# Selective Cyclooxygenase-2 Inhibition: Biological and Clinical Effects

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Until three decades ago various hypotheses had been proposed to explain the mode of action of non-steroidal anti-inflammatory drugs, i.e., mithochondrial oxidative phosphorylation, uncoupling protein synthesis, or inhibition of various enzymes. However, no unifying and convincing theory was formulated.

The common mode of action of this group of drugs was described for the first time by Piper and Vane in 1969 [1]: they demonstrated that the release of a prostaglandin (thromboxane$A_2$) during induction of anaphylaxis in isolated guinea pigs lungs was inhibited by aspirin. This evidence, together with the observation that aspirin and other NSAIDs inhibit PG release in various tissues, led the investigators to formulate the hypothesis that these drugs' therapeutic effects were based on prostaglandin biosynthesis inhibition [2].

## Mechanism of action

In 1971 Sir John Vane [2] demonstrated that aspirin and other NSAIDs block PG synthesis by inhibiting a single enzyme, cyclooxygenase, that catalyzes the first step of the pathway that produces PG starting from arachidonic acid [3]. It is a bifunctional enzyme with two distinct catalytic sites: cyclooxygenase and peroxidase. At the cyclooxygenase site arachidonic acid is converted to $PGG_2$, an unstable product, which undergoes peroxidation to $PGH_2$ at the peroxidase site. $PGH_2$ is another unstable product that can be processed into stable and biologically active eicosaonoids – such as $PGE_2$, $PGI_2$ (prostacyclin), $PGF_2$ and $TXA_2$ – by different PG synthetases [3].

Although most tissues are able to synthesize $PGG_2$ and $PGH_2$, the peculiar PG produced by a given cell type may vary in different tissues depending on the different specific PG synthetase present in that cell. For example, lung and spleen synthesize the whole series of enzymes, while platelets contain only the thromboxane-synthetase, and endothelial cells present mainly the prostacyclin-synthetase.

In healthy individuals, prostaglandins play a number of different roles in homeostatic and physiological processes, including gastrointestinal mucosa protection, renal and vascular homeostasis, uterine function, embryo implantation and labor, and regulation of both the sleep-wake cycle and body temperature [4]. On the other hand, $PGE_2$ is one of the main mediators of the inflammatory response and lowers nociceptor thresholds, being also a pyretic agent [2].

## COX-1 and COX-2

Until 1991, when the two COX isoforms were discovered, it was believed that COX was constitutively expressed in different tissues at fairly constant levels and that PG synthesis increased during inflammation due to a higher release of the precursor, arachidonic acid [5]. In 1990 it was reported that bacterial lipopolysaccharide induced human monocytes to increase COX activity without affecting phospholipase functions [6]. Moreover, glucocorticoid dexamethasone could block LPS-induced PG release completely by inhibiting the induction of COX expression in monocytes without interfering with basal PG biosynthesis [6]. Thus, the existence of at least two isoforms of COX was suggested, one of which could be induced by LPS and inhibited by glucocorticoids.

The second COX isoform, COX-2, was characterized for the first time in 1991, 20 years after the discovery of NSAIDs' mechanism of action. It was postulated that the differences between COX-1 and COX-2 could have functional and therapeutic implications. The difference between the two isoforms is evident at their gene level: COX-1 promoter region has the characteristics of a "housekeeping" gene, i.e., one that is constitutively expressed [2]. In contrast, COX-2 promoter contains TATA and CAAT elements, common in highly regulated genes, particularly in those involved in inflammation. In addition, it has been reported that in some human cell types, nuclear factor kB is an important mediator of COX-2 transcription [7]. Moreover,

NSAIDs = non-steroidal anti-inflammatory drugs
PG = prostaglandin

COX = cyclooxygenase
LPS = lipopolysaccaride

COX-2 expression is also post-transcriptionally regulated, for example by interleukin-1 [8].

COX-1 and COX-2 are both homodimeric and share a 60% homology in the amino acid sequence. Each dimer has three independent folding units: an epidermal growth factor-like domain, a membrane binding domain, and an enzymatic domain where the cyclooxygenase and the peroxidase sites are separated but adjacent. Both isoforms are membrane associated and integrate into the lipid bilayer internal leaflet. The proximity with cell membranes makes the interaction with the substrate (arachidonic acid) possible [5].

## Expression of COX-1 and COX-2

Tissue expression studies performed with northern blot, immunohistochemistry and *in situ* hybridization established that COX-1 is a constitutively expressed enzyme, present in virtually all tissues and cell types except red blood cells [4]. COX-1 is involved in the production of PGs, which are responsible for physiological and homeostatic functions. COX-1 concentrations remain fairly constant although the enzyme can be up-regulated two to fourfold by different growth factors and hormones [4,9].

One of the main functions of COX-1 is to maintain the integrity of gastric and duodenal mucosa [10]. This cytoprotective property is carried through $PGI_2$ vasodilator action at the gastric and duodenal level. All NSAIDs are capable of inhibiting COX-1 [3].

