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Pharmacology

Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: The human pharmacology of a selective inhibitor of COX-2

(prostaglandins/platelets/monocytes/ibuprofen/celecoxib)

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ABSTRACT **Prostaglandins (PG) are synthesized by two isoforms of the enzyme PG G/H synthase [cyclooxygenase (COX)]. To examine selectivity of tolerated doses of an inhibitor of the inducible COX-2 in humans, we examined the effects of celecoxib on indices of COX-1-dependent platelet thromboxane (Tx) A_2 and on systemic biosynthesis of prostacyclin *in vivo*. Volunteers received doses of 100, 400, or 800 mg of celecoxib or 800 mg of a nonselective inhibitor, ibuprofen. Ibuprofen, but not celecoxib, significantly inhibited TxA_2-dependent aggregation, induced *ex vivo* by arachidonic acid (83 ± 11% vs. 11.9 ± 2.2%; $P < 0.005$) and by collagen. Neither agent altered aggregation induced by thromboxane mimetic, U46619. Ibuprofen reduced serum TxB_2 (−95 ± 2% vs. −6.9 ± 4.2%; $P < 0.001$) and urinary excretion of the major Tx metabolite, 11-dehydro TxB_2 (−70 ± 9.9% vs. −20.3 ± 5.3%; $P < 0.05$) when compared with placebo. Despite a failure to suppress TxA_2-dependant platelet aggregation, celecoxib had a modest but significant inhibitory effect on serum TxB_2 4 hr after dosing. By contrast, both ibuprofen and celecoxib suppressed a biochemical index of COX-2 activity (endotoxin induced PGE_2 in whole blood *ex vivo*) to a comparable degree (−93.3 ± 2% vs. −83 ± 6.1%). There was no significant difference between the doses of celecoxib on COX-2 inhibition. Celecoxib and ibuprofen suppressed urinary excretion of the prostacyclin metabolite 2,3 dinor 6-keto $PGF_{1\alpha}$. These data suggest that (*i*) platelet COX-1-dependent aggregation is not inhibited by up to 800 mg of celecoxib; (*ii*) comparable COX-2 inhibition is attained by celecoxib (100–800 mg) and ibuprofen (800 mg) after acute dosing; and (*iii*) COX-2 is a major source of systemic prostacyclin biosynthesis in healthy humans.**

Prostaglandins (PGs) are autacoidal lipid mediators of importance in physiological responses, inflammation and thrombosis (1). They are formed from arachidonic acid by the catalytic activity of prostaglandin G/H synthase, also known colloquially as cyclooxygenase (COX) (2). This rate-limiting, committed step in the formation of prostaglandins results in the formation of an unstable endoperoxide intermediate, PGH_2. In turn, PGH_2 serves as substrate for cell-specific isomerases and synthases to produce the prostaglandins PGE_2, PGD_2, prostacyclin (PGI_2), and thromboxane (Tx) A_2 (3, 4). It is now recognized that there are two related but distinct gene products that possess COX activity, termed COX-1 and COX-2 (5–7). COX-1 is expressed constitutively in most tissues (8). It is thought to release prostaglandins involved in cellular "house-keeping" functions, such as the maintenance of gastrointestinal integrity and vascular homeostasis (9). COX-2 is undetectable in most tissues in the absence of stimulation but is induced as an intermediate–early gene in a limited repertoire of cells, notably in monocytes, macrophages, neutrophils, and endothelial cells (10–12). Among the stimulants of COX-2 induction are bacterial lipopolysaccharides (LPS), growth factors, cytokines, and phorbol esters (13–16). Increased expression of COX-2, but not of COX-1, has been demonstrated in rheumatoid synovial tissues *in vivo* (17). Conversely, expression of this isoform is inhibited by glucocorticoids and by the anti-inflammatory cytokines interleukin 10 and interleukin 4 (18–20). These observations have led to the hypothesis that COX-2 expression mediates the enhanced prostanoid release, which characterizes the inflammatory response (21). COX-2 also has been implicated in tumorigenesis (22).

