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REVIEW

Role of cyclooxygenase-2 in the carcinogenesis of gastrointestinal tract cancers: A review and report of personal experience

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Abstract

Selective cyclooxygenase (COX)-2 inhibitors (coxibs) were developed as one of the anti-inflammatory drugs to avoid the various side effects of non-steroidal anti-inflammatory drugs (NSAIDs). However, coxibs also have an ability to inhibit tumor development of various kinds the same way that NSAIDs do. Many experimental studies using cell lines and animal models demonstrated an ability to prevent tumor proliferation of COX-2 inhibitors. After performing a randomized study for polyp chemoprevention study in patients with familial adenomatous polyposis (FAP), which showed that the treatment with celecoxib, one of the coxibs, significantly reduced the number of colorectal polyps in 2000, the U.S. Food and Drug Administration (FDA) immediately approved the clinical use of celecoxib for FAP patients. However, some coxibs were recently reported to increase the risk of serious cardiovascular events including heart attack and stroke. In this article we review a role of COX-2 in carcinogenesis of gastrointestinal tract, such as the esophagus, stomach and colorectum, and also analyze the prospect of coxibs for chemoprevention of gastrointestinal tract tumors.

Key words: Cyclooxygenase-2 (COX-2); Selective COX-2 inhibitors; Esophageal cancer; Gastric cancer; Colorectal cancer

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DEVELOPMENT OF SELECTIVE COX-2 INHIBITORS

The administration of non-steroidal anti-inflammatory drugs (NSAIDs), one of the most prevalent antipyretics and analgesics, is also known to reduce the risk of cancer development in the gastrointestinal tract organs including the esophagus, stomach and colorectum[1, 2]. Vane[3] indicated in 1971 that NSAIDs act upon cyclooxygenase (COX), a rate-limiting enzyme in the arachidonate metabolism. The enzyme catalyzes the biosynthesis of prostaglandin H_2, the precursor of derivatives such as prostaglandins, prostacyclin, and thromboxanes. Up to now there have been at least two isoenzymes of COX reported, COX-1 and COX-2. COX-1 is constitutively expressed in many tissues and it controls homeostasis by maintaining physiological levels of prostaglandins, while COX-2, induced by cytokines, mitogens, and growth factors, is responsible for inflammatory reactions and tumor development. Recently, COX-3, was reported to be related with pain and fever, and identified as an alternative splice of COX-1[4].

COX-2 and PGE2 play an important role in tumorigenesis from the development to invasion and metastasis of carcinoma through various mechanisms. COX-2 expression promotes cell proliferation by the activation of EGFR[5] and inhibit apoptosis by up-regulation of bcl-2[6], and suppresses host immune response[7]. Furthermore, COX-2 induces angiogenesis with VEGF and bFGF expression[8], and facilitates a metastatic potential by up-regulation of uPA and MMP-2[9, 10]. Theoretically, NSAIDs may be a candidate for chemopreventive agents against tumorigenesis by inhibiting COX-2. In fact, two large-scale randomized, double-blind trials demonstrated that aspirin, a representative of NSAIDs, could prevent colorectal adenoma[11, 12].

But the regular use of NSAIDs causes severe adverse effects including gastrointestinal bleeding, a reduction of the renal blood flow, and dysfunction of platelets because they inhibit both COX-1 and COX-2. To avoid these side effects of NSAIDs the development of selective COX-2 inhibitors was gradually aroused after the discovery of

Table 1 Selective COX-2 inhibitors (coxibs) and chemoprevention in gastrointestinal tract tumors

Generic name	Brand name	PhCo[b]	Esophagus				Stomach			Colorectum			
			Cancer cell line	CIA[c]	reflux-induced animal	Human (BE[d])	Cancer cell line	CIA[c]	MIA[e]	Cancer cell line	CIA[c]	MIA[e]	Human (FAP[f])
Tricyclic													
Celecoxib	Celebrex	Pfizer			(23)			(46,47)	(75)	(76,77)	(81,82)	(54)	(56)
MF-tricyclic	EC[a]	Merck			(21)							(53,87)	
Rofecoxib	Vioxx	Merck				(24)						(55)	
Tilmacoxib	Japan Tobacco			(20)						(78)		(88,89)	
Valdecoxib Bextra	Pfizer												
Etoricoxib Arcoxia	Merck												
Methanesulphonamide													
NS-398	EC[a]	Taisho	(18,19,70,71)				(44,45,72)		(49)	(72)	(83)		
Nimesulide	Mesulid	Helsinn					(73)	(48)			(84)	(90)	
Flosulide		Schering	(70)										
Others													
Nabumetone	Relafen	Glaxo Smith Kline									(85)	(91)	
Meloxicam	Mobic	Boehringer Ingelheim								(79,80)	(86)		
Etodolac	Lodine	Wyeth					(74)			(74)			
Lumiracoxib	Prexige	Novartis											

