Effects of Specific Inhibition of Cyclooxygenase-2 on Sodium Balance, Hemodynamics, and Vasoactive Eicosanoids

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ABSTRACT

Conventional nonsteroidal anti-inflammatory drugs inhibit both cyclooxygenase (Cox) isoforms (Cox-1 and Cox-2) and may be associated with nephrotoxicity. The present study was undertaken to assess the renal effects of the specific Cox-2 inhibitor, MK-966. Healthy older adults (n = 36) were admitted to a clinical research unit, placed on a fixed sodium intake, and randomized under double-blind conditions to receive the specific Cox-2 inhibitor, MK-966 (50 mg every day), a nonspecific Cox-1/Cox-2 inhibitor, indomethacin (50 mg t.i.d.), or placebo for 2 weeks. All treatments were well tolerated. Both active regimens were associated with a transient but significant decline in urinary sodium excretion during the first 72 h of treatment. Blood pressure and body weight did not change significantly in any group. The glomerular filtration rate (GFR) was decreased by indomethacin but was not changed significantly by MK-966 treatment. Thromboxane biosynthesis by platelets was inhibited by indomethacin only. The urinary excretion of the prostacyclin metabolite 2,3-dinor-6-keto prostaglandin F_1α was decreased by both MK-966 and indomethacin and was unchanged by placebo. Cox-2 may play a role in the systemic biosynthesis of prostacyclin in healthy humans. Selective inhibition of Cox-2 by MK-966 caused a clinically insignificant and transient retention of sodium, but no depression of GFR. Inhibition of both Cox isoforms by indomethacin caused transient sodium retention and a decline in GFR. Our data suggest that acute sodium retention by nonsteroidal anti-inflammatory drugs in healthy elderly subjects is mediated by the inhibition of Cox-2, whereas depression of GFR is due to inhibition of Cox-1.

The clinical benefits and adverse effects of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) derive from inhibition of the enzyme cyclooxygenase (Cox), the first step in the conversion of arachidonic acid to prostaglandins (PGs), thromboxane (TX) A_2, and prostacyclin (PGI_2). For many years, only a single form of Cox was recognized. This isozyme, now referred to as Cox-1, is constitutively expressed in platelets, the gastric mucosa, and most tissues, where it is thought to exert “housekeeping” functions, such as vascular homeostasis and gastric cytoprotection (Smith et al., 1996). The amino acid sequence of human Cox-2 is 60% homologous to Cox-1. This isozyme is commonly termed “inducible,” because it is transiently expressed in response to inflammatory mediators, tumor promoters, and growth factors (Hla and Neillon, 1992; Jones et al., 1993; Smith et al., 1996). However, these definitions are likely to oversimplify more complex regulatory mechanisms that govern expression of the two isoforms. Thus, Cox-2 is present in the kidney and the brain in the absence of inflammation (Harris et al., 1994; Guan et al., 1997; Komhoff et al., 1997; Yang et al., 1997), whereas growth factor induction and developmental regulation of the Cox-1 gene have been reported.

Conventional NSAIDs inhibit both Cox-1 and Cox-2 with limited selectivity (Patrignani et al., 1994). It is generally assumed, albeit with few supporting data, that their anti-inflammatory and analgesic activity is mediated via Cox-2 inhibition (Zhang et al., 1997). Inhibition of Cox-1, by contrast, is thought to be responsible for the gastric toxicity and bleeding complications associated with NSAID treatment. It is unclear whether NSAID-induced nephrotoxicity is attrib-
utable to inhibition of Cox-1 or Cox-2. The intrarenal distribution and regulation of Cox-2 by sodium intake strongly suggest a role for this enzyme in renal physiology (Harris et al., 1994; Guan et al., 1997; Komhoff et al., 1997; Yang et al., 1997) and emphasize the need to clarify the renal effects of selective Cox-2 inhibitors that are at an advanced stage of clinical development. Notably, one such compound, flurbiprofen, has been withdrawn from clinical development because of a high incidence of peripheral edema (Emery, 1996). The present study was undertaken to assess the renal effects of the specific Cox-2 inhibitor, MK-966, during a 2-week administration to elderly subjects. This is a target population for these drugs and one that is the most susceptible to NSAID-induced nephrotoxicity. Sodium excretion and other indices of renal function were assessed under conditions of controlled sodium intake. We hypothesized that the effect of MK-966 on urinary sodium excretion would be comparable to that of a dual Cox-1/Cox-2 inhibitor, such as indomethacin.