Inhibition of prostaglandin synthesis is thought to account for both the therapeutic and adverse effects associated with the administration of conventional nonsteroidal antiinflammatory drugs (NSAIDs) (23, 24). These compounds do not discriminate markedly between the two isozymes. For example, their IC_{50} selectivity ratios for COX-2/COX-1 *in vitro* tend to be <10 (25, 26). It is thought that the therapeutic effects of these agents are related to the inhibition of COX-2 at sites of inflammation whereas the adverse gastrointestinal effects and bleeding associated with NSAIDs are attributed to inhibition of COX-1 in gastric epithelium and in platelets, respectively (27). Although both COX isoforms have similar catalytic activities and conserve critical amino acid residues, they differ particularly in the topography of the substrate binding pocket at the active site (28). Thus, it has been possible to develop small molecules that differ radically in their potencies for inhibition of COX-2 vs. COX-1 *in vitro* (29). Celecoxib is highly selective for COX-2 *in vitro* (30), with a selectivity ratio of >375 in a baculovirus expression system (31). However, it is unclear how usefully such *in vitro* assay systems may predict selectivity for COX-2 in humans.

TxA_2 is the major product of platelet COX-1 (5, 32), and inhibition of serum TxB_2, its inactive hydrolysis product, reflects inhibition of this isoform *ex vivo.* Actual Tx synthesis *in vivo* is reflected by urinary Tx metabolite excretion (33). Monocytes and macrophages express COX-2 when induced by LPS *in vitro* (34), producing PGE_2 and TxA_2 as major products in a time-dependant manner. Inhibition of COX-1 with aspirin followed by stimulation of whole blood with LPS results in COX-2-dependant formation of PGE_2 (35). Thus, inhibition of PGE_2 formation may be used to reflect COX-2 inhibition *ex vivo*. We report that celecoxib and ibuprofen potently and

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Abbreviations: PG, prostaglandin; COX, cyclooxygenase; LPS, lipopolysaccharide; Tx, thromboxane; NSAID, nonsteroidal antiinflammatory drugs.

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reversibly inhibit COX-2 *ex vivo* in volunteers. Although ibuprofen inhibits COX-1 and COX-2 to a comparable degree, inhibition of COX-1 is much less pronounced, although detectable, with celecoxib. Unlike the conventional NSAID, celecoxib did not inhibit platelet function but suppressed excretion of both the hydration product, 6-keto $PGF_{1\alpha}$, and the major urinary metabolite of prostacyclin, 2,3 dinor-6-keto $PGF_{1\alpha}$ (PGI-M).

METHODS

Study Design. The study protocol was approved by the Institutional Review Board of the University of Pennsylvania Health System (Philadelphia) and by the Advisory Council of the General Clinical Research Center (University of Pennsylvania, Philadelphia). All volunteers were healthy individuals who refrained from smoking and using any medication for 2 weeks before the start of the study. Volunteers with a history of coagulation disorders, a bleeding tendency, drug allergy, or gastrointestinal disorders were excluded from participation in the study.

Subjects. Thirty-seven subjects, 22 females and 15 males, aged from 21–49 years (median age 28), were randomized under double blind conditions to receive a single dose of celecoxib (100, 400, or 800 mg), 800 mg of ibuprofen, or placebo. There were seven subjects in each group. The drugs were administered between 0700 and 0800 hr on the study morning. Volunteers remained in the General Clinical Research Center for the duration of the study. Routine hematology, biochemistry, and urinalysis were assessed at baseline and at 24 hr after administration of the drugs on completion of the study.

Platelet Aggregation. Platelets were harvested as described (36). In brief, platelet-rich plasma was prepared by centrifugation of citrated blood sample at $160 \times g$ for 10 min followed by centrifugation of platelet-poor plasma at $900 \times g$ for 10 min at room temperature. Platelet number was adjusted to 2×10^8 platelets/ml with platelet-poor plasma. Platelet aggregation was assessed at 37°C in platelet-rich plasma by using a Biodata PAP-4 Aggregometer (Biodata, Hatboro, PA). Platelet responses to three agonists, collagen (0.5 and 2 μg/ml), arachidonate (20 M), and U46619 (4 μM), were assessed at baseline, 3, 8, and 24 hr after administration of the inhibitors. For each agonist, maximum aggregation was calculated as the percentage of maximal light transmission achieved within 5 min of addition of the agonist. Collagen and U46619 were purchased from Biodata. The arachidonic acid and all deuterated internal standards were obtained from Cayman Chemicals (Ann Arbor, MI).