ECa, experimental compound; PhCob, Pharmaceutical company; CIAc, carcinogen-induced animal; BEd, Barrett's esophagus; MIAe, mutation-induced animal; FAPf, familial adenomatous polyposis; Numbers in parentheses show reference numbers

COX-2 in the early 1990s[13]. Some drugs were discovered as a result of a search for selective COX-2 inhibitors, others were revealed as being COX-2 selective after the discovery of COX-2. There are three classes of selective COX-2 inhibitors (Table 1), the first one being 1,2-diarylcyclopentenes (so-called tricyclic compounds), such as celecoxib and rofecoxib; the second one being methanesulphonamide compounds, such as NS-398 and nimesulide; and the third one being NSAIDS-derivates, such as meloxicam and etodolac. Some selective COX-2 inhibitors, which demonstrate chemopreventive effects on gastrointestinal cancers in experiments and human studies, are already commercialized as anti-inflammatory drugs, but no drug except for celecoxib is presently allowed for use in chemoprevention. In this paper we review the role of COX-2 in the carcinogenesis of gastrointestinal tract cancers and also discuss the prospect of selective COX-2 inhibitors for chemoprevention of gastrointestinal tract cancers.

COX-2 IN CARCINOGENESIS OF GASTROINTESTINAL TRACT CANCER

Esophageal cancer

Recently, the incidence of Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC) has been rapidly increasing in individuals of Western countries, particularly, among white males. The sequence of events leading from gastroesophageal reflux disease (GERD) to EAC is thought to involve the development of inflammation-stimulated hyperplasia and metaplasia, followed by multifocal dysplasia and adenocarcinoma. The up-regulation of COX-2 expression in human tissue of esophagitis, BE and EAC has been demonstrated. The incidence of COX-2 protein expression gradually increases with the development of esophageal lesions, from 75% in metaplasia, to 83% in low-grade dysplasia and up to 100% in high-grade dysplasia and EAC[14]. Combined reflux of the duodenal contents with gastric juice contributes to the development of these diseases[15] and BE patients have higher bile acid levels in the stomach than healthy controls and GERD patients without BE[16]. These observations strongly indicate that duodenal juice including bile is associated with the inflammation-metaplasia-adenocarcinoma sequence. In particular, bile acid is likely to play a pivotal role. Zhang *et al*[17] reported that COX-2 was expressed in the esophageal mucosa using a duodenogastroesophageal reflux model and bile acids, not only unconjugated but also conjugated ones, induced COX-2 mRNA, followed by COX-2 protein and PGE2 production.

The suppressive effects of a COX-2 inhibitor, NS398, on the epithelium of BE have been demonstrated in two independent *in vitro* studies[18, 19]. An increase in apoptosis and a suppression of cell proliferation are supposed to be responsible for the inhibition of cancer cells in these articles. Furthermore, some selective COX-2 inhibitors have been reported to prevent the development of esophageal cancer using *in vivo* animal models. N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats was prevented by the administration of another selective COX-2 inhibitor, JTE-522[20]. The study was carried out using a carcinogen-induced rodent model, whereas two studies have been reported using an esophageal reflux model. Buttar *et al*[21] showed the preventive effect on EAC of MF-tricyclic in a rat model of BE and EAC induced by duodenogastroesophageal reflux. In their report, MF-tricyclic prevented the development of EAC, but did not suppress the prevalence of BE. On the other hand, celecoxib suppressed not only the development of EAC, but also that of BE in our study.

We have investigated the effect of celecoxib on esophageal adenocarcinogenesis by using duodenoesophageal reflux model, established by Miwa and his colleagues [22, 23]. Male Fisher 344 rats underwent a duodenoesophageal reflux procedure and were divided into two groups. One

Table 2 Incidences of inflammatory changes, Barrett's esophagus, and adenocarcinoma in a rodent duodenoesophageal reflux model

Wk	Group	*n*	RT#	BCH$	Incidence (%) of Barrett's esophagus	Incidence (%) of Adeno-carcinom
10	Control	10	100[c]	100[c]	10	0
	Celecoxib	5	40	40	0	0
20	Control	10	100[c]	100[c]	40	0
	Celecoxib	5	40	40	20	0
30	Control	10	100[c]	100[c]	50	10
	Celecoxib	5	40	40	40	0
40	Control	19	100[c]	100[c]	89[a]	47[b]
	Celecoxib	8	38	38	25	0

RT#, Regenerative thickening; BCH$, Basal cell hyperplasia; [a]$P<0.005$ and [c]$P<0.05$, respectively, control *vs* celecoxib group, Fisher's exact test.