Although we expected that selective inhibition of Cox-2 would fail to reduce urinary 11-dehydro-TXB₂ (TX-M) and serum TXB₂ indices of Cox-1-dependent thromboxane biosynthesis by platelets, we also wished to address the hypothesis that prostacyclin biosynthesis, as reflected by urinary excretion of 2,3-dinor-6-keto PGF₁α (PGI-M), its major metabolite in vivo (FitzGerald et al., 1981; Brash et al., 1983), also would be unaffected by inhibition of Cox-2.

Materials and Methods

Human Subjects. The study protocol was approved by the Institutional Review Board of the University of Pennsylvania, the Advisory Committee of the General Clinical Research Center at the Hospital of the University of Pennsylvania, and the Southern Institutional Review Board (Miami, FL). Thirty-six subjects were enrolled in the study. Stratification by gender and race (African Americans versus Caucasians/others) ensured a balance for these baseline characteristics. Subjects eligible for inclusion in the study were between 59 and 80 years of age. They were judged to be in good health for their age based on medical history, physical examination, and routine hematology and biochemistry. Subjects requiring pharmacologic treatment for hypertension or diabetes mellitus were excluded from participation in the study. Other exclusion criteria included serum creatinine > 2 mg/dl and creatinine clearance < 50 ml/min. All participants gave written informed consent, refrained from smoking, and did not consume any medications containing aspirin or other NSAIDs for at least 2 weeks before and during the trial.

All study subjects were confined to the General Clinical Research Center or the Clinical Pharmacology Associates Research Unit for a minimum of 17 days and adhered to a fixed 200-mEq sodium (−0.8 g/kg protein, 60–80 mEq potassium, −350 mg of magnesium, 800 mg of calcium, isocaloric) diet, prepared by the metabolic kitchen. This was started at least 5 days before dosing and continued for the entire duration of the study. Only patients in sodium balance on the metabolic ward (based on weight within 1.0 kg for 2 consecutive days and 24-h urinary sodium between 180 and 220 mEq) were allowed to commence the study.

Study discontinuation was required for all subjects whose serum creatinine, blood pressures, or body weight increased above prespecified safety values.

Interventions. Study subjects were randomized under double-blind conditions to receive 50 mg of MK-966 every day (q.d.; 8:00 AM), 50 mg of indomethacin t.i.d. (8:00 AM, 3:00 PM, and 11:00 PM), or matching placebo for 2 weeks. The dosage of MK-966 was selected on the basis of previous studies. A dose-ranging study in postsurgical dental pain showed that a single dose of 50 mg was the minimal dose required to provide maximal analgesic efficacy (Ehrich et al., 1996a).

In addition, in a pilot study of the treatment of osteoarthritis of the knee, 25 mg once daily for 6 weeks was indistinguishable from 125 mg for all primary efficacy endpoints. Thus, the dose chosen for this study is twice the maximum dose needed for chronic treatment of osteoarthritis (Ehrich et al., 1997). The dose of indomethacin chosen for the study is the standard dose used for chronic anti-inflammatory, analgesic treatment.

Efficacy and Safety Assessments. Twenty-four-hour urine collections (8:00 AM to 8:00 AM) were performed daily for the assessment of sodium and potassium excretion. Glomerular filtration rate (GFR) was assessed by iohexol clearance on day −1 and day 14. Creatinine clearance also was measured throughout the study. Urinary excretion of N-acetyl-β-glucosaminidase (Boehringer Mannheim Biochemica, Indianapolis, IN), an index of renal proximal tubular dysfunction, was measured on day −2 and day 13. Blood pressure (three measurements after 10-min supine rest) was measured every 4 h (from 8:00 AM to 8:00 PM). Body weight was measured daily (8:00 AM) on a calibrated scale.