Biochemical Analyses. Whole blood samples without anticoagulant were drawn at baseline and at 2, 4, 6, and 24 hr after drug ingestion for measurement of TxB_2 as described (37). Heparinized blood, drawn at baseline and at 2, 4, 6, and 24 hr after drug administration, was treated with 10 μg/ml LPS (*Escherichia coli* serotype 026:B6) after addition of aspirin (10 μg/ml) as described (35). Plasma was separated by centrifugation and was kept at −70°C until assayed for PGE_2. Aspirin and LPS *E. coli* 026:B6 were purchased from Sigma. Urine samples were saved at baseline (−2–0 hr) and at 0–2, 2–4, 4–6, 6–12, and 12–24 hr after dosing for the measurement of 11-dehydro thromboxane (Tx-M) and PGI-M, the major urinary metabolites of thromboxane and prostacyclin, respectively, indices of their systemic formation *in vivo* (38). Selected samples also were analyzed for urinary 6-keto $PGF_{2\alpha}$, an index that reflects predominantly renal biosynthesis of prostacyclin. Plasma samples were analyzed for celecoxib by HPLC. The assay involved the extraction of SC-58635 and the internal standard, SC-59751, from 30 μl of human plasma by using an Isolute Confirm 130-mg Solid Phase Extraction Column (Jones Chromatography, Lakewood, CO). The extract was analyzed by HPLC by using a 15-cm × 3.9-mm × 4-mm Novapak C18 column (Waters) equipped with a 15- × 3.2- × 7-mm RP-18 New Guard cartridge, and fluorescence detection (excitation = 240 nm, emission = 380 nm). The HPLC mobile phase was acetonitrile: 0.01 M sodium phosphate (50:50, vol/vol). The standard curve ranged from 10.0 to 5,000 ng SC-58635/ml. A 1:10 dilution quality control sample was validated. Acceptable precision (coefficient of variation) and accuracy (analytical recovery) were obtained from both between and within run studies with quality control samples in the range 10.0 to 5,000 ng SC-58635/ml. Stability through three freeze/thaw cycles also was proven. Blood hemolysis had no effect on the quantitation. A run size of 72 standards, quality controls, and unknowns was established.

GC/Mass Spectrometry Analyses. All analyses by GC/mass spectrometry were performed on a Fissons MD-800 (VG Organic, Manchester, U.K.) equipped with a split/splitless injector operated in the splitless mode at 260°C. The interface was maintained at 300°C, and the ion source was maintained at 260°C. The mass spectrometer was operated in the negative ion, chemical ionization mode, using ammonia as the reagent gas. All solvents used in sample preparation for GC/mass spectrometry were of HPLC grade and were obtained from J. T. Baker. The LK 60 silica gel plates (0.25 mm thick) were from Whatman. Analysis of prostanoids was performed as described (39–42).

Statistical Analysis. Results are expressed as mean and SEM. Statistical comparisons were made by using analysis of variance with subsequent application of Duncan's Multiple Range test, as appropriate.

RESULTS

Clinical. Celecoxib was well tolerated at all doses given. No volunteer withdrew from the study or experienced serious adverse events. There were no clinically significant changes identified in hematology, biochemistry, urinalysis, supine vital signs, or physical examination during the course of the study period.

Platelet Aggregation *ex Vivo*. As expected, ibuprofen reversibly inhibited platelet function. The aggregation response to collagen was significantly inhibited with 800 mg ibuprofen 3 hr after dosing ($P < 0.05$) but returned toward normal 8 hr after drug administration. Collagen induced aggregation showed no consistent trend with time after placebo or celecoxib administration. Neither celecoxib nor ibuprofen inhibited aggregation induced by U46619. Aggregation responses to arachidonate 20 μM were significantly inhibited at 3 [-83 ± 6.5 (ibuprofen) vs. $-2 \pm 1\%$ (placebo) expressed as percent change from baseline; $P < 0.005$] and 8 hr ($-41 \pm 15\%$ vs. $-2.2 \pm 3.4\%$; $P < 0.05$) after dosing with ibuprofen but returned to baseline 24 hr after drug administration. Celecoxib did not significantly inhibit the aggregation response to arachidonic acid (Fig. 1).