group was given commercial chow (control group), while the other group was given experimental chow containing celecoxib (celecoxib group). The animals were sacrificed sequentially, at 10th, 20th, 30th and finally 40th wk after surgery. In the control group, esophagitis, BE and EAC were first observed at 10th wk, 20th wk and 30th wk, respectively. Their incidences sequentially increased and at the 40th wk reached 100%, 89% and 47%, respectively. In the celecoxib group, the esophagitis was mild and the incidence of BE was significantly lower at each week ($P<0.001$), in comparison with the control group, and EAC was not identified throughout the experiment ($P<0.05$) (Table 2). COX-2 expression was up-regulated at the 10th and 20th wk ($P<0.05$, respectively) (Figure 1). PGE_2 level and proliferative activity were also up-regulated in both groups, but they were lower in the celecoxib group than in the control group ($P<0.05$) (Figures 1 and 2). Apoptosis increased after the celecoxib treatment ($P<0.05$) (Figure 2). Celecoxib thus proved to be effective for preventing reflux esophagitis, BE and EAC by suppressing PGE_2 production in a rodent model.

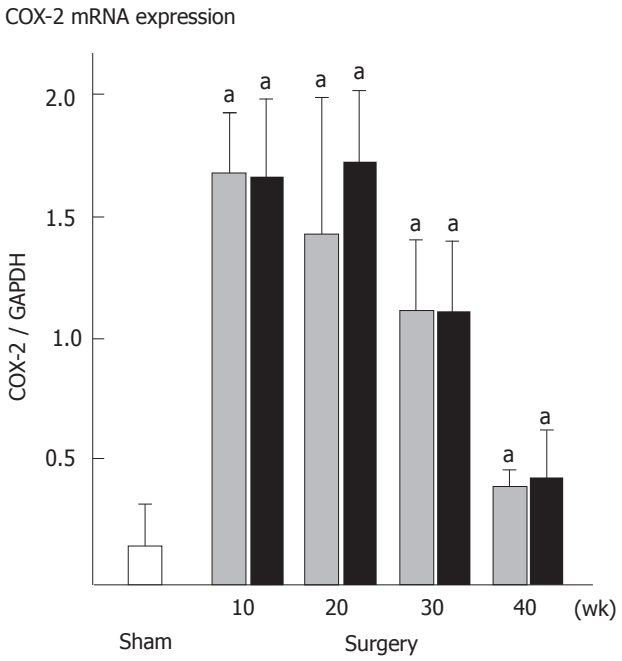
Our results showed surges of COX-2 and PGE_2 between the beginning and the 20th wk in the control group, thus suggesting that the COX-2 expression played an important role in the early phase of the esophageal carcinogenesis in the inflammation-metaplasia-adenocarcinoma sequence. The fact that the suppression of PGE_2 continued throughout the experiment in the celecoxib group may explain that celecoxib suppressed not only the development of EAC, but also that of BE. These data led to perform a clinical chemoprevention study for the patients with BE. Kaur *et al*[24] administered 25-mg/day rofecoxib to twelve patients with BE for 10 days and reported that COX-2 expression, PGE_2 contents and PCNA of epithelium of BE were 3-fold, 2-fold, and 2-fold higher than those of epithelium of normal esophagus, respectively, and all biomarkers decreased after treatment by 77%, 59%, and 62.5%, respectively. Furthermore, a Chemoprevention for Barrett's Esophagus Trial (CBET) was started in 2003 as a phase IIb, multicenter, randomized, double-masked, placebo-controlled study of celecoxib in patients with Barrett's dysplasia[25].

Gastric cancer

Though the incidence of gastric cancer has recently decreased in the United State of America and Western European countries, it is still a major cause of cancer death in many countries, such as Eastern Asia, Eastern Europe, and Latin America. Gastric cancer develops in a multistep process from normal gastric mucosa to chronic active gastritis, to gastric atrophy and intestinal metaplasia, and finally to dysplasia and cancer[26]. According to recent epidemiologic evidence, it is very likely that *Helicobactor pylori* (*H pylori*) plays an important role in this carcinogenic sequence. It is shown that *H pylori* induces COX-2 mRNA/protein levels with the production of PGE_2 in premalignant and malignant lesions[27, 28]. A chronic infection of *H pylori* causes gastritis due to COX-2, iNOS, and other cytokines, but the precise mechanism of *H. pylori* involvement in gastric carcinogenesis remains to be elucidated. Normal gastric mucosa scarcely expresses COX-2, but the expression of COX-2 increases through the multistep process of gastric carcinogenesis. Sun *et al*[29] reported the positive rates of COX-2 by immunohistochemistry in superficial gastritis, gastric atrophy, intestinal metaplasia, dysplasia, and cancer to be 10.0%, 35.7%, 37.8%, 41.7%, and 69.5%, respectively. In addition to these findings several studies have strongly suggested COX-2 expression to be a relatively early event in the sequence of gastric carcinogenesis[30, 31].