Six-hour urine collections (from 8:00 AM to 2:00 PM) were performed on day −2, day 1, and day 13 for measurement of TX-M, an index of Cox-1-dependent TX formation by platelets (FitzGerald et al., 1983; Catella et al., 1986a; Catella and FitzGerald, 1987), 6-keto-PGF₁α, an index of the renal biosynthesis of prostacyclin (Catella et al., 1986b), and PGI-M, an index of total body biosynthesis of prostacyclin (FitzGerald et al., 1981, 1983; Brash et al., 1983; Catella et al., 1986b). Urinary eicosanoids were measured by negative ion chemical ionization–gas chromatography/mass spectrometry (NICI-GC/MS) using authentic deuterated standards (Lawson et al., 1985, 1986; Catella et al., 1988; Catella and FitzGerald, 1990). In a subgroup of subjects, NICI-GC/MS also was applied to the measurement of serum TXB₂, an index of the capacity of the platelets to convert arachidonic acid through the Cox-1 pathway during whole-blood clotting (Patrono et al., 1980). Serum TXB₂ was measured at baseline and 4 h postdosing on day 1. Deuterated Tx-M, 6-keto-PGF₁α, and TXB₂ were obtained from Cayman Chemical Co., Inc. (Ann Arbor, MI). Deuterated PGI-M was obtained from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA).

Statistical Analysis. The primary hypothesis of the study was that MK-966 and indomethacin would produce similar levels of mean reduction in urinary sodium excretion from baseline during the first 72 h of treatment. This assertion was evaluated by an ANOVA appropriate for a three-factor experimental design with repeated measures over Time (Pre- versus Post-) and nonrepeated classifications of Treatment and Center. Model specification allowed for tests of significance of second-order interactions for Treatment × Center and Treatment × Time. Homogeneity of variance and normality assumptions were evaluated by the Shapiro-Wilk test and Levene’s test, respectively. In addition to means and S.D. values for pre- and posttreatment time points, means of posttreatment measures adjusted for their corresponding values and associated S.E. values were tabulated for all variables except for serum TXB₂ (no ANOVA was performed on this variable because of the small sample size).

A sample size of 12 subjects per treatment group was recruited for this study. We anticipated that if the mean reduction in urinary sodium excretion during the first 72 h for patients receiving 50 mg of MK-966 q.d. was equal to that for patients receiving 50 mg of indomethacin i.t.i.d., then this sample size would provide 80% probability that a 90% confidence interval for the difference would fall within the prespecified similarity limits of ±0.5 mEq.

Results

Age, weight, systolic and diastolic blood pressure, and serum creatinine and creatinine clearance of the three treatment groups at baseline are reported in Table 1.

All treatments were well tolerated. All subjects completed
the study according to the protocol, with the exception of one patient in the indomethacin group, who was discontinued prematurely because of increasing anxiety. One subject on MK-966 had a mild increase of serum transaminases that resolved upon drug discontinuation.

Both active treatments (MK-966 and indomethacin) significantly reduced net urinary sodium excretion during the first 72 h of treatment compared with baseline (Fig. 1). MK-966 and indomethacin were not different \( p = 0.35 \) with respect to their effects on this parameter. In both groups, the sodium-retaining effect was short-lived, largely disappearing by day 3 (Fig. 2), and the urinary sodium excretion at day 7 was not different from baseline for all treatment groups. However, in indomethacin-treated subjects, sodium excretion declined again on day 14 (least-squares mean change from baseline = \(-36.6 \pm 13.4\) mEq/24 h; \( p < .05 \)). A trend toward delayed sodium retention also was present in the placebo (least-squares mean change from baseline = \(-10 \pm 12.9\) mEq/24 h) and in the MK-966 group (least-squares mean change from baseline = \(-8.1 \pm 12.7\) mEq/24 h). Also, the between-treatment comparisons showed that the effect of indomethacin on sodium excretion at day 14 was not different from placebo \( (p = .169) \) or MK-966 \( (p = .134) \). The change from baseline for the daily average urinary sodium excretion during the 14 days of treatment was significantly greater with indomethacin when compared with both MK-966 \( (p < .05) \) and placebo \( (p < .05) \). The mean change over the 14 days of MK-966 treatment was not different from placebo.