In contrast to COX-1, COX-2 is a predominantly inducible enzyme that is barely, if at all, expressed in most cells under basal conditions [6,11]. COX-2 expression can be up-regulated 20-fold by various stimuli related to cell damage or inflammation [Table 1]. Macrophages, monocytes, synoviocytes, chondrocytes, fibroblasts and endothelial cells dramatically increase COX-2 synthesis in response to inflammatory stimuli such as LPS, cytokines and growth factors [3]. COX-2 mRNA up-regulation has been detected in synoviocytes, monocytes and endothelial cells of patients with rheumatoid arthritis and osteoarthritis but not in synovial tissue from healthy subjects [12]. COX-2 up-regulation has also been observed in the lung during inflammatory responses, in the brain during febrile episodes, or related to ischemic damage, seizures and pain [3,11,13]. Moreover COX-2 is up-regulated in gastrointestinal tract inflammation, as in response to *Helicobacter pylori*-induced ulcer in the stomach or in inflammatory bowel diseases [5].

Although COX-2 physiological benefits are yet to be established, some physiological roles have recently been suggested. COX-2 has been detected in the thick ascending limb of the loop of Henle and in the macula densa [11]. At this site, by stimulating renin release, COX-2 could play a part in hydroelectrolytic balance and arterial pressure regulation [13]. COX-2 is also structurally expressed in the cortex, the hippocampus, the female reproductive tract, the adult male rat vas deferens, and the pancreatic islet where, under basal conditions, is the dominant isoform [11,13].

**Table 1.** Summary of the structure, distribution and regulation of COX-1 and COX-2

| | COX-1 | COX-2 |
|---|---|---|
| cDNA | Chromosome 9; 22 kB | Chromosome 1; 8.3 kB |
| mRNA | 2.8 kB | 4.5 kB |
| Protein | 72 KDa: 599 amino acids | 72 KDa: 604 amino acids |
| Homology | -60% | |
| Regulation | Predominantly constitutive: increased 2 to-4-fold by inflammatory stimuli | Predominantly inducible (10 to-20-fold) |
| Tissue expression | Most tissues, but particularly platelets, stomach, kidney | Induced by inflammatory stimuli and mitogens in macrophages, monocytes, synoviocytes, chondrocytes, fibroblasts, endothelial cells. Constitutive in certain organs (CNS, macula densa, testes) |

Adapted from Brooks et al. [4]

Various experimental findings have demonstrated a role for COX-2 in tumor pathogenesis, especially in colorectal cancer [11]. Epidemiological studies have shown a decreased risk for colorectal cancer in subjects taking NSAIDs regularly [13]. Recently, an overexpression of COX-2 and COX-2 mRNA was demonstrated in colon adenomas and carcinomas. In an experimental model on mice prone to develop colonic polyps similar to those associated with human familial polyposis, anti-COX-2 specific inhibitors appeared to reduce adenomas [14].

NSAIDs block COX-1 and COX-2 about halfway down the channel by hydrogen bonding to an arginine at position 120. COX-2 active site differs from COX-1 since valine replaces isoleucine at position 523. This replacement leads to the formation of a side pocket that represents the most significant difference between the two isoforms [5]. Thus, the binding site in COX-2 is 25% larger than in COX-1: compounds able to occupy the secondary pocket and too big to enter COX-1 channel may be COX-2 selective inhibitors.

## NSAID and gastrointestinal toxicity

NSAID therapy is associated with several adverse effects: gastrointestinal toxicity, platelet function impairment with increased risk of bleeding, bronchospasm, and hydrosaline retention. Evidence shows that NSAIDs block both COX-1 and COX-2. COX-1 inhibition, with the ensuing lower production of PG involved in tissue homeostasis, is apparently accountable for most side effects, especially gastrotoxicity.

NSAIDs are one of the most prescribed class of drugs all over the world. They expose patients to the risk of gastrointestinal toxicity, ranging from mild dyspepsia to

mucosal erosions and peptic ulcers, that can be complicated by bleeding, perforation and strictures. Less common side effects are esophagitis, small bowel ulcerations, small bowel strictures, diverticular disease, and exacerbation of chronic inflammatory bowel disease [15].

In the United States, NSAIDs are responsible yearly for at least 103,000 cases of hospitalization due to serious gastrointestinal complications among patients with rheumatoid arthritis and osteoarthritis. Among these patients, 16,500 died as the result of NSAID-related gastrointestinal adverse effects, thus representing the 15th most common cause of death in that country [16]. Gastrointestinal toxicity is mainly related to NSAIDs' systemic rather than topical effects [17]. In fact, the use of enteric-coated preparations, by parenteral or rectal administration, failed to prevent the development of ulcers.