Since Ristimaki *et al*[32] first described an elevated expression of COX-2 in gastric carcinoma in 1997, numerous studies have reported the relationship between COX-2 expression and gastric cancer. According to a review article, COX-2 mRNA is up-regulated in 51% to 76% (median 73%) of the tumors by Northern blot or RT-PCR, while COX-2 protein is overexpressed in 67% to 83% (median 73%) by immunoblotting and 43% to 100% (median 62%) by immunohistochemistry[33]. The COX-2 expression is more frequent in intestinal-type than in diffuse-type gastric cancer[34-36], and it also correlates with noncardia cancer[37], tumor size[38], depth of invasion[36, 38, 39], lymph node metastasis[38-42], lymphatic invasion[41, 42], clinical stage[41-42], and angiogenesis[39, 43].

Sawaoka *et al*[44, 45] demonstrated the inhibitory effects of a COX-2 inhibitor, NS-398, on the gastric cell line expressing COX-2 (MKN45) and on its xenograft in nude mice *in vivo*. Hu *et al*[46] examined the chemopreventive effect of indomethacin and celecoxib, using a rat model. They induced gastric cancer by the administration of 100 μg/ml MNNG to Wistar rats for 40 wk and reported the incidence and the tumor multiplicity of gastric cancer of 10 mg celecoxib group to be 18.8% and 0.19, which was significantly lower than 75.0% and 1.0 of the control group, but indomethacin did not show any such preventive effect. Curiously, indomethacin strongly inhibited PGE_2 production in comparison with celecoxib. They supposed that chemopreventive effects of the celecoxib may not be mediated by the inhibition of the COX-2 activity or prostaglandins production alone and thus carried out another experiment to elucidate the cell kinetics[47]. They indicated that both drugs suppressed cell proliferation, but celecoxib



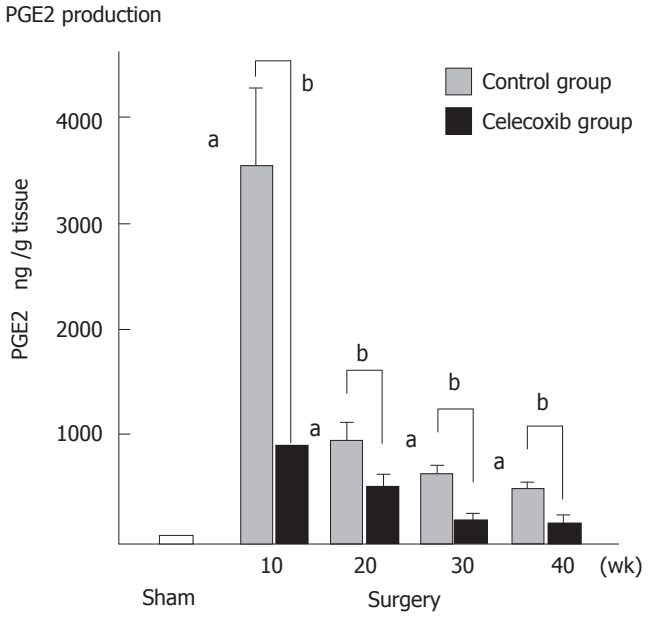
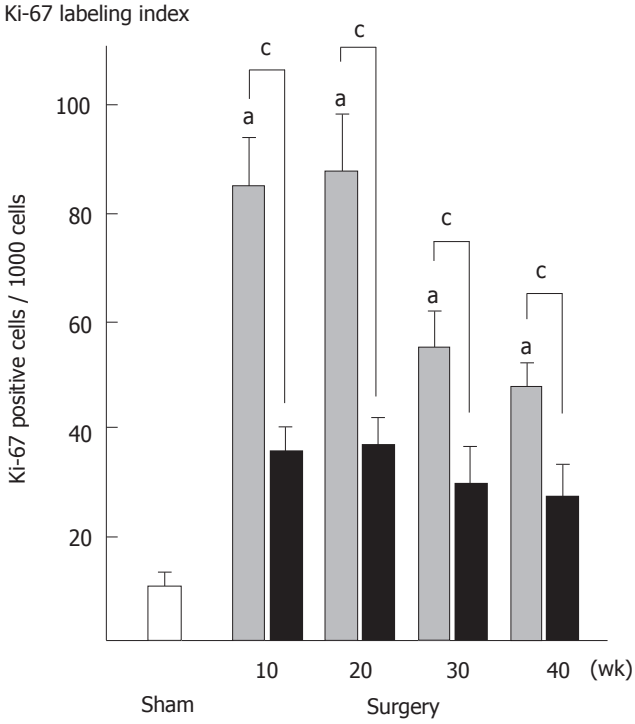