There were no significant changes in any treatment group in the daily average urinary excretion of potassium during the first week or during the entire 2-week treatment period. Urinary excretion of potassium on days 7 and 14 also was unchanged by the three treatments. The urinary potassium excretion during the first 72 h of treatment was not modified by placebo or indomethacin. However, a decrease (from \(146.5 \pm 23.6\) mEq/72 h to \(136.8 \pm 21.6\) mEq/72 h) was observed in the MK-966 group. Even though statistically significant by ANOVA \( (p < .05) \), this drop failed to reach significance by univariate analysis. Also, there was no difference between MK-966 and placebo \( (p = .246) \) or MK-966 and indomethacin \( (p = .152) \) in the between-treatment comparisons.

Body weight was not changed significantly by either of the active treatments or by placebo. At day 14, systolic blood pressure increased slightly from \(129.4 \pm 9.6\) mm Hg to \(135.0 \pm 13.0\) mm Hg in the MK-966 group, from \(124.9 \pm 12.9\) mm Hg to \(130.3 \pm 11.9\) mm Hg in the indomethacin group, and from \(125.2 \pm 11.2\) mm Hg to \(126.9 \pm 16.4\) mm Hg in the placebo group. None of these changes reached statistical significance in the within-treatment ANOVA or in the between-treatment comparisons. A similar trend toward a rise in diastolic blood pressure occurred at day 14. The least-squares mean change from baseline at day 14 was \(1.7 \pm 1.5\), \(2.6 \pm 1.5\), and \(1.6 \pm 1.6\) mm Hg in the placebo, MK-966, and indomethacin groups, respectively. All changes failed to attain significance.

The GFR, as assessed by iohexol clearance, decreased after 2 weeks of indomethacin, but was not changed significantly by MK-966 treatment or placebo (Fig. 3). The effect of indomethacin on iohexol clearance was significantly different from that of placebo \( (p = .014) \) and MK-966 \( (p = .004) \). Creatinine clearance was also decreased after 2 weeks of indomethacin (from \(86.03 \pm 21.01\) ml/min to \(75.52 \pm 26.51\) ml/min; \( p < .05 \)), but was not affected significantly by MK-966 treatment (from \(90.90 \pm 23.20\) ml/min to \(95.03 \pm 21.48\) ml/min; \( p \) is not significant) or placebo (from \(98.13 \pm 24.26\) ml/min to \(90.92 \pm 31.82\) ml/min; \( p \) is not significant). The effect of indomethacin on creatinine clearance was signifi-

### Table 1: Baseline Characteristics

<table>
<thead>
<tr>
<th>Placebo</th>
<th>MK 966</th>
<th>Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>64.6 (4.5)</td>
<td>67.9 (6.8)</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>165.4 (9.7)</td>
<td>168.2 (11.5)</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>76.3 (11.5)</td>
<td>89.4 (13.2)</td>
</tr>
<tr>
<td><strong>Diastolic BP (mm Hg)</strong></td>
<td>72.0 (6.6)</td>
<td>75.9 (5.2)</td>
</tr>
<tr>
<td><strong>Systolic BP (mm Hg)</strong></td>
<td>125.2 (11.2)</td>
<td>129.4 (9.6)</td>
</tr>
<tr>
<td><strong>Serum creatinine (mg/dl)</strong></td>
<td>0.81 (0.18)</td>
<td>0.84 (0.15)</td>
</tr>
<tr>
<td><strong>Creatinine clearance (ml/min)</strong></td>
<td>98.1 (24.3)</td>
<td>90.9 (23.2)</td>
</tr>
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BP, blood pressure. \( n = 12 \) for all three columns.
cantly different from that of MK-966 (p < .05) but was not different from placebo (p = 0.57).