Topical damage is related to NSAIDs' weak acidic property. In the highly acid gastric lumen, these drugs remain in the non-ionized lipophilic form, which favors their migration through the gastric mucosa. Subsequently, they enter the surface epithelial cells where they can be dissociated into the ionized form accountable for cell damage and necrosis [17]. Moreover, topical injury can be caused by an indirect mechanism mediated by the biliary excretion of active NSAID metabolites and their subsequent reflux in the gastric lumen. However, the main mechanism implicated in NSAIDs' gastrointestinal injury is related to COX-1 mediated PG synthesis inhibition, leading to the impairment of mucosal resistance to endogenous and exogenous noxious factors [17]. The main PG-related mucosal protective factors are represented by normal blood flow, mucus and bicarbonate production and epithelial proliferation.

After describing the second COX isoform, pharmaceutical researches focused their attention on the characterization of compounds that could selectively inhibit COX-2 (considered responsible for clinical manifestations during inflammatory responses), sparing COX-1, essential for gastrointestinal integrity and other homeostatic functions.

## COX isoform selectivity

Several *in vitro* assays have been proposed to evaluate COX-1 and COX-2 relative NSAID inhibition. Selectivity towards these two isoenzymes is expressed in terms of concentration required to inhibit 50% of COX activity (IC50). A large degree of variability in COX-1/COX-2 IC50 ratio has been observed depending on the different experimental conditions used [4]. In 1994 Patrignani et al. [18] developed a more physiological assay where human whole blood was used to assess COX-1 and COX-2 inhibition both *in vitro* and *ex vivo*. This assay was proposed by the International Consensus Meeting on the Mode of Action of COX-2 Inhibition (ICMMAC) as the best currently available assay for COX-isoform selectivity evaluation [4].

In human whole blood, COX-2-mediated production of LPS-induced $PGE_2$ is measured in heparinized samples after 24 hours of incubation in the presence or absence of the testing drug. COX-1 inhibition is tested by the parallel measurement of seric TXB2 after 60 minutes of blood clotting [18]. Using this assay, conventional NSAIDs and new compounds can be grouped into four categories [19]: selective COX-1 inhibitors, non-selective COX-1 inhibitors, relative selective COX-2 inhibitors, and high selective (specific) COX-2 inhibitors [Table 2]. A specific COX-2 inhibitor is a drug that, by using human whole-blood assay, inhibits COX-2 but not COX-1 activity across the entire therapeutic dose range. So far, two specific COX-2 inhibitors, celecoxib and rofecoxib, have been approved for marketing by the Food and Drug Administration.

**Table 2.** COX isoform selectivity assessed in whole-blood assays *in vitro* by COX inhibitors

| Inhibitor | COX-1/COX-2 $IC_{50}$ ratio | |
|---|---|---|
| Aspirin | 0.01 | |
| S-Indobufen | 0.043 | Selective COX-1 inhibitors |
| Valeryl salicylate | <0.240 | |
| Ibuprofen | 0.50 | |
| Naproxen | 0.56 | |
| S-Ketoprofen | 0.61 | |
| Flurbiprofen | 1.00 | Non-selective COX inhibitors |
| Sodium salicylate | 1.03 | |
| 6-MNA* | 1.49 | |
| Indomethacin | 1.90 | |
| Piroxicam | 3.12 | |
| Meloxicam | 11.16 | |
| Nimesulide | 17.69 | Relatively selective COX-2 inhibitors |
| Diclofenac | 18.90 | |
| SC-58125** | 143.30 | Highly selective (specific) COX-2 inhibitors |
| Rofecoxib | 410.00 | |

Adapted from P. Patrignani [19]
* 6-MNA is the active metabolite of nabumetone
** SC-58125 is the prototype of celecoxib

### • Rofecoxib

Rofecoxib (Vioxx, Merck & Co., USA) MK-966; 4-[4-(methylsulfonil)phenyl]-3-phenyl-(5H)-furanone, is a compound that selectively inhibits COX-2 activity in a dose-dependent manner and does not show any significant inhibition of COX-1 activity when administered in single doses of up to 1,000 mg or in multiple doses of 25–375 mg daily for 14 days [20]. It inhibits COX-2 with a 410-fold selectivity compared to COX-1 [19]. When directly compared to other NSAIDs, rofecoxib shows a minimal COX-1 isoform inhibition (IC50=5–9%) [21].

Rofecoxib was approved by the FDA in May 1999 for the relief of signs and symptoms of osteoarthritis, the management of acute pain in adults, and the treatment of primary dysmenorrhea. For osteoarthritis the recommended starting dosage is 12.5 mg up to a maximum of 25 mg once daily; 50

FDA = Food and Drug Administration

mg for a maximum of 5 consecutive days results in effective control of acute pain or primary dysmenorrhea [22]. Rofecoxib 50 mg showed analgesic properties in post-surgical dental pain similar to ibuprofen 400 mg and superior to placebo [23].

In a 6 week double-blind randomized placebo-controlled multicenter trial [24], 25 mg and 125 mg rofecoxib were administered to determine the efficacy of specific COX-2 inhibition in 219 patients with osteoarthritis of the knee. Rofecoxib, compared to placebo, at dosages of 25 mg and 125 mg once daily induced symptom relief, including pain, and improved physical functions. Improvement with both doses of rofecoxib was already evident at week 1 and sustained through week 6.

