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REVIEW ARTICLE

Recent Advances in Nonsteroidal Anti-Inflammatory Drugs

Shinichi Kawai[1,2], Fumiaki Kojima[2] and Natsuko Kusunoki[1]

ABSTRACT

Recent advances in basic and clinical researches of nonsteroidal anti-inflammatory drugs (NSAIDs) are reviewed. Concerning arachidonic acid cascade, recent studies revealed that not only cyclooxygenase (COX) but also terminal enzymes such as prostaglandin E synthase are very important in the understanding of the pathogenesis of inflammation and mechanisms of action of NSAIDs. We also found that some conventional NSAIDs and a selective COX-2 inhibitor exert pro-apoptotic effect on synovial fibroblasts and several cancer cells by COX-independent mechanisms. Clinical indications for use of NSAIDs are broad and include the following : rheumatic diseases ; painful and/or febrile conditions ; and prevention from thrombotic diseases such as myocardial infarction. In addition, recently developed selective COX-2 inhibitors have been found to be nearly as effective for the same conditions as conventional NSAIDs except with regard to the prevention of thrombosis. The incidence of severe gastrointestinal events in patients treated with selective COX-2 inhibitors has been proven to be lower than patients treated with conventional NSAIDs. However, there was no difference in renal complications between the two groups. Instead, an increased incidence of myocardial infarction after administration of selective COX-2 inhibitors may occur in patients with risk factors for atherosclerotic or thrombotic complications. Further basic and clinical studies remain to be investigated in the future.

KEY WORDS

apoptosis, gastrointestinal complications, myocardial infarction, prostaglandin E synthase, selective cyclooxygenase-2 inhibitors

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are drugs with activity against inflammatory symptoms but without any glucocorticoid action. In 1971, Vane[1] discovered that inhibition of cyclooxygenase (COX) was the major mechanism of action for aspirin and some other NSAIDs. COX-2, an isozyme of COX, was subsequently discovered,[2-4] and selective COX-2 inhibitors with an expected lower incidence of adverse reactions have been approved and released in recent years. This paper introduces recent advances in basic and clinical research on NSAIDs.

MECHANISMS OF ACTION OF NSAIDS

INHIBITION OF COX

As the features of COX-1 and COX-2 have already been explained in many reviews, a simple overview including our recent findings is discussed as follows. Arachidonic acid is released from the cell membrane phospholipids by phospholipase (PL) A_2. COX is a rate-limiting enzyme that metabolizes arachidonic acid to various physiologically active substances such as prostaglandins (PGs). One of the major routes of metabolism for arachidonic acid is transformation from arachidonic acid into PGG_2 by the enzymatic activity of COX, and further metabolism to PGH_2 by the peroxygenase activity of this enzyme. COX-2 is also suggested to have the same enzymatic activities as those of COX-1. NSAIDs demonstrate anti-inflammatory action by inhibiting COX, thereby inhibiting the production of inflammatory chemical mediators such as PGE_2 and PGI_2.

A part of the arachidonic acid cascade and metabolic pathways in the syntheses of PGs and thromboxane (TX) are shown in Figure 1. In recent years, cDNAs for the enzymes involved in the metabolic process after PGH_2 have been cloned and the