Figure 1 COX-2 mRNA expression and PGE2 production of esophageal mucosa. [a]$P < 0.005$ *vs* sham; [b]$P < 0.01$ between the two groups , Mann-Whitney U test



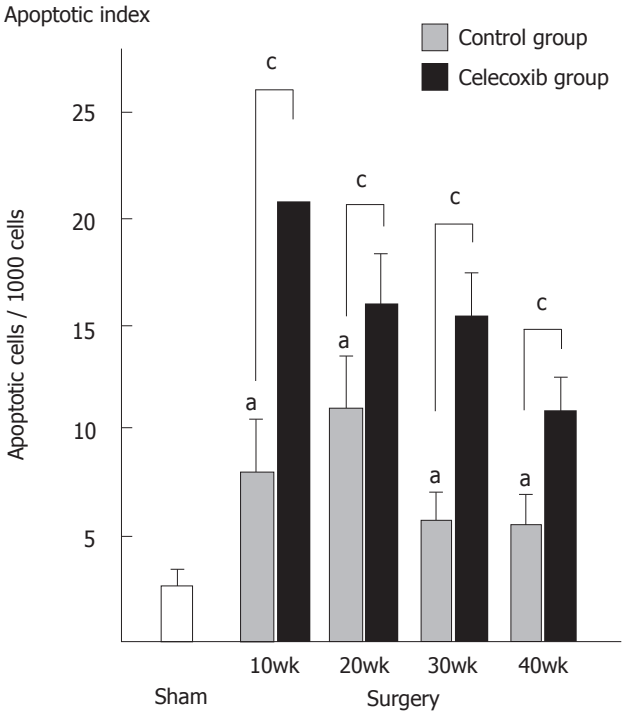


Figure 2 Cell kinetics of esophageal mucosa in a duodenoesophageal reflux model. [a]$P < 0.05$ *vs* sham; [c]$P < 0.05$ between the two groups , Mann-Whitney U test

increased the apoptosis of gastric cell in a dose-dependent manner, whereas indomethacin did not effect apoptosis, thus suggesting that celecoxib inhibits gastric carcinogenesis by the COX-2 independent pathway, such as by the inhibition of the NF-1-κB signaling pathway. Furthermore, Nam *et al*[48] examined the effect of nimesulide on gastric carcinogenesis using an N-methyl-N-nitrosourea (NMU)-induced and an *H pylori*-infected mouse model, demonstrating that gastric tumors developed in 68.8% of mice given both MNU and *H pylori*, whereas the tumor incidence in the mice receiving nimesulide in addition to MNU and *H pylori* was 27.8%.

More recently COX-2 was proven to have a strong relationship with gastric tumorigenesis in a study using transgenic mice[49]. In the transgenic model expressing both COX-2 and microsomal prostaglandin E synthase (mPGES)-1, the animals developed inflammation-associated hyperplastic gastric tumors in the proximal glandular stomach. In addition, NS-398 treatment for four weeks completely suppressed the gastric hypertrophy, thereby reducing the mucosal thickness in the same model. We previously established a rodent duodenogastric reflux model, in which gastric cancer developed for 50 to 60 wk without any chemical carcinogens[50]. We have now started an experiment to prove the chemopreventive effects of

Table 3 Chemopreventive effects of coxibs on intestinal tumors using animal models

Drug				Outcomes		Reference	
Name	Concentration	Term	Animal model	Inhibition rate (%)	*P* value	Reporter (#)	Year
carcinogen-induced rat model							
Celecoxib	1500 ppm	5-16 wk	F344 rat, AOM[a]	40 (ACF)	$P < 0.001$	Reddy *et al* (92)	1996
NS-398	1 mg/kg•bw	5-11 wk	F344 rat, AOM[a]	34 (ACF)	$P < 0.05$	Yoshimi *et al* (83)	1997
	10 mg/kg•bw			47 (ACF)	$P < 0.01$		
Celecoxib	1500 ppm	5-50 wk	F344 rat, AOM[a]	93 (colon tumor)	$P < 0.00001$	Kawamori *et al* (81)	1998
Nimesulide	200 ppm	6-30 wk	ICR mouse, AOM[a]	36 (adenocarcinoma)	NS	Fukutake *et al* (84)	1998
	400 ppm		50 (adenocarcinoma)		$P < 0.05$		
Celecoxib	500 ppm	5-58 wk	F344 rat, AOM[a]	55 (adenocarcinoma)	$P < 0.001$	Reddy *et al* (82)	2000
		1000 ppm	5-58 wk	62 (adenocarcinoma)	$P < 0.001$		
		1500 ppm	5-58 wk	77 (adenocarcinoma)	$P < 0.0001$		
		1500 ppm	22-58 wk	47 (adenocarcinoma)	$P < 0.01$		
Nabumetone	750 ppm	for 18 wk	F344 rat, AOM[a]	15 (ACF)	$P < 0.05$	Roy *et al* (85)	2001
	1500 ppm			37 (ACF)	$P < 0.01$		

to be continued

Table 3 (continued)