In a 6 week double-blind study performed on 809 patients with osteoarthritis of the knee or hip, rofecoxib (12.5–25 mg/day) showed an efficacy clinically comparable with ibuprofen (800 mg three times daily) and significantly greater than placebo [25]. A one-year randomized double-blind active comparator-controlled trial [26] was performed to compare the clinical efficacy of rofecoxib (25 and 50 mg daily) to that of diclofenac (150 mg daily) in the treatment of osteoarthritis of the knee or hip. Of 784 patients enrolled, 448 completed the study. Both doses of rofecoxib demonstrated an efficacy comparable to that of diclofenac. In all three treatment groups response was seen within 2 weeks and sustained at a consistent level for up to 1 year of treatment. Discontinuation of therapy due to lack of efficacy or adverse events was similar among the three groups.

An 8 week double-blind placebo-controlled trial [27] demonstrated rofecoxib's efficacy in patients with rheumatoid arthritis. In this study 658 patients were enrolled and randomized to placebo or rofecoxib 5, 25 and 50 mg daily. Patients were stratified based on concurrent methotrexate use. Efficacy and tolerability were evaluated after 2, 4 and 8 weeks of treatment. At doses of 25 and 50 mg rofecoxib induced significant clinical improvement compared with placebo. Patients who were taking 25 and 50 mg respectively achieved an American College of Rheumatology 20 response of 43.9% and 49.7%. Clinical efficacy was evident at week 2 and sustained throughout the 8 week trial. Rofecoxib showed the same efficacy and safety in methotrexate users as in non-users.

Based on the hypothesis that COX-2 specific inhibition might be associated with a lower incidence of endoscopic gastroduodenal damage, a study was performed where high dosages of rofecoxib were compared to conventional dosages of ibuprofen and aspirin as well as placebo [28]. In this trial 170 healthy volunteers were randomized to rofecoxib 250 mg, ibuprofen 2,400 mg, aspirin 2,600 mg and placebo. Upper gastrointestinal endoscopy was performed at baseline and after 8 days. In patients who took rofecoxib and placebo, erosion and ulcerations were significantly lower than in patients taking the other two NSAIDs ($P<0.001$).

Another two studies, in the United States [29] and Europe [30], were performed to confirm the hypothesis that COX-2 specific inhibition was associated with a lower gastroduodenal injury compared to equally effective doses of ibuprofen. In these trials, 742 and 775 patients were enrolled in the USA and Europe respectively and randomized to rofecoxib 25 mg or 50 mg once daily, ibuprofen 800 mg 3 times daily, or placebo. Endoscopy was performed at baseline and at 6, 12 and 24 weeks. The incidence of ulcers and erosions with both doses of rofecoxib was similar to that observed with placebo and significantly lower than with ibuprofen. By measuring fecal red blood cell loss and intestinal permeability, two double-blind studies designed to assess gastrointestinal injury demonstrated the safety of 25 mg and 50 mg rofecoxib compared to other NSAIDs [5]. Other rofecoxib-associated side effects were upper respiratory tract infections, diarrhea, headache, nausea and lower extremity edema [29].

### • Celecoxib

Celecoxib (Celebrex™, G.D. Searle & Co., Pfizer); SC-58635; 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-y] benzenesulfonamide, selectively inhibits COX-2 activity. The prototype of celecoxib (SC-58125) inhibits COX-2 with a 143.3-fold selectivity as compared to COX-1 [19].

Celecoxib's marked selectivity is confirmed by its inability to affect $TXB_2$ production in platelets, a measure of COX-1 activity, even at supratherapeutic doses of 1,200 mg/day [31]. Celecoxib is quickly absorbed and reaches peak plasma concentrations 2–3 hours after administration. High fat meals can delay absorption by about an hour. Plasma protein binding is about 97% at therapeutic plasma concentrations and half-life elimination is at about 8–12 hours [32]. Less than 1% of the dose is found unmodified in urine.

In December 1998, the FDA approved celecoxib for the symptomatic treatment of osteoarthritis and rheumatoid arthritis. A phase II placebo-controlled trial [33] that evaluated 293 patients with osteoarthritis for 2 weeks and 330 patients with rheumatoid arthritis for 4 weeks demonstrated the analgesic and anti-inflammatory efficacy of celecoxib at dosages of 100–200 mg/twice daily and 200–400 mg/twice daily respectively.

In a perspective randomized double-blind 12 week trial [34], based on the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), celecoxib (100–200 mg b.i.d.) proved to be statistically comparable to naproxen (500 mg b.i.d.) and superior to placebo in improving the functional status of 1,003 patients with symptomatic osteoarthritis of the knee.

A randomized double-blind 6 month trial in 655 patients with rheumatoid arthritis [35] showed that celecoxib (200 mg b.i.d.) had a clinical efficacy similar to that of diclofenac (75 mg b.i.d.). Another randomized multicenter placebo-controlled double-blind 12 weeks trial in 1,149 patients with rheumatoid arthritis [36] demonstrated that at doses of 100–200–400 mg twice daily, celecoxib showed an analgesic and

anti-inflammatory effect comparable to that of naproxen 500 mg twice daily.