Renal tubular function was assessed by measurement of urinary N-acetyl-β-glucosaminidase excretion. None of the treatments significantly altered this parameter.

Urinary excretion of TX-M, an index of Cox-1-dependent thromboxane biosynthesis by platelets, was decreased by 59.9 ± 16.6% (percentage of change least-squares mean ± S.E.M.) on indomethacin, but was not changed significantly by MK-966 (+1.74 ± 15.2% change least-squares mean ± S.E.M.) or placebo (+11.1 ± 13.8% change least-squares mean ± S.E.M.). The decrease in TX-M excretion was statistically significant only in the indomethacin group (p < .05) by ANOVA (Fig. 4). There were no differences between the effects of MK-966 and placebo treatments (p = .552), whereas the effects of indomethacin on TX-M excretion were significantly different both from placebo (p = .002) and MK-966 (p = .017).

Selectivity of MK-966 for the Cox-2 isozyme also was confirmed by measurement of serum TXB₂ in a subgroup of subjects. Serum TXB₂, an index of the capacity of the platelets to synthesize TX via Cox-1, was not changed significantly by MK-966 (+10.8 ± 21.3% change from baseline; n = 3) or placebo (−42 ± 21.8% change from baseline; n = 5), whereas it was nearly completely inhibited by indomethacin (−96.3 ± 1.4% change from baseline; n = 5) 4 h after dosing on day 1.

Urinary 6 keto-PGF₁α, and PGI-M were inhibited by indomethacin and MK-966, but were not affected significantly by placebo (Figs. 5 and 6). The least-squares mean change from baseline for urinary PGI-M was −64.8 ± 10.8 (p < .05), −73.6 ± 9.1 (p < .05), and −0.27 ± 9.1 pg/mg creatinine (p < .05) in the indomethacin, MK-966, and placebo groups, respectively. There were no differences between MK-966 and indomethacin with respect to their inhibitory effects on urinary PGI-M excretion (p = .55). Similarly, the least-squares mean change from baseline for urinary 6-keto PGF₁α was −25.3 ± 7.0 (p < .05), −27.1 ± 6.3 (p < .05), and 2.03 ± 6.4 pg/mg creatinine (p < .05) in the indomethacin, MK-966, and placebo groups, respectively.

Discussion

It is unclear whether NSAID-induced renal toxicity is attributable to inhibition of Cox-1 or Cox-2. Both Cox isoforms are constitutively expressed in the kidney. However, Cox-1 mRNA is widely distributed, whereas expression of Cox-2 in the rat renal cortex is localized to the macula densa and surrounding cells of the cortical thick ascending limbs (Harris et al., 1994; Guan et al., 1997; Komhoff et al., 1997; Yang et al., 1997), which play a key role in the regulation of vascular tone and renin release. In the rabbit kidney, Cox-2 mRNA expression is present in the macula densa and in the interstitial cells of the outer medulla (Guan et al., 1997). In these cells, which may play an important role in regulating salt and water excretion, Cox-2 expression predominates over Cox-1 (Guan et al., 1997). In contrast to the rat and rabbit, Cox-2 is not expressed in the macula densa in human kidney, but is predominantly expressed intraglomerularly in podocytes (Komhoff et al., 1997). Thus, Cox-2 in humans might regulate glomerular hemodynamics by contracting the podocytes. Yang et al. (1997) have reported that expression of Cox-2 is regulated in a cell-specific fashion in response to

![Fig. 3. GFR, assessed by iohexol clearance, at baseline and after 14-day treatment (mean ± S.D.; *p < .05).](image)