Apc gene mutant mouse model							
MF-tricyclic	3,5 mg/kg•d	3-11 wk	ApcΔ716	52 (intestinal polyp)	$P = 0.0037$	Oshima *et al* (53)	1996
	14 mg/kg•d			62 (intestinal polyp)	$P < 0.0001$		
Nimesulide	400 ppm	4-15 wk	ApcΔ850 (Min)	48 (intestinal polyp)	$P < 0.05$	Nakatsugi *et al* (90)	1997
Celecoxib	150 ppm	30-80 d	ApcΔ850 (Min)	29 (intestinal polyp)	$P < 0.01$	Jacoby *et al* (54)	2000
	500 ppm		29 (intestinal polyp)				
	1500 ppm		71 (intestinal polyp)				
JTE-522	0.001 (%)	4-12 wk	ApcΔ474	9 (intestinal polyp)	NS	Sasai *et al* (88)	2000
	0.01 (%)			32 (intestinal polyp)	$P < 0.05$		
Nabumetone	900 ppm	5-15 wk	ApcΔ850 (Min)	50 (small bowel polyp)	$P < 0.05$	Roy *et al* (91)	2001
				65 (large bowel polyp)	$P < 0.05$		
MF-tricyclic	13 mg/kg/d	3-7 wk	ApcΔ850 (Min) + Msh2-/-	48 (intestinal polyp)	$P < 0.001$	Lal *et al* (87)	2001
Rofecoxib	0.0025 (%)	3-11 wk	ApcΔ716	36 (intestinal polyp)		Oshima *et al* (55)	2001
	0.0075 (%)			55 (intestinal polyp)			
JTE-522	0.01 (%)	4-12 wk	ApcΔ474	49 (large adenoma)	$P < 0.01$	Sunayama *et al* (89)	2001
				-28 (small adenoma)	NS		

AOM[a], azoxymethane; Reporter (#), Name of reporter and (#) shows reference number

meloxicam on gastric tumors including gastric adenoma and adenocarcinoma using this model and have preliminarily confirmed a suppressive effect on gastric lesions (data not shown).

Colorectal cancer

Colorectal cancer is one of the most popular cancers and its incidence is increasing with high mortality rates in westernized countries. The relationship between the carcinogenesis and COX-2 is most intensively elucidated in both basic and clinical research about colorectal polyps, adenoma, and cancer. Before the discovery of COX-2, numerous studies about inhibitory effects of NSAIDs on intestinal tumorigenesis were performed using chemical carcinogen-induced animal models and *Apc* gene mutant mouse models[51, 52]. The *Apc* gene plays an important role in colon cancer development. An epoch-making paper was published by Oshima *et al*[53] in 1996 about the contribution of COX-2 to carcinogenic sequence in Wnt/Apc/Tcf pathway. They induced COX-2 mutations in $Apc^{\Delta 1716}$ knockout mice, which led to the development of numerous polyps in the intestine. In COX-2-/- $Apc^{\Delta 1716}$ and COX-2+/- $Apc^{\Delta 1716}$ mice, the number of polyps dramatically decreased by 86% and 66%, respectively, in comparison to that in the littermate COX-2+/+ $Apc^{\Delta 1716}$ mice. They also reported in the same paper that MF-tricyclic suppressed number of polyps in $Apc^{\Delta 1716}$ mice. This is the first report that COX-2 inhibitor reduced the number of intestinal polyps. Following this finding several COX-2 inhibitors have been reported to succeed in polyp reduction in knockout *Apc*

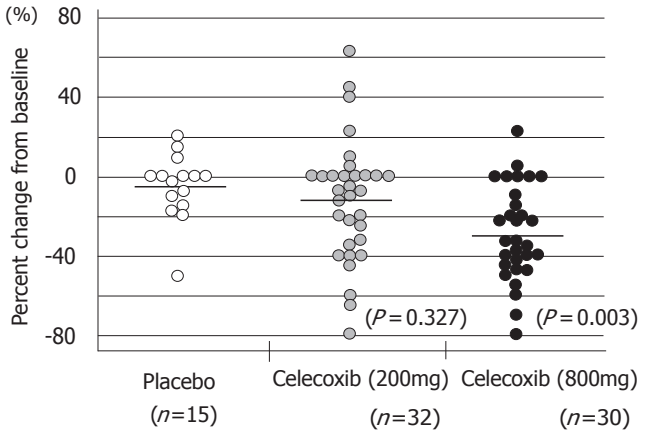


Figure 3 Percentage change from the baseline in the number of colorectal polyps in FAP patients

mice (Table 3).