[1]Division of Rheumatology, Department of Internal Medicine, Toho University School of Medicine, Tokyo and [2]Institute of Medical Science, St. Marianna University School of Medicine, Kanagawa, Japan.
Correspondence: Shinichi Kawai, M.D., Ph.D., Professor of Internal Medicine, Division of Rheumatology, Toho University School of Medicine, 6–11–1 Omori-Nishi, Ota-ku, Tokyo 143–8541, Japan.
Email: skawai@med.toho-u.ac.jp
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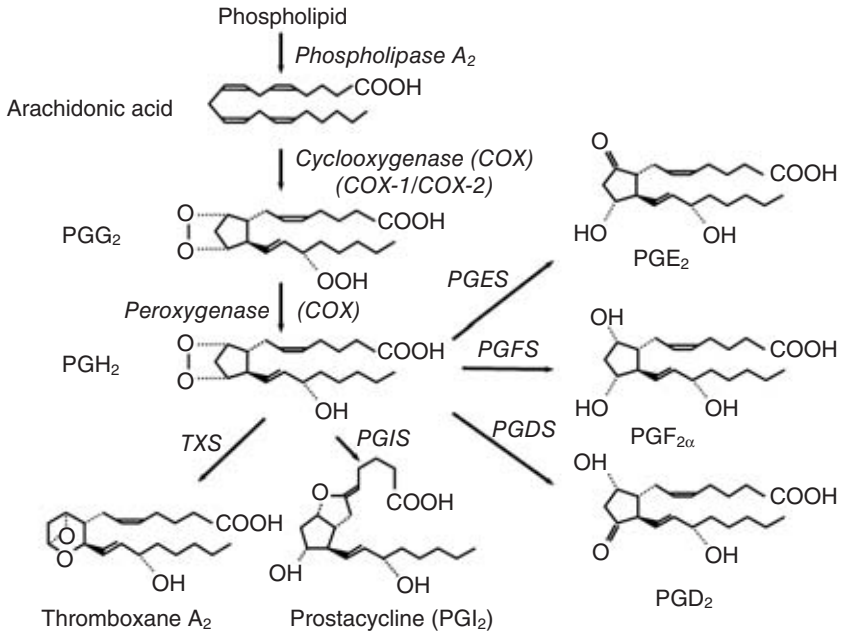

Fig. 1 A scheme of metabolic pathways from arachidonic acid to various kinds of prostaglandins (PGs) and thromboxane (TX). PGES: PGE synthase.

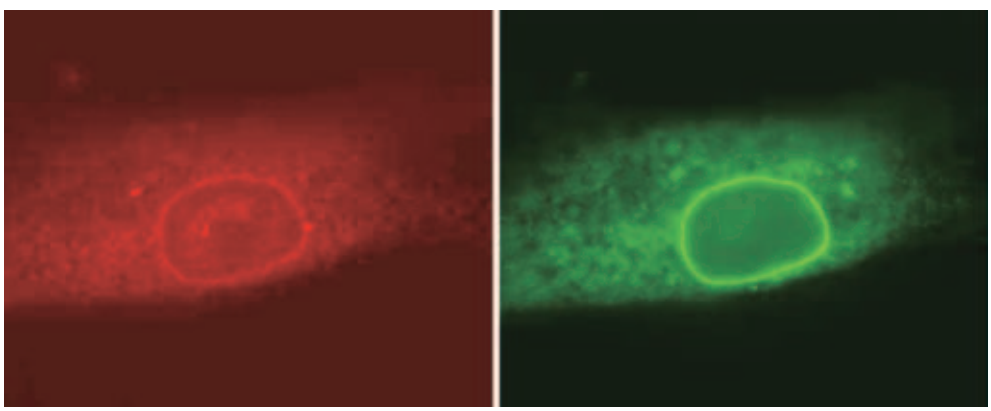

Fig. 2 Co-location of membrane-associated prostaglandin E synthase (mPGES) and cyclooxygenase (COX)-2 in interleukin-1β-stimulated rheumatoid synovial fibroblasts.

structures of the proteins have been identified.[5] These enzymes are labeled in the following manner: PGE synthase (PGES) for PGE_2; PGDS for PGD_2; PGFS for $PGF_{2\alpha}$; PGIS for PGI_2 (prostacyclin); and TXS for TX productions. Isozymes of PLA_2, COX, and the various PG or TX synthases are involved in the metabolism of arachidonic acid, and it has been found that they are closely linked with each others.[5] Recently, we showed that the expression of microsomal or membrane-associated PGES (mPGES, also recently called as mPGES-1) in the synovial fibroblasts from patients with rheumatoid arthritis (RA) were induced by stimulation of interleukin (IL) -1β.[6] COX-2 expression is also induced simultaneously, and these enzymes are co-located intracellularly (Fig. 2) and cooperate to maintain PGE_2 production. It was also found that IL-1β -induced mPGES expression in RA synovial fibroblasts is enhanced by PGE_2 itself, showing an autoregulatory action.[7] A scheme of this action is shown in Figure 3. In the near future, mPGES could be a novel target for a novel anti-inflammatory agent with a higher specific action for suppression of PGE_2 production than COX-2 inhibitors.