Serum TxB_2. Inhibition of serum TxB_2 was >95% inhibited between 2, 4, and 6 hr after dosing with ibuprofen ($P < 0.001$) with recovery evident at 24 hr. Although doses of celecoxib tended also to suppress serum TxB_2 to a minor degree, this change was statistically different from that observed after placebo ($-28 \pm 4.8\%$ vs. $-5.8 \pm 3.2\%$; $P < 0.05$) only with 800 mg, at 4 hr after dosing (Fig. 2*A*). There was a variable relationship between the plasma concentration of SC-58635 and the degree of inhibition of serum TxB_2. However, even at plasma concentrations of >500 ng/ml, <10% of samples exhibited >50% inhibition of serum TxB_2 (Fig. 2*B*).

Pharmacokinetics. The area under the plasma concentration-time curve ($AUC_{(0-24)}$) and the average maximal plasma and (C_{max}) concentrations of celecoxib attained in the study are shown in Table 1. The dose-response effect was nonlinear

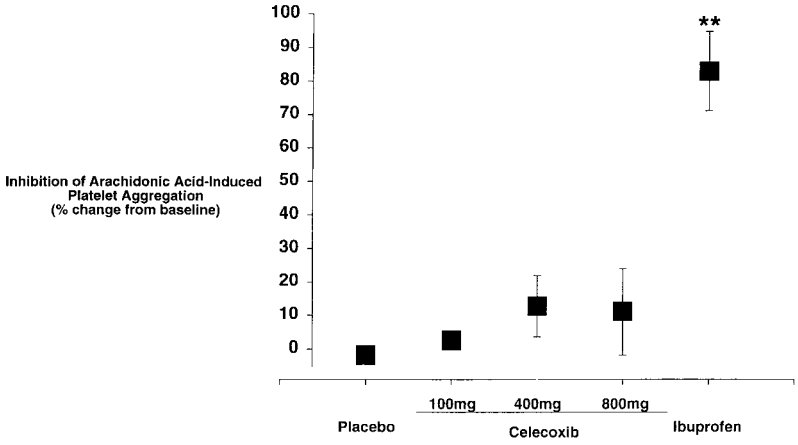

FIG. 1. Inhibition of arachidonic acid-induced platelet aggregation *ex vivo* in volunteers 3 hr after dosing with placebo, 800 mg ibuprofen, and various doses of celecoxib. **, $P < 0.01$ for comparisons with placebo.

across the range of doses administered. The median time to maximum plasma concentration (T_{max}) was 4.2 hr.

Urinary Tx-M. Ibuprofen significantly reduced urinary Tx-M; maximal effects were observed 4–6 hr (−70.2 ± 4%) and 6–12 hr (−70.4 ± 6.3%) after administration ($P < 0.05$). Suppression of Tx-M by celecoxib at the 100 mg, 400 mg, and 800 mg was not statistically different from that by placebo (−16 ± 5.3%, −28.4 ± 8%, and 43 ± 5.3% vs. −20.6 ± 9.9%), respectively (Fig. 3).

Levels of Whole Blood Monocyte COX-2 Activity. PGE_2 in stimulated whole blood varied with placebo administration, showing an average decrease of 35% compared with baseline at each time interval. Nonetheless, ibuprofen caused significantly greater inhibitory effect 2, 4, and 6 hr after treatment compared with placebo. The maximal inhibitory effect occurred at 4 hr after dosing [−93.3 ± 2% vs. −93.3 ± 2% vs. −35.9 ± 10.45 (placebo)]. Celecoxib produced similar inhibitory effects on this index of monocyte COX-2 activity, but the effect was not dose-dependent (−75.3 ± 8.6% vs. −86.3 ± 3.1 vs. −83.2 ± 6.1%; 100, 400, and 800 mg, respectively) (Fig. 4*A*). Interindividual variability of response was again evident in the relationship of plasma concentration of celecoxib to inhibition of COX-2 activity. However, in contrast to the relationship with serum TxB_2, the plasma concentration-response relationship was steeper (Fig. 4*B*). For example, at plasma concentration >500 ng/ml, 75% of samples reflected >80% inhibition of this *ex vivo* index of COX-2 activity whereas only 16% of samples exhibited 40% inhibition of COX-1 activity.