Moreover, for post-surgical dental pain celecoxib (200 mg as a single dose) showed analgesic properties superior to placebo ($P<0.05$) for total pain relief, time to perceptible pain relief and time to use of rescue medication [23].

Gastrointestinal tract tolerability was tested in 128 healthy subjects randomized into 32 groups to receive placebo, celecoxib (100 or 200 mg b.i.d.) or naproxen (500 mg b.i.d.) for 6½ days. Scheduled endoscopic evaluations revealed the presence of at least one gastric ulcer in 19% of the naproxen-treated group, while no ulcers were detected in any of the subjects receiving either placebo or celecoxib [33].

In the study conducted by Emery et al. [35] involving 655 patients with rheumatoid arthritis treated with celecoxib 200 mg/twice a day or diclofenac 75 mg/twice a day for 24 weeks, gastroduodenal safety was evaluated by upper endoscopy in 430 patients. Gastroduodenal ulcers were detected in 4% of celecoxib-treated subjects and in 15% of subjects receiving diclofenac ($P<0.001$). Moreover, the withdrawal rate for any gastrointestinal-related adverse event was 3 times higher in the diclofenac-treated group (16%) than in the celecoxib-treated group (6%) ($P<0.001$). The most frequently reported adverse events were diarrhea (12% for celecoxib vs. 14% for diclofenac), abdominal pain (11% vs. 21%), dyspepsia (10% vs. 13%) and headache (9% vs. 6%), reaching a statistically significant difference for abdominal pain only.

Simon and co-workers [36] published the results of a 12 week endoscopic trial on rheumatoid arthritis patients treated with placebo, celecoxib (100, 200, 400 mg b.i.d.) or naproxene (500 mg b.i.d.). The incidence of endoscopically detected gastroduodenal ulcers in placebo-treated patients was 4 of 99 (4%), and the incidence across all dosages of celecoxib was not significantly different ($P>0.40$): 9 of 148 (6%) with 100 mg twice a day, 6 of 145 (4%) with 200 mg twice a day, and 8 of 130 (6%) with 400 mg intake twice a day. No statistically significant difference was observed among the groups ($P>0.40$). On the contrary, the incidence of gastroduodenal ulcer was 36 of 137 patients (26%) treated with naproxene, significantly higher than that observed with either placebo or celecoxib ($P<0.001$).

Goldstein et al. [37] pooled the results of 14 controlled randomized double-blind trials involving 11,008 patients with osteoarthritis and rheumatoid arthritis (2–24 weeks of treatment), and in a separate analysis reported a long-term open label trial (5,155 patients treated for up to 2 years) to assess the efficacy and safety of celecoxib (25–400 b.i.d.) compared with different NSAIDS – naproxen (500 mg b.i.d.), diclofenac (50–75 b.i.d.) and ibuprofen (800 t.i.d.). The results of this study showed a statistically significant reduction of the absolute risk for severe gastrointestinal complications (bleeding, perforation, or obstruction) in the celecoxib-treated patients compared with traditional NSAID-treated patients. No difference in the risk incidence was found between celecoxib and placebo-treated subjects. Unlike COX-1, COX-2 is overexpressed in human colorectal cancer. Celecoxib showed a chemopreventive action in carcinogen-induced colon tumorigenesis [14] and in nude mice implanted with a transformed human colon cancer cell line (HCA-7).

In a double-blind placebo-controlled study [38], 77 patients with familial adenomatous polyposis were randomized to receive celecoxib (100–400 mg twice a day) or placebo for 6 months. Patients treated with 400 mg twice a day showed a 28% reduction in the mean number of colorectal polyps ($P=0.003$) and a 30.7% in the polyp burden (the sum of polyp diameters) ($P=0.001$) as compared to placebo. Recently, the FDA approved celecoxib as the first drug treatment for familial adenomatous polyposis.

Harris et al. [39] demonstrated, in 7,12-dimethyl-benz(a)anthracene induced mammary carcinogenesis in female Sprague-Dawley rats, a reduction in the incidence (68%), multiplicity (86%) and volume of tumors (81%) ascribable to celecoxib. The results differed statistically with placebo ($P<0.001$) and were of greater amplitude than with ibuprofen.

## COX-2 inhibitors and renal function

NSAIDs can affect renal function in many different ways. Most commonly observed effects include a decline in renal perfusion, glomerular filtration rate, and potassium and sodium excretion, which may result in weight gain, peripheral edema and anti-hypertensive therapy impairment [40].

A randomized double-blind 2 week study [41] was performed in 36 healthy subjects to assess the renal effects of rofecoxib 50 mg once daily versus indomethacin 50 mg three times daily, and placebo. A transient but consistent decline in urinary sodium excretion was observed with both rofecoxib and indomethacin during the first 72 hours of treatment, while GFR was reduced by indomethacin but non-significantly affected by rofecoxib.