In 1991, COX-2 was discovered as an isozyme of COX that is expressed after stimulation with mitogens, while COX-1 is constitutively expressed by

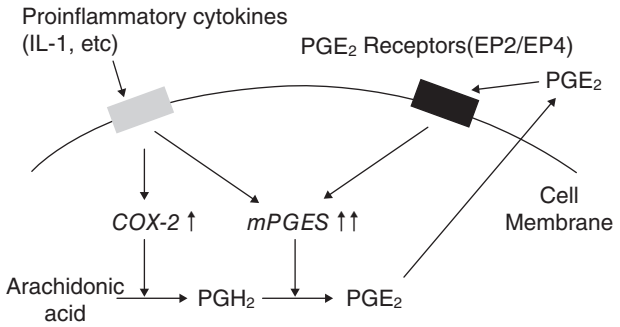

Fig. 3 A scheme of autoregulation of prostaglandin (PG) E_2 production *via* enhanced expression of membrane-associated prostaglandin E synthase (mPGES) by PGE_2 itself in proinflammatory cytokine-induced rheumatoid synovial cells.

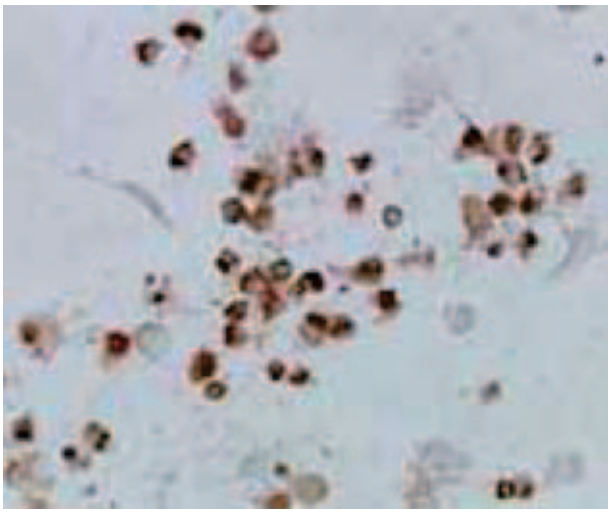

Fig. 4 Celecoxib-induced apoptosis in rheumatoid synovial fibroblasts. Apoptotic cells were detected by TUNEL assay.

many different kinds of cells. For example, TXA_2 is produced in platelets by only COX-1, and has a protective effect by promoting platelet adhesion when bleeding occurs. PGI_2 and PGE_2 are produced by COX-1 in the gastric mucosa, where they protect the gastro-intestinal mucosa by maintaining blood flow and increasing mucus secretion. In other words, the PGs that are produced by COX-1 act as host defenses. On the other hand, COX-2 expression is induced at sites of inflammation and the PGs that are produced act in the promotion of inflammation as already described above. Thus, selective COX-2 inhibitors are theoretically suggested to be NSAIDs with fewer adverse reactions.

ACTIONS OTHER THAN COX INHIBITION

The major mechanism of action of NSAIDs has been explained as COX inhibition, however there are also phenomena that cannot be explained by this effect alone. Recently, it was found that treatment with high concentrations of several conventional NSAIDs (such as indometacin and diclofenac) induce apoptosis in the synovial fibroblasts of RA patients.[8] Moreover, there was a positive correlation between PPARγ activation and pro-apoptotic action induced by these NSAIDs. We also reported that induction of apoptosis was only found in relatively lower concentrations of celecoxib incubated with the RA synovial fibroblasts (Fig. 4), whereas various other selective COX-2 inhibitors including etodolac, meloxicam, nimesulide, NS398, and rofecoxib, even with higher concentrations were not identified.[9] In this case, however, celecoxib did not stimulate PPARγ activation in these cells. Although the mechanism of the celecoxib-induced apoptosis is still unclear, in addition to acting as anti-inflammatory drug, celecoxib or some conventional NSAIDs may also act as a disease modifying anti-rheumatic drug.