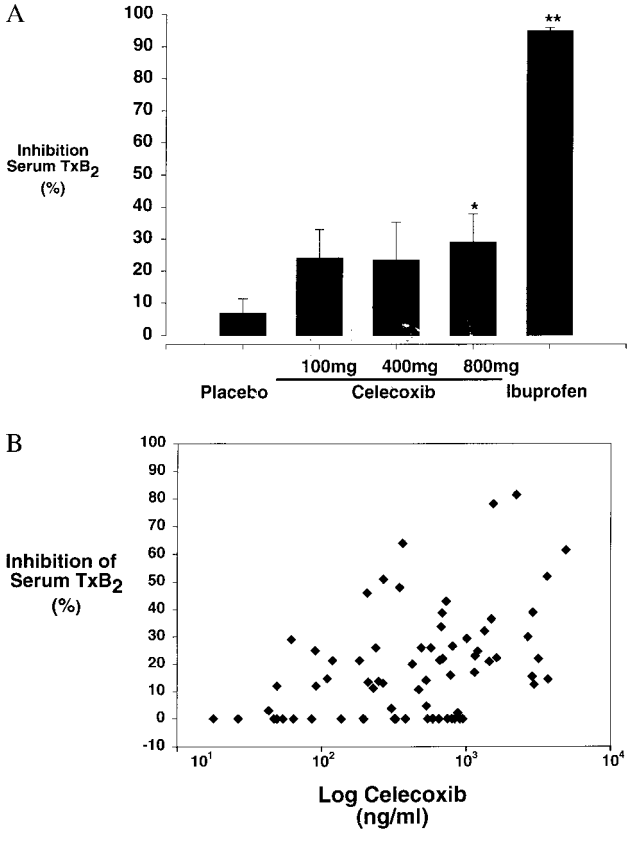

FIG. 2. (A) Inhibition of serum TxB_2, an index of COX-1 activity *ex vivo* in volunteers 4 hr after receiving placebo, 800 mg ibuprofen, or various doses of celecoxib. *, $P < 0.05$; **, $P < 0.01$ for comparisons with placebo. (*B*) The relationship between log plasma concentration of celecoxib taken 2, 4, 6, and 24 hr after dosing and inhibition of serum TxB_2 expressed as the percentage change from baseline values. A shallow but variable dose response effect is evident (see *Results*).

Urinary 2,3 dinor-6 keto $PGF_{1\alpha}$ and 6-keto $PGF_{1\alpha}$. Urinary excretion of 2,3-dinor 6-keto $PGF_{1\alpha}$, an index of systemic prostacyclin biosynthesis, remained unchanged after placebo. However, it was reduced to a similar degree by 400and 800 mg of celecoxib as well as by ibuprofen compared with placebo at both 4–6 and 6–12 hr after dosing (Table 2; $P < 0.01$). There were no significant differences between the effects of celecoxib and ibuprofen on PGI-M excretion. The effects of 800 mg celecoxib had tended to revert back toward predosing values (288 ± 82 pg/mg creatine) in the sample collected 12–24 hr after dosing with 800 mg (155.4 ± 69 pg/mg creatine) but was still significantly depressed ($P < 0.05$). Urinary 6-keto $PGF_{1\alpha}$ also was depressed by celecoxib 800 mg. Thus, predosing values (172 ± 73.4 pg/mg creatine) fell to 88.5 ± 17.4 pg/mg creatinine ($P < 0.05$) 4–6 hr after dosing, partially recovering to 109.9 ± 27.6 pg/mg creatine 12–24 hr after dosing.

DISCUSSION

The results of this study demonstrate that single doses of celecoxib, a highly selective COX-2 inhibitor *in vitro*, are well tolerated by healthy volunteers. All doses inhibited LPS-stimulated monocyte PGE_2 formation *ex vivo*, an index of COX-2 activity, to a degree that approximated that attained after 800 mg ibuprofen, a therapeutic dose of a nonselective, conventional NSAID. Although interindividual differences in response were apparent and the biochemical selectivity of Celecoxib for COX-2 was relative, rather than absolute, in humans, it did not influence TxA_2-dependant platelet aggregation *ex vivo*. Surprisingly, celecoxib and ibuprofen had comparable suppressive effects on the excretion of PGI-M. Celecoxib also suppressed urinary 6-keto $PGF_{1\alpha}$. This implies

Table 1. Pharmacokinetics of celecoxib in healthy volunteers

Dose	$AUC_{(0-24)}$, ng/hr/ml	C_{max} at 4 h, ng/ml	T_{max}, hours
100 mg, mean ± SEM	4,513.6 ± 531.2	549.9 ± 65.0	4.1 ± 0.6
400 mg, mean ± SEM	11,282.4 ± 1,339.0	970.6 ± 115.0	4.3 ± 0.9
800 mg, mean ± SEM	23,109.0 ± 6,318.0	2,928.6 ± 437.0	4.0 ± 0.1

a major role for COX-2 in the biosynthesis of both systemic and renal PGI_2 under physiological conditions in young volunteers.