Swan and colleagues [42] conducted a single-dose cross-over study and a randomized parallel-group multiple-dose study to determinate the effect of rofecoxib on renal function in elderly patients (60–80 years of age). In the first phase of the study 15 patients were randomized to receive a single dose of rofecoxib (250 mg), indomethacin (75 mg) or placebo. In the second phase, multiple doses of rofecoxib (12.5 or 25 mg daily), indomethacin (50 mg 3 times daily), or placebo were administered to 60 patients. All patients received a low sodium diet. The results showed that both single doses of rofecoxib and indomethacin decreased the GFR by 0.23 and 0.18 ml/sec respectively. Rofecoxib, administered in multiple doses of 12, 5 and 25 mg daily, decreased GFR by 0.14 and 0.13 ml/sec while indomethacin decreased GFR by 0.10 ml/sec. In this study the effects of

GFR = glomerular filtration rate

rofecoxib on renal function were similar to those observed with the non-selective NSAID indomethacin.

Celecoxib's effects on renal function were evaluated in 29 healthy elderly subjects in a double-blind randomized crossover trial [43]. The study demonstrated a lower decrease in the GFR with celecoxib (200–400 b.i.d.) compared to naproxen (500 mg b.i.d.), which became statistically significant on day 6 ($P=0.004$). Similar effects of the two drugs were observed in reducing sodium, PGE2 and 6-keto-prostaglandin F1α urinary excretion.

A randomized double-blind 1 week trial on 40 normotensive salt-depleted subjects compared the renal effects of celecoxib (200 or 400 b.i.d.) with naproxen (500 mg b.i.d.) and placebo [44]. Both drugs decreased urine output as well as sodium, lithium and potassium excretion on day 1.

Evidence suggests that COX-2 inhibitors may impair renal function and cause sodium retention especially in patients with mild pre-existing renal failure and in elderly subjects [45]. Therefore, the same degree of caution followed with conventional NSAIDs should be used with COX-2 inhibitors.