Both celecoxib and refecoxib, two popular drugs as the first generation of selective COX-2 inhibitors, are now commercially available for orthopedic diseases. Both drugs have been shown to have chemopreventive effects on intestinal polyps in *Apc* mutant mouse models. Jacoby *et al*[54] performed two experiments of adenoma prevention (early phase) and regression (late phase) by celecoxib using the *Min* mice model. They showed that celecoxib decreased not only tumor size and multiplicity in the prevention study, but also caused a decrease in the size of established polyps in the regression study. In the rofecoxib study using $Apc^{\Delta 716}$ mice model, the drug successfully decreased the number and size of polyps in a dose-dependent manner[55].

The *Apc* gene mutation is also responsible for familial adenomatous polyposis (FAP). Some articles have demonstrated the chemopreventive effects of NSAIDs on colorectal polyps of FAP patients[51]. The successful outcomes of selective COX-2 inhibitors in animal models enabled us to start a clinical study of chemoprevention of FAP. Steinbach *et al*[56] of the University of Texas, Anderson Cancer Center, in Houston, reported that treatment with celecoxib significantly reduced the number of colorectal polyps in patients with FAP in 2000. I also joined this trial, which was performed as a double-blind, placebo-controlled study and was supported by a contract with the U.S. National Cancer Institute, and Searle Pharmaceuticals. All patients underwent total colonoscopy at the beginning and end of the study. All polyps observed by endoscopy were photographed and videotaped. Several members in the study group assessed the number and size of the polyps using these records in a completely blind manner. A statistical analysis was independently carried out by a biomathematician. Seventy-seven FAP patients were randomly assigned to treatment with celecoxib (100 or 400 mg twice daily) or a placebo for six months. Twice daily treatment with 400 mg celecoxib brought a 28% reduction in the number of polyps, a 100-mg dose led to an 11.9% reduction. In contrast, the polyp counts in patients who received placebo fell by only 4.5%. (Figure 3). At least a 25% reduction in polyps was experienced by 53% of the patients in the 400-mg treatment group, compared with 31% of the 100-mg group and 7% of the placebo group (Figure 4). The incidence of adverse events was similar among the groups.

Corresponding to these results, the U.S. Food and Drug Administration (FDA) immediately approved the clinical use of celecoxib for FAP patients, since it was considered to be a potentially useful adjunct to current management by suppressing polyp formation in patients with a residual rectum after colectomy and in patients with an intact colon who are awaiting a colectomy. Several years later the preventive effects on duodenal polyps in FAP patients were established by the same group[57]. Thereafter, three large trials of the chemopreventive effect on the recurrence of neoplastic polyps of the large bowel in patients with a history of colorectal adenoma have been initiated. The APPROVe (Adenomatous Polyp Prevention On Vioxx) was designed to examine the effects of treatment with rofecoxib in April 2000. The APC （Adenoma Prevention with Celebrex）cancer trial and the PreSAP（Prevention of Spontaneous Adenomatopus Polyps) cancer trial started using celecoxib in December 1999 and March 2001, respectively. Unexpectedly, all the trials now have been stopped because of an observed increased risk in cardiovascular (CV) events.

HEAD WIND AGAINST COX-2 INHIBITORS

In spite of the advances and successes of COX-2 inhibitors, recently some pharmaceutical companies have abandoned the development or marketing of such inhibitors. The Vioxx Gastrointestinal Outcomes Research Study (VIGOR study) foreshadowed a current tough situation of COX-2 inhibitors. The VIGOR study was originally designed to assess whether rofecoxib is associated with a lower incidence of clinically important upper gastrointestinal (GI) events (gastroduodenal perforation or obstruction, upper GI bleeding, and symptomatic gastroduodenal ulcers) than is naproxen, a nonselective NSAID, among 8 076 patients with rheumatoid arthritis[58]. As expected, 2.1 confirmed the incidence of GI events per 100 patient-years occurred with rofecoxib, in comparison to 4.5 per 100 patient-year with naproxen (relative risk, 0.5; $P<0.001$). However, the VIGOR study also showed the relative risk of developing a confirmed adjudicated thrombotic CV event (myocardial infarction, unstable angina, cardiac thrombus, resuscitated cardiac arrest, sudden or unexplained death, ischemic stroke, and transient ischemic attacks) with rofecoxib treatment in comparison to that with naproxen to be 2.38 ($P=0.002$). On the other hand, another similar study, the Celecoxib Long-term Arthritis Safety Study (CLASS) yielded different results[59]. The CLASS was conducted to determine whether celecoxib is associated with a lower incidence of significant upper GI toxic effects and other adverse effects in comparison with conventional NSAID, ibuprofen or diclofenac. For all 8 059 patients enrolled in the CLASS, the annualized incidence rates of upper GI ulcer complications alone and combined with symptomatic ulcers of celecoxib vs NSAIDs were 0.76% *vs* 1.45% ($P=0.09$) and 2.08% *vs* 3.54% ($P=0.02$), respectively, whereas there was no significant difference in the CV event (myocardial infarction, stroke, and death) rates between celecoxib and NSAIDs. It was later reported