![Fig. 4. Urinary excretion of TX-M at baseline and after 13-day treatment (mean ± S.D.; *p < .05).](image)

![Fig. 5. Urinary excretion of 6 keto-PGF₁α, at baseline and after 13-day treatment (mean ± S.D.; *p < .05).](image)
altered sodium intake, whereas Cox-1 expression is not influenced by dietary salt. Restriction of dietary sodium increases Cox-2 in the rat renal cortex, particularly in the macula densa, suggesting that this enzyme may play an important role in the homeostatic regulation of renal perfusion and glomerular hemodynamics. In contrast, a high-salt diet increases expression of Cox-2 in the renal medulla, supporting a role for Cox-2 in the regulation of sodium and water excretion (Yang et al., 1997).

Cox-2 also plays an important role in renal development in mice (Dinchuk et al., 1995; Morham et al., 1995). Deletion of the Cox-2 gene in mice results in severe nephropathy (Dinchuk et al., 1995; Morham et al., 1995). However, the renal localization and the temporal pattern of expression of the two Cox enzymes in human fetal kidney suggest that murine Cox-2 knockout data may not be applicable to humans (Komhoff et al., 1997).

Little is known about the selective roles of the Cox isoforms in humans. However, the studies in animals raise the possibility of adverse renal effects of selective Cox-2 inhibition in humans. To investigate this possibility we selected a dose of a Cox-2 inhibitor that has been shown to be biochemically selective (Ehrich et al., 1996b) and an effective analgesic in humans (Ehrich et al., 1996a). The dose of MK-966 chosen for this study is twice the maximum dose needed for chronic treatment of osteoarthritis (Ehrich et al., 1997).

Nephrotoxicity induced by conventional NSAIDs is most commonly characterized by impaired GFR and/or an acute decrease in sodium excretion (Murray and Brater, 1993; Nies, 1998). The decline in sodium excretion may result from reduced renal blood flow or from a direct inhibition on sodium absorption independent of renal hemodynamics. This study demonstrates that, in healthy elderly subjects, the decline in GFR observed during short-term NSAID therapy appears attributable to inhibition of Cox-1. In this population, an early, transient decline in sodium excretion occurred without a change of GFR, suggesting that it resulted from inhibition of Cox-2 in the renal tubules.

Sodium retention induced by MK-966 at a dose that was selective for inhibition of Cox-2 was short-lived and was not accompanied by a significant rise in blood pressure or body weight. The effects of selective Cox-2 inhibition in subjects with hypertension and/or impaired renal function remain to be established. Therefore, although the results of the present study indicate that short-term administration of MK-966 is not associated with impaired GFR or renal toxicity, they pertain only to healthy, elderly patients on a fixed intake of sodium.

The study population followed a constant 200-mEq sodium diet. This daily sodium intake is estimated to represent the typical American diet, even though it greatly exceeds the recommended minimum requirement of 20 mEq daily. The effects of Cox-2 inhibition under renoprival conditions that would activate the renin-angiotensin system are unknown and merit further investigation.

The effects on urinary excretion of TX-M and serum TXB₂ demonstrate that the dose of MK-966 that we studied (50 mg q.d.) has no effect on Cox-1-dependent thromboxane biosynthesis by platelets. By contrast, specific Cox-2 inhibition resulted in partial suppression of both renal and extrarenal biosynthesis of prostacyclin. Urinary excretion of 6 keto-PGF₁α, an index of renal biosynthesis of prostacyclin (Catella et al., 1986b), and PGI-M, an index of total body biosynthesis of prostacyclin (FitzGerald et al., 1981, 1983; Brash et al., 1983; Catella et al., 1986b), both were inhibited similarly by MK-966 and indomethacin. Cox-2 is constitutively expressed in the rat, rabbit, and human kidney (Harris et al., 1994; Guan et al., 1997; Komhoff et al., 1997; Yang et al., 1997), and it is conceivable that it contributes to prostacyclin synthesis by the kidney under physiological conditions. However, systemic infusion of prostacyclin increases urinary 6 keto-PGF₁α, as well as urinary PGI-M (Brash et al., 1983).