## References


1. Piper PJ, Vane JR. Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature* 1969;223:29–35.
2. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* 1971;231:235–7.
3. Vane J. Differential inhibition of cyclooxygenase isoforms. An explanation of the action of NSAIDs. *J Clin Rheum* 1998;4:S3–10.
4. Brooks P, Emery P, Evans JF, Fenner H, Hawkey CJ, Patrono C, Smolen J, Breedveld F, Day R, Dougados M, Ehrich EW, Gijon-Baños J, Kvien TK, Van Rijswijk MH, Warner T, Zeidler H. Interpreting the clinical significance of the differential inhibition of cyclooxygenase-1 and cyclooxygenase-2. *Rheumatology* 1999;38:779–88.
5. Hawkey CJ. COX-2 inhibitors. *Lancet* 1999;353:307–14.
6. Masferrer JL, Zweifel BS, Seibert K, Needelman P. Selective regulation of cellular cyclooxygenase by dexamethasone and endotoxin in mice. *J Clin Invest* 1990;86:1375–9.
7. Crofford LJ, Tan B, Mc Carthy CJ, Hla T. Involvement of nuclear factor KB in the regulation of cyclooxygenase-2 expression by interleukin-1 in rheumatoid synoviocytes. *Arthritis Rheum* 1997;40:226–36.
8. Ristimaki A, Garfinkel S, Wessendorf J, Maciag T, Hla T. Induction of cyclooxygenase-2 by interleukin-1 alpha: evidence for post-transcriptional regulation. *J Biol Chem* 1994;269:11769–75.
9. Vane JR, Botting RM. New insights into the mode of action of anti-inflammatory drugs. *Inflamm Res* 1995;44:1–10.
10. Whittle BJR, Higgs GA, Eakins KE, Moncada S, Vane JR. Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. *Nature* 1980;284:271–3.
11. Lipsky PE. The clinical potential of cyclooxygenase-2-specific inhibitors. *Am J Med* 1999;106:51–7S.
12. Kang RY, Freire.Moar J, Sigal E, Chu CQ. Expression of cyclooxygenase-2 in human and animal model of rheumatoid arthritis. *Br J Rheumatol* 1996;34:309–22.
13. Crofford LJ, Lipsky PE, Brooks P, Abramson SB, Simon LS, Van de Putte LBA. Basic biology and clinical application of cyclooxygenase-2 inhibitors. *Arthritis Rheum* 2000;43:4–13.
14. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxigenase-2 inhibitor, against colon carcinogenesis. *Cancer Res* 1998;58:409–12.
15. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1999;340:1888–99.
16. Singh G, Triadafilopulos G. Epidemiology of NSAIDs-induced GI complications [Abstract]. *J Rheumatol* 1999;40(Suppl):593.
17. Schoen RT, Vender RJ. Mechanism of nonsteroidal anti-inflammatory drug-induced gastric damage. *Am J Med* 1989;86:449–58.
18. Patrignani P, Panara MR, Greco A, Fusco O, Natoli C, Iacobelli S, Cipollone I, Ganci A, Creminon C, Maclouf T, et al. Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. *J Pharmacol Exp Ther* 1994;271:1705–12.
19. Patrignani P. Non steroidal anti-inflammatory drugs, COX-2 and colorectal cancer. *Toxicol Lett* 2000;112–113:493–98.
20. Scott LJ, Lamb HM. Rofecoxib. *Drugs* 1999;58:505.
21. Schwartz JI, Van Hecken A, DeLepeleire I. Comparative inhibitory activity of rofecoxib (MK-966™), naproxen and COX-2 vs COX-1 in healthy female volounteers [abstract no. 857]. In XIV European League Against Rheumatism Congress: 6–11 June 1999, Glasgow.
22. Morrison BW, Daniels SE, Koley P, Cantu N, Seideberg B. Rofecoxib, a specific cyclooxygenase 2 inhibitor, in primary dysmenohrrea: a randomized controlled trial. *Obstet Gynecol* 1999;31:504–8.
23. Malmstrom K, Daniels S, Kotey P, Seidenberg BC, Desjardin PJ. Comparison of Rofecoxib and Celecoxib, two cyclooxygenase-2 inhibitors, in postoperative dental pain: a randomized, placebo- and active comparator-controlled clinical trial. *Clin Ther* 1999;21:1653–63.
24. Ehrich EW, Schnitzer TJ, McIlwain H, Levy R, Wolfe F, Weisman M, Zeng Q, Morrison B, Bolognese J, Seidenberg B, Gertz BJ. Effect of specific COX-2 inhibition in osteoarthritis of the knee: a 6 week double blind, placebo controlled pilot study of rofecoxib. *J Rheumatol* 1999;26:2438–47.
25. Day R, Morrison B, Luza A, Castaneda O, Strusberg A, Nahir M, Helgetveit KB, Kress B, Daniels B, Bolognese J, Krupa D, Seideberg B, Ehrich E. A randomized trial of the efficacy and tolerability of the COX-2 inhibitor rofecoxib vs ibuprofen in patients with osteoarthritis: Rofecoxib/Ibuprofen Comparator Study Group. *Arch Intern Med* 2000;160:1781–7.
26. Cannon GW, Caldwell JR, Holt P, Mc Lean B, Seidenberg B, Bolognese J, Ehrich E, Mukhopadhyay S, Daniels B. Rofecoxib, a specific inhibitor of cyclooxygenase-2, with clinical efficacy comparable with that of diclofenac sodium. *Arthritis Rheum* 2000; 43:978–87.
27. Schnitzer TJ, Truitt K, Fleischmann R, Dalgin P, Block J, Zeng Q, Bolognese J, Seidenberg B, Ehrich EW. The safety profile, tolerability, and effective dose range of rofecoxib in the treatment of rheumatoid arthritis. *Clin Ther* 1999;21:1688–702.
28. Lanza FL, Rack MF, Simon TJ, Quan H, Bolognese JA, Hoover ME, Wilson FR, Harper SE. Specific inhibition of cycloxygenase-2 with MK-0966 is associated with less gastroduodenal damage than either aspirin and ibuprofen. *Aliment Pharmacol Ther* 1999;13:761–7.
29. Laine L, Harper S, Simon T, Bath R, Johanson J, Schwartz H, Stern S, Bolognese J. A randomized trial comparing the effect of rofecoxib, a cyclooxygenase 2-specific inhibitor, with that of ibuprofen on the gastroduodenal mucosa of patients with osteoarthritis. *Gastroenterology* 1999;117:776–83.
30. Hawkey C, Laine L, Simon T, Beaulieu A, Maldonado-Cocco J, Acevedo E, Shahane A, Quan H, Bolognese J, Mortensen E. Comparison of the effect of rofecoxib (a cyclooxygenase 2 inhibitor), ibuprofen and placebo on the gastroduodenal mucosa of patients with osteoarthritis. *Arthritis Rheum* 2000;43:370–7.
31. Leese PT, Hubbard RC, Karim A, Isakson PC, Yu SS, Geis GS. Effects of celecoxib, a novel cyclooxygenase-2 inhibitor, on platelet function in healthy adults: a randomized, controlled trial. *J Clin Pharmacol* 2000;40:124–32.
32. Clemett D, Goa KL. Celecoxib. A review of its use in osteoarthritis, rheumatoid arthritis and acute pain. *Drugs* 2000;59:957–80.
33. Simon LS, Lanza FL, Lipsky PE, Hubbard RC, Talwalker S, Schwartz BD, Isakson PC, Geis GS. Preliminary study of the safety and efficacy of SC-58635, a novel cyclooxygenase 2 inhibitor. Efficacy and safety in two placebo-controlled trials in osteoarthritis and rheumatoid arthritis, and studies of gastrointestinal and platelet effects. *Arthritis Rheum* 1998;41:1591–602.
34. Zhao SZ, McMillen JI, Markenson JA, Dedhiya SD, Zhao WW, Osterhaus JT, Yu SS. Evaluation of the functional status aspects of health-related quality of life of patients with osteoarthritis treated with Celecoxib. *Pharmacotherapy* 1999;19:1269–78.
35. Emery P, Zeidler H, Kvien TK, Guslandi M, Naudin R, Stead H, Verburg KM, Isakson PC, Hubbard RC, Geis GS. Celecoxib versus diclofenac in

long-term management of rheumatoid arthritis: a randomised double-blind comparison. *Lancet* 1999;354:2106–11.