COX-2 SELECTIVITY

After the establishment of the COX-2 theory, development of novel COX-2 inhibitors was rapid. Even among conventional NSAIDs, however, there exist some with considerable COX-2 selectivity, such as etodolac and meloxicam. These COX-2 inhibitors which have been marketed in Japan and other countries show a low rate of serious gastrointestinal complications.[10] On the other hand, celecoxib and rofecoxib are drugs designed with specific binding to COX-2 molecules and have already been marketed in many major foreign countries except Japan.

The method of measuring COX-2 selectivity varies widely among reports. In general, if clinical usefulness is to be considered, a method that uses human cells is most appropriate.[11] Etodolac, meloxicam, celecoxib, rofecoxib, valdecoxib and etoricoxib all show selectivity for COX-2 based on multiple reports using human cells. These drugs are clinically available as COX-2 inhibitors approved in other countries, whereas only etodolac and meloxicam have been approved in Japan. There is also an argument that so-called coxibs must be differentiated from non-coxib COX-2 inhibitors that were developed earlier. Regarding such controversy, Vane *et al.*[12] have suggested that the degree of selective inhibition of COX-2 should be the most important factor. If COX-2 selectivity is observed, drugs must be labeled as selective COX-2 inhibitors or COX-1 sparing NSAIDs. Indeed, it should be stressed that coxib is the term for a chemical structure, just like oxicam, for example. In addition, adjective terms for COX-2 inhibitors, such as preferential, selective, and specific, do not have any significant meaning.

Table 1 A comparison between large-scale clinical trials of celecoxib and rofecoxib

Clinical Trials	CLASS (Silverstein) *JAMA* 284: 1247, 2000	VIGOR (Bonbardier) *NEJM* 343: 1520, 2000
Subjects	OA・RA (*n*=8,059)	RA (*n*=8,076)
Drugs	Celecoxib (800 mg/day)	Rofecoxib (50 mg/day)
Control Drugs	Ibuprofen (2400 mg/day) Diclofenac (150 mg/day)	Naproxane (1000 mg/day)
Severe GI Complications		
Reduction Rate	50% (p=0.09)	60% (p=0.005)
・Aspirin User (20%)	equal	
・non-Aspirin (80%)	60%	
Myocardial Infarction	equal	Increased (0.4% *vs* 0.1%)

CLINICAL APPLICATIONS OF SELECTIVE COX-2 INHIBITORS

NSAIDs have a long history of use dating back to the era of herbal medicines, and their indications are extremely broad, including such conditions as rheumatic and musculoskeletal diseases, painful illnesses, fever, and prevention from thrombotic and atherosclerotic events (myocardial and cerebral infarctions, etc). In addition, indications for several kinds of tumors, Alzheimer's disease, and other conditions have recently been discussed. The clinical application of NSAIDs or COX-2 inhibitors for these indications are outlined below.

RHEUMATIC DISEASES AND MUSCULOSKELETAL DISEASES

The following is a discussion of the results of a clinical study on celecoxib that was conducted on RA patients[13] in the U.S.A. The percentages of patients with RA who achieved equal and more than 20% improvements are estimated using criteria established by American College of Rheumatology (ACR).[14] Compared with placebo, celecoxib was superior at all doses (200−800 mg/day), and the results were also either equal or superior as compared with naproxen (1,000 mg/day). Rofecoxib had an effect on RA that was almost the same as conventional NSAIDs.[15] In other words, when, compared to anti-rheumatic drugs such as methotrexate, NSAIDs even selective COX-2 inhibitors were clearly inferior.[16] In addition, most reports on the clinical efficacy of selective COX-2 inhibitors for osteoarthritis were nearly equivalent to conventional NSAIDs.