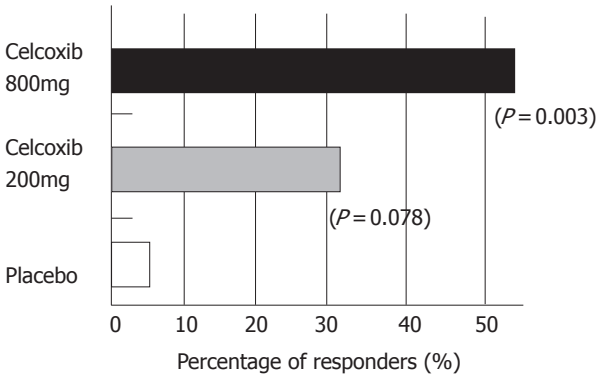


Figure 4 Percentage of responders who showed a 25% or more decrease in the mean number of colorectal polyps

that the adjusted odds ratio for myocardial infarction (MI) among celecoxib users, relative to persons who did not use NSAIDs, was 0.43 in comparison with 1.16 among rofecoxib users, and the use of rofecoxib was associated with a significantly higher odds of MI in comparison wtih the use of celecoxib (adjusted odds ratio for rofecoxib *vs* celecoxib, 2.72, $P = 0.01$) in a study comparing rofecoxib with celecoxib regarding the risk of MI incidence[60].

Merck withdrew rofecoxib from the market in September, 2004 because of an increased risk of serious CV events, including heart attack and stroke, among study patients taking rofecoxib compared to patients receiving placebo (the APPROVe). Japan Tobacco Incorporation has already declined to develop JT-522 for clinical use after phase II study in September, 2003. Regarding celecoxib, in an APC cancer trial, Pfizer demonstrated an increased CV risk over placebo, while the PreSAP cancer trial revealed no greater cardiovascular risk than the placebo. The outcomes of two trials were completely different, but Pfizer nevertheless decided to stop them. The US FDA issued a Public Health Advisory, which stated that the long-term use of NSAIDs and selective COX-2 inhibitors might increase the risk of severe CV events (myocardial infarction, strokes, *etc*) at the beginning of 2005. According to the conclusions of an advisory panel, Pfizer decided to withdraw valdecoxib from the market in April, 2005. Recently, Shaya *et al*[61] performed an observational cohort study to examine the CV risk of COX-2 inhibitors compared with nonspecific NSAIDs except naproxen in Maryland Medicaid enrollees, a high-risk population. But they did not find that COX-2 inhibitors increased CV risk over nonnaproxen NSAIDs. Whether or not selective COX-2 inhibitors really increase the risk of CV events compared with other NSAIDs remains unknown and still controversial.

COX-1 is constitutively expressed in most tissues and cells, such as the kidney, stomach, platelets, and vascular endothelium, while COX-2 expression is induced in fibroblasts, endothelial cells, monocytes, and ovarian follicles[62, 63]. Accordingly, COX-1 alone is expressed in platelets. Ironically, because the selective COX-2 inhibitors hardly suppress COX-1 inducing thromboxane A2, which activates aggregation of platelets, CV risk might be increased among the users of COX-2 inhibitors[64]. In this sense, drugs belonging to the intermediate class of COX-1/COX-2 inhibitors (moderately selective COX-2 inhibitors), such as meloxicam and etodolac, might be reassessed in the near future. But it is very beneficial for most patients that selective COX-2 inhibitors undoubtedly reduce GI disorders about in half compared with NSAIDs[58, 59]. Physicians should select COX-2 inhibitors or NSAIDs, after carefully considering which events are most important for each patient, namely GI or CV events. Recently, COX-2 inhibitors have been found to have new pharmacological advantages. Pyo *et al*[65] reported that NS-398 enhanced the effect of radiation on the COX-2 expressing cells. It was also shown that COX-2 inhibitors had a synergistic antitumor effect in combination with several chemotherapeutic agents, including gemcitabine or 5FU in pancreatic cancer[66], and paclitaxel and carboplatin in non-small-cell lung cancer[67]. Furthermore, the combination of celecoxib and an angiotensin-converting enzyme inhibitor enhanced the antitumor effect through insulin-like growth factor I receptor pathway[68] and low doses of celecoxib was useful for chemoprevention of intestinal polyps in omega-3 polyunsaturated fatty acid-rich diet [69]. These facts are very encouraging to both researchers and clinicians regarding COX-2 inhibitors, thus offering hope for their eventual use in the future.

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