The two COX isoforms are distinct gene products prone to differential patterns of regulation (5–9, 13–17). Although their subcellular localization may be similar (43), it is thought that they have distinct roles in human biology and during development (44). Given its induction by growth factors and tumor promoters (14, 15), attention has focused on COX-2 as the likely source of prostaglandin formation during inflammation (17, 21), embryogenesis (44), and tumor growth (45). The COX isoforms exhibit marked conservation of their primary sequences (46). Differences in the topography of their active sites (47, 48) have permitted development of compounds highly selective for inhibition of COX-2 (49, 50). Biochemical selectivity for COX-2 inhibition has been assessed by assays based on measuring either oxygen consumption or prostaglandin formation (51), involving either nonhuman or human isozymes in a variety of expression systems (52, 53). Ranking of "conventional" NSAIDs for COX-2 inhibitory activity has reflected this variability (24–26). Presently, several compounds have emerged from such screening that exhibit a particularly high degree of selectivity for COX-2 (IC_{50}s for COX-2/COX-1 >500). At least two such compounds are at an advanced stage of clinical development. However, such inhibitors have been highly effective in models of inflammation (21, 60, 54, 55) and cancer (56) and exhibit minimal gastrotoxicity in clinical trials (57); there is little information as to their actual biochemical and functional selectivity for inhibition of COX-2 at doses tolerated by humans.

To address the hypothesis that celecoxib is highly selective for COX-2 in humans, we took advantage of assays (35, 37, 56) that reflect isozyme activity *ex vivo.* In the case of COX-2, pretreatment of heparinized blood with aspirin is used to inhibit constitutively expressed COX-1. Blood esterases deacetylate and inactivate aspirin rapidly. Thus, it does not interfere with PGE_2 formation by COX-2, which subsequently is induced by LPS. Although considerable baseline variability is apparent in this assay (35, 58), both celecoxib and ibuprofen

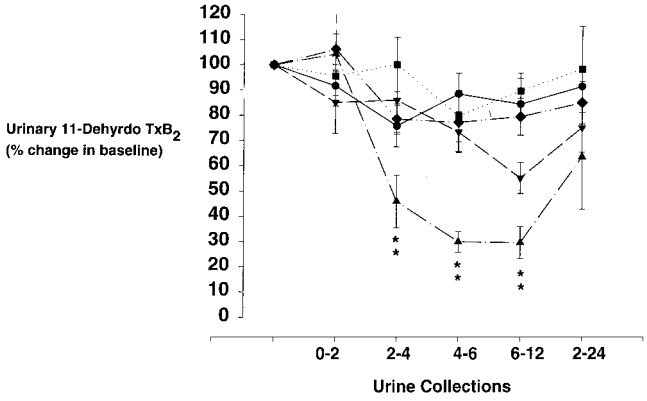

FIG. 3. Urinary excretion of 11-dehydro TxB_2, a major TxB_2 metabolite largely derived from platelets after placebo (■—■), 800 mg of ibuprofen (▲—▲), and celecoxib at 100 (●—●), 400 (◆—◆), and 800 mg (▼—▼).