OTHER PAINFUL CONDITIONS AND FEVER

Pain is one of the most important joint symptoms in RA, but the extent to which COX-2 is involved in the manifestation of pain is unknown. In general, COX-2 has been shown to be involved in the perception of central pain, while COX-1 is involved in peripheral pain. However, a report[17] that compared selective COX-2 inhibitors (rofecoxib 50 mg and celecoxib 200 mg) with a conventional drug (ibuprofen 400 mg) for dental pain revealed that selective COX-2 inhibition achieved an analgesic effect equal or better than conventional drugs which inhibit both COX-1 and COX-2. The plasma half-life of ibuprofen is shorter than that of rofecoxib or celecoxib, and as can be clearly expected, the efficacy of each drug was consistent with changes in the plasma concentration. The mechanism for induction of systemic fever involves stimulation of the hypothalamic regions in the brain by PGE_2 via COX-2, and the antipyretic effect of selective COX-2 inhibitors was also suggested to be the same as that of conventional drugs.

ANTITHROMBOTIC AND ANTIPLATELET EFFECTS

This effect is obtained by a reduction of TXA_2 production through inhibition of COX-1, so selective COX-2 inhibitors cannot be used for this purpose. In fact, this action is recognized as an indication for low-dose aspirin only,[18] and even other conventional NSAIDs that inhibit COX-1 are not satisfactory for this purpose.[19] In contrast to other conventional NSAIDs, aspirin inhibits COX-1 irreversibly by acetylation, and a potent and prolonged effect is obtained even at low doses. With selective COX-2 inhibitors, there is the converse possibility of complications related to thrombosis as mentioned below.

PATENT DUCTUS ARTERIOSUS IN PREMATURE INFANTS

When NSAIDs are used during pregnancy, there is the risk of ductus arteriosus occlusion in the fetus, but this action can also be used to treat patent ductus arteriosus in premature infants. This indication has only been approved for the non-selective COX inhibitor, injectable indometacin, but recent experiments have shown that PGI_2 produced by COX-2 is linked to ductus arteriosus patency. There is thus the possibility that further investigations will lead to the use of selective COX-2 inhibitors for this condition.

OTHER DISEASES SUCH AS TUMORS

There are several epidemiologic studies that indicate a lower incidence of various malignant diseases in NSAID users. There are also many basic studies that have shown NSAIDs can inhibit the proliferation of various cancer cell lines *in vitro*, therefore implicating an anti-tumor effect by NSAIDs. In general, high concentrations of NSAIDs are required to produce this anti-tumor effect, and thus selective COX-2 inhibitors that are known to have few adverse drug reactions are attracting attention because higher doses could be administered.[20] In a clinical study,[21] patients with familial adenomatous polyposis received either of 2 doses of celecoxib or placebo for 6 months, and the number of colorectal polyps was significantly reduced in the group that took 400 mg of celecoxib twice daily. Recently, Sonoshita *et al.*[22] showed that PGE_2 promotes polyp formation in the intestines of mice via the EP2 receptor, suggesting that at least part of the anti-tumor effect of COX-2 inhibitors is mediated via inhibition of PGE_2. However, as previously mentioned, the induction of apoptosis[9] in RA synovial fibroblasts is specific to celecoxib. Furthermore, only celecoxib among 6 selective COX-2 inhibitors has been found to induce apoptosis of colon cancer cell lines.[23] Therefore, we suggest that this reduction of number of polyps is not only due to COX-2 inhibition but that there is a possibility of direct pro-apoptotic action also being involved.

Epidemiological studies indicate that NSAIDs decrease the risk of developing Alzheimer's disease (AD).[24] Their beneficial effects may be due to interference in the chronic inflammatory reaction that takes place in AD. So far, however, clinical trials designed to inhibit inflammation or COX-2 activity by conventional NSAIDs or selective COX-2 inhibitors have failed in the treatment of AD patients.

ADVERSE DRUGS REACTIONS OF SELECTIVE COX-2 INHIBITORS

GASTROINTESTINAL COMPLICATIONS

Several clinical studies have shown that the incidence of severe gastrointestinal complications is lower for etodolac and meloxicam when compared with conventional NSAIDs.[10] However, since the development of celecoxib and rofecoxib were initially designed as COX-2 specific inhibitors, there has been progress in clinical research, and more rigorously conducted studies are being published.

