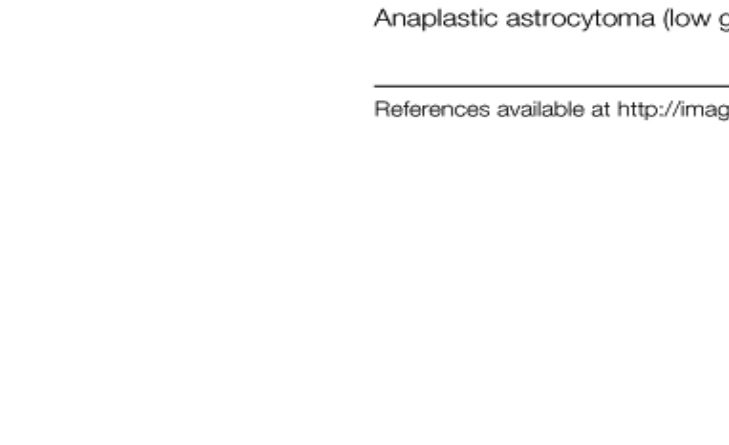

Figure 4: Increased expression of COX2 in human cancers.

Table 1: COX2 expression in malignant or premalignant human tumours.

Premalignant or malignant lesion	COX2 expression (%)
Colorectal	80–90
Gastric	80
Oesophageal	70
Hepatocellular (liver cirrhosis)	54 (81)
Pancreatic	67
Head and neck	80
Non-small-cell lung cancer	70
Breast (ductal carcinoma-in-situ)	40 (60)
Prostatic	83–93
Bladder	86
Cervix	43
Endometrial	37
Cutaneous basal cell	25
Cutaneous squamous cell	80
pPNET	100
Glioblastoma multiforme	71–74
Anaplastic astrocytoma (low grade)	44 (30)

References available at http://image.thelancet.com/extras/03oncl205webfr.pdf