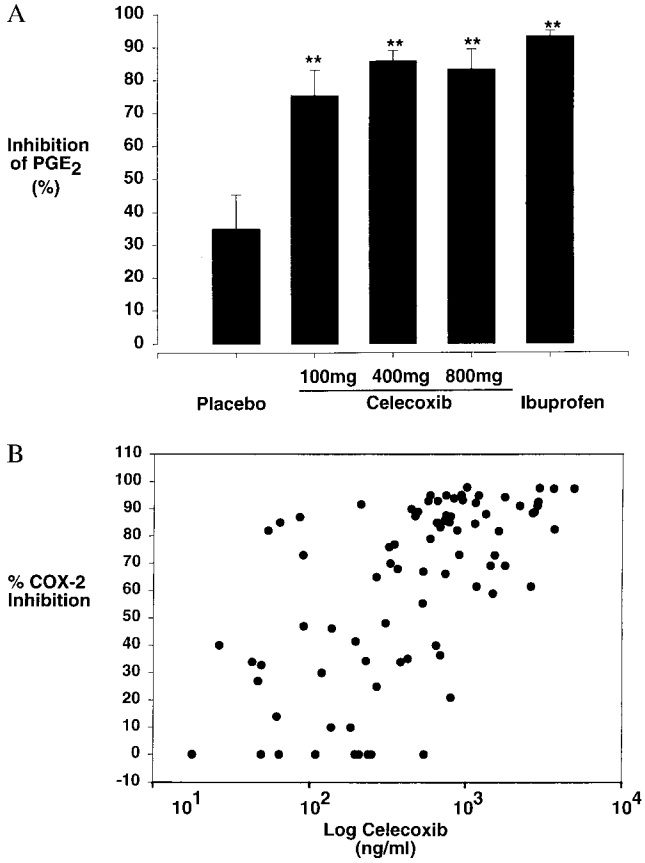

FIG. 4. (*A*) Inhibition of LPS-stimulated plasma PGE_2, an index of COX-2 activity, *ex vivo* in volunteers receiving placebo, 800 mg of ibuprofen, and various doses of celecoxib. **, $P < 0.01$ when compared with placebo. (*B*) The relationship between LPS-stimulated plasma PGE_2 *ex vivo*, an index of COX-2 activity, and log plasma concentrations of celecoxib 2, 4, 6, and 24 hr after dosing. PGE_2 is expressed as a percentage of predosing values. A steep but variable dose-response is evident. **, $P < 0.01$ for comparisons with placebo.

significantly suppressed this index of COX-2 activity *ex vivo*. Of importance, celecoxib does not induce COX-2 expression in monocytes *in vitro* (P. Isakson, personal communication). Although the average maximal plasma concentration of celecoxib related to dose, there was no evident distinction between the maximal effect of acute doses in the range 100–800 mg with respect to inhibition of COX-2. This accords with the failure to discriminate between most indices of symptomatic relief afforded by chronic administration of these doses of celecoxib to patients with osteoarthritis (57). Although interindividual variability in the dose-response relationship was apparent, the relationship of plasma concentration to enzyme inhibition was sigmoidal. The IC_{50} for COX-2 inhibition by celecoxib in *in vitro* systems ranges from 0.025 to 0.075 μM (31), which is lower than the range (1–3 μM) at which clinical efficacy in arthritis is expected (57). This is expected because the *in vitro* assays are performed in the absence of plasma

Table 2. Urinary PGI-M (pg/mg creatinine) after treatment with placebo, 400 and 800 mg of celecoxib, or 800 mg of ibuprofen

Treatment	Hours after dosing 4–6 h	6–12 h
Placebo	117.1 ± 49.4	126.2 ± 63.8
Celecoxib, 400 mg	34.2 ± 7.3**	25.1 ± 5.4**
Celecoxib, 800 mg	22.8 ± 8.8**	27.0 ± 11.8**
Ibuprofen, 800 mg	51.3 ± 18.8*	39.8 ± 6.3**

**, $P < 0.01$; *, $P < 0.05$ for comparisons with placebo.

proteins that bind the drug. It also raises the possibility that near-maximal inhibition of COX-2 is necessary for anti-inflammatory efficacy, although this remains to be established.

The biochemical selectivity of celecoxib for COX-2 was assessed by measurement of indices of COX-1 activity. Platelets possessing only the COX-1 isozyme are known to be the predominant source of TxB_2 formed in serum (5, 37, 58). Dose-dependant inhibition of serum TxB_2 *ex vivo* by aspirin (59) and Tx synthase inhibitors (60) has been reported. All doses of celecoxib in this study had some inhibitory effect on serum TxB_2. However, this effect was modest compared with celecoxib's effects on COX-2 activity. It is not surprising that TxA_2-dependent platelet aggregation induced by arachidonic acid was prevented by an efficient COX-1 inhibitor (ibuprofen) but not by celecoxib. It has been demonstrated that the relationship between inhibition of the capacity of platelets to form TxA_2 (serum TxB_2) and the TxA_2-dependant platelet aggregation is nonlinear; a residual 5–10% capacity to form TxA_2 is sufficient to sustain aggregation (61). Neither celecoxib nor ibuprofen affected Tx-independent platelet aggregation induced by the TxA_2 receptor agonist U44619. We also examined the effect of celecoxib on urinary 11-dehydro TxB_2, an index of TxA_2 biosynthesis *in vivo* (33). Previous studies have demonstrated that platelets are the predominant but not exclusive contributors to the excretion of Tx-M in healthy volunteers (33, 62). Ibuprofen, as expected, suppressed Tx-M, reflective of its action as a reversible inhibitor of COX-1 and COX-2. Celecoxib 800 mg also tended to suppress Tx-M, although this failed to attain statistical significance. Thus, although single doses of celecoxib exhibit relative rather than absolute biochemical specificity for inhibition of COX- 2, they demonstrated functional specificity, at least with respect to the platelet. Whether this also will be true for other sites of COX-1 function, such as gastric epithelium and the brain, remains to be determined.