First, a clinical study on celecoxib in which short-term gastrointestinal safety data were determined by endoscopy showed that the incidence of gastroduodenal ulcers after 12 weeks of treatment (even at the maximum dose of 800 mg/day) was not different from that in the placebo group.[13] Next, the CLASS and VIGOR studies on celecoxib[25] and rofecoxib[26], respectively, were reported almost simultaneously. These were both large-scale randomized controlled studies that enrolled 8,000 or more patients (Table 1). In the CLASS study, serious gastrointestinal complications were decreased by approximately 50% in the celecoxib group compared with the reference ibuprofen or diclofenac groups, although there was no significant difference. In the VIGOR study, however, serious gastrointestinal complications were significantly decreased by approximately 60% in the rofecoxib group compared with the naproxen group. In the CLASS study, 20% of the patients were treated concomitantly with low-dose aspirin for the prevention of myocardial infarction. When only the patients on concomitant aspirin therapy were compared, there was no difference between the two groups. However, the decrease in the rate of serious gastrointestinal complications among patients without concomitant treatment with aspirin was the same as in the VIGOR study.

COX-2 has been detected in experimental gastric ulcer tissue of rats. Since COX-2 is involved in ulcer healing, administration of selective COX-2 inhibitors to rats after ulceration was reported to cause prolonged ulcer healing.[27] It was also suggested by analysis of the CLASS data that selective COX-2 inhibitors do not necessarily reduce gastrointestinal complications compared with conventional drugs.[28] However, if the various clinical studies conducted to date are compiled, it seems that selective COX-2 inhibitors caused a lower rate of gastrointestinal complications as a whole when compared with conventional NSAIDs.

RENAL IMPAIRMENT

In a clinical study on RA patients, the same percentages of edema were observed in the celecoxib and naproxen groups.[13] There were no cases of edema in the placebo group, which suggested that selective COX-2 inhibitor also caused renal blood flow impairment and accumulation of sodium as found in other conventional drugs. COX-2 is constitutionally expressed by some renal cells,[29] and this could be a factor in renal impairment. In addition to such mild impairment, serious renal impairment due to selective COX-2 inhibitors has also been reported.[30] This is another topic for further investigation.

MYOCARDIAL INFARCTION AND OTHER THROMBOTIC DISEASES

Although the VIGOR and CLASS studies revealed a decrease in serious gastrointestinal damage side effects, there was also evidence warning of new adverse drug reactions associated with selective COX-2 inhibitors. The incidence of myocardial infarction was significantly increased by approximately 4-fold in the rofecoxib group compared with the naproxen group in the VIGOR study (Table 1). Further analysis of the CLASS study[31] has also revealed that ischemic heart disease was increased with the use of celecoxib, but

there were many contrary objections for this report because this analysis was conducted using comparison with placebo of other clinical trials. However, occurrence of thrombosis after treatment with celecoxib has been reported[32] in 4 patients with connective tissue diseases including anti-phospholipid antibody syndrome.

PGI_2 production in patients with atherosclerosis is not only via COX-1, but also via COX-2.[33] In other words, because COX-2 inhibitors reduce PGI_2 production by inhibition of COX-2 in the vascular epithelium without inhibiting COX-1 in platelets, there is the possibility that thrombosis may be augmented by use of any kinds of selective COX-2 inhibitors compared with conventional NSAIDs. Whelton *et al.*[34] reported that rofecoxib increased systolic blood pressure in aged patients with osteoarthritis more than that of users of celecoxib.

In the end of September 2004, Merck announced withdrawal of rofecoxib from the market throughout the world. This decision was due to the result of the increased incidence of myocardial infarction in rofecoxib group when compared with placebo group in the long-term clinical study to see the prevention of polyps. Safety of celecoxib and other selective COX-2 inhibitors, or even conventional NSAIDs are just under discussion. Although further detailed studies are needed, it seems to be better for patients who are predisposed to thrombosis to avoid use of selective COX-2 inhibitors.

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