36. Simon LS, Weaver AL, Graham DY, Kivitz AJ, Lipsky PE, Hubbard RC, Isakson PC, Verburg KM, Yu SS, Zhao WW, Geis GS. Anti-inflammatory and upper gastrointestinal effects of celecoxib in rheumathoid arthritis: a randomized control trial. *JAMA* 1999;282:1921–8.
37. Goldstein JL, Silverstein FE, Agrawal NM, Hubbard RC, Kaiser J, Maurath CJ, Verburg KM, Geis GS. Reduced risk of upper gastrointestinal ulcer complications with celecoxib, a novel COX-2 inhibitor. *Am J Gastroenterol* 2000;95:1681–90
38. Steinbach G, Lynch PM, Phillips RKS, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su L, Levin B. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000;342:1946–52.
39. Harris RE, Alshafie GA, Abou-Issa H, Seibert K. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. *Cancer Res* 2000;60:2101–3.
40. Brater DC. Effects of nonsteroidal anti-inflammatory drugs on renal function: focus on cyclooxygenase-2-selective inhibition. *Am J Med* 1999;107(6A):65–71S.
41. Catella-Lawson F, Mcadam B, Morrison BW, Kapoor S, Kujubu D, Antes L, Lasseter KC, Quan H, Gertz BJ, Fitzgerald GA. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoid. *J Pharmacol Exp Ther* 1999;289:735–41.
42. Swan SK, Rudy DW, Lasseter KC, Ryan CF, Buechel KL, Lambrecht LJ, Pinto MB, Dilzer SC, Obrda O, Sundblad KJ, Gumbs CP, Ebel DL, Quan H, Larson PJ, Schwartz JL, Musliner TA, Gertz BJ, Brater DC, Yao SL. Effect of cyclooxygenase-2 inhibition on renal function in elderly persons receiving a low-salt diet. A randomized, controlled trial. *Ann Intern Med* 2000;133:1–9.
43. Whelton A, Schulman G, Wallermark C, Drower EJ, Isakson PC, Verburg KM, Geis GS. Effects of celecoxib and naproxen on renal function in the elderly. *Arch Intern Med* 2000;160:1465–70.
44. Rossat J, Maillard M, Nussberger J, Brunner HR. Renal effect of selective cyclooxygenase-2 inhibition in normotensive salt-depleted subjects. *Clin Pharmacol Ther* 1999;66:76–84.
45. Stubanus M, Riegger GAJ, Kammerl MC, Fischeredere M, Kramer BK. Renal side-effects of cyclooxygenase-type-2 inhibitor use. *Lancet* 2000;355:753

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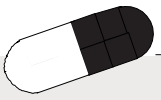
*I do not object to people looking at their watches when I am speaking. But I strongly object when they start shaking them to make sure they are still going.*

*Normal Birkett, English barrister and judge (1883–1962)*

## *Capsule*



# Physical activity and risk of stroke in women

Persuasive evidence has demonstrated that increased physical activity is associated with substantial reduction in risk of coronary heart disease. However, the role of physical activity in the prevention of stroke is less well established.

Hu et al. tried to examine the association between physical activity and risk of total stroke and stroke subtypes in women. The study group comprised 72,488 female nurses aged 40 to 65 who did not have diagnosed cardiovascular disease or cancer at baseline in 1986 and who completed detailed physical activity questionnaires in 1986, 1988, and 1992. The Main Outcome Measure was incident stroke occurring between baseline and 1 June 1994, compared among quintiles of physical activity level as measured by metabolic equivalent tasks (METs) in hours per week. During 8 years (560,087 person-years) of follow-up, the researchers documented 407 incident cases of stroke (258 ischemic strokes, 67 subarachnoid hemorrhages, 42 intracerebral hemorrhages, and 40 strokes of unknown type). In multivariate analyses controlling for age, body mass index, history of hypertension, and other covariates, increasing physical activity was strongly inversely associated with risk of total stroke. Relative risks (RRs) in the lowest to highest MET quintiles were 1.00, 0.98, 0.82, 0.74, and 0.66. The inverse gradient was seen primarily for ischemic stroke. Physical activity was not significantly associated with subarachnoid hemorrhage or intracerebral hemorrhage. After multivariate adjustment, walking was associated with reduced risk of total stroke (RRs across increasing walking MET quintiles, 1.00, 0.76, 0.78, 0.70, and 0.66; *P* for trend = .01) and ischemic stroke (RRs across increasing walking MET quintiles, 1.00, 0.77,0.75, 0.69, and 0.60; *P* for trend .02). Brisk or striding walking pace was associated with lower risk of total and ischemic stroke compared with average or casual pace.

These data indicate that physical activity, including moderate-intensity *exercise such as walking*, is associated with substantial reduction in risk of total and ischemic stroke in a dose-response manner.

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