Prostacyclin is the major COX product of macrovascular endothelium. Urinary excretion of PGI-M reflects predominately extrarenal formation of this eicosanoid in healthy volunteers (63). Physical (64) or chemical (65) stimulation of the vasculature in humans results in increased excretion of PGI-M, as does systemic infusion of the eicosanoid (66). Administration of conventional NSAIDs and aspirin suppresses PGI-M excretion in volunteers (36, 67, 68). COX-1 is expressed constitutively in vascular endothelial cells and in smooth muscle cells (69, 70). However, COX-2 may be induced in these tissues by LPS and cytokines (69–71). Recently, Topper *et al.* (72) have shown that laminar, but not turbulent, shear will up-regulate COX-2 in endothelial cells. In the present study, ibuprofen, as expected, caused a marked reversible, inhibitory effect on PGI-M excretion. Surprisingly, celecoxib also depressed PGI-M excretion to comparable degree at doses (400 and 800 mg) that inhibited monocyte COX-2 activity to the same extent as 800 mg ibuprofen. We also found that celecoxib suppressed urinary 6-keto $PGF_{1\alpha}$. Although this index predominantly reflects renal biosynthesis of prostacyclin, in contrast to the metabolite, these distinctions are not absolute (38). Similar effects on both urinary 6-keto $PGF_{1\alpha}$ and PGI-M with a structurally distinct COX-2 inhibitor—Vioxx (MK 966)—under chronic dosing conditions in elderly humans recently have been reported‡. Thus, this appears to be a feature of this class of compounds, rather than a COX-2-independent effect of celecoxib alone.

Although these results clearly implicate COX-2 as a major source of PGI_2 biosynthesis in humans, the effects of this compound and a structurally distinct COX-2 inhibitor on this eicosanoid under chronic dosing conditions in therapeutically relevant populations remain to be determined. PGI_2 is a potent vasodilator and platelet inhibitor. However, its importance *in vivo* has remained speculative in the absence of a pharmacological antagonist of the PGI_2 receptor (IP). Recently, Narumiya and colleagues (73) have generated data in mice deficient in the IP receptor that suggest an important role for PGI_2 in mediating inflammation and in preventing thrombosis. It has been demonstrated that PGI-M excretion is increased in patients with syndromes of platelet activation, such as unstable angina, severe atherosclerosis, and, periprocedurally, during angioplasty (64, 74, 75). Given the role of cytokines, growth factors, and thrombin in the induction of COX-2 by vascular tissues *in vitro*, it might be expected that this isozyme also would contribute to the augmented PGI_2 biosynthesis observed in these conditions.

Celecoxib exhibits relative rather than absolute biochemical selectivity for COX-2 *ex vivo* at doses tolerated in humans. Nonetheless, it does not modify TxA_2-dependent platelet aggregation, reflective of its modest inhibitory effect on COX-1. Ibuprofen and 800 mg celecoxib inhibit COX-2 *ex vivo* and suppress urinary PGI-M to a comparable degree. This appears to extend to the class of COX-2 inhibitors. It remains to be determined whether this effect is sustained during chronic dosing in age groups at risk for cardiovascular disease. If this is so, trials much larger than those necessary to detect efficacy and safety in arthritis (57) will be necessary to determine whether cardiovascular consequences of inhibiting PGI_2 biosynthesis will modulate the anti-inflammatory benefit to be derived from chronic administration of COX-2 inhibitors in humans.

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