Therefore, the suppressive effects of both indomethacin and MK-966 on 6 keto-PGF₁α may merely reflect inhibition of the extrarenal contribution to excretion of the hydrolysis product in urine.

An additional finding of this study was the suggestion that extrarenal biosynthesis of prostacyclin also was mediated by Cox-2. This was unexpected because Cox-1, but not Cox-2, is expressed constitutively by endothelial and vascular smooth muscle cells in vitro (Smith et al., 1996). One possible explanation may be related to the finding that laminar shear stress up-regulates Cox-2 in vascular endothelium in vitro (Topper et al., 1996). A further contribution from Cox-2 might be expected in advancing age and in syndromes of platelet activation and inflammation, where prostacyclin biosynthesis, as reflected by excretion of urinary PGI-M, is augmented (FitzGerald et al., 1984; Reilly and FitzGerald, 1986; Bernard et al., 1991). Thus, prothrombotic and inflammatory stimuli induce Cox-2 expression and prostacyclin generation by vascular tissues in vitro (Hla and Neilson, 1992; Jones et al., 1993). Furthermore, the arachidonic acid in microparticles shed from activated platelets can up-regulate Cox-2 expression in endothelial cells and be used as a substrate for increased prostacyclin biosynthesis (Barry et al., 1997). It is also formally possible that the inhibition of urinary PGI-M by MK-966 reflects a property of MK-966 in addition to, but distinct from, its capacity to inhibit Cox-2. MK-966 might inhibit β-oxidation of prostanooids, therefore shifting the metabolism of prostacyclin toward other products. The possibility that MK-966 might directly reduce the renal clearance of PGI-M also has not been excluded.
Prostacyclin is the major Cox product of macrovascular endothelium in vitro. Although it is a potent modulator of platelet function and vascular tone in vivo, its importance in vivo has been speculative. Thus, although the effects of prostacyclin appear to be mediated by a single-membrane G protein-coupled receptor, the absence of receptor antagonists have made it difficult to assess the role of this eicosanoid in integrated systems. However, Narumiya and coworkers (Murata et al., 1997) have reported that inactivation of the prostacyclin receptor gene results in an increased susceptibility to thrombosis in vivo. Although these results provide the first evidence for the homeostatic antithrombotic effect of endogenous prostacyclin in vivo, infusion of exogenous prostacyclin is an effective platelet inhibitor in vivo, albeit limited by gastrointestinal and vasoactive side effects (Fitzgerald et al., 1979; Belch et al., 1995). Pharmacological enhancement of endogenous prostacyclin is also an effective antithrombotic strategy in vivo. Thus, adenosinergic delivery of Cox-1 to canine coronary vasculature prevents platelet activation in vivo (Wu, 1997). Similarly, these results are consistent with experimental data that demonstrate that the antithrombotic effect of combining thromboxane synthase inhibitors with thromboxane receptor antagonists is largely attributable to augmented prostacyclin formation (Fitzgerald et al., 1988). Presently, the implications of prostacyclin suppression in vivo are unclear. Results from prostacyclin receptor knockout mice would suggest that prevention of prostacyclin formation might be expected to contribute to, if not explain, the anti-inflammatory and analgesic effects of such compounds (Murata et al., 1997). Prostacyclin formation by the vasculature is of functional importance in limiting the response to a thrombotic insult in mice, and we have shown previously that urinary excretion of PGI-M is increased in syndromes of platelet activation (Fitzgerald et al., 1986). It remains to be established whether treatment with specific Cox-2 inhibitors will suppress this response.

In conclusion, our results suggest that Cox-2 plays a role in the biosynthesis of prostacyclin under physiological conditions in humans, at least in healthy, elderly subjects on a controlled intake of sodium. In these subjects, MK-966 inhibited Cox-2 selectively, and this resulted in a clinically insignificant and transient reduction of sodium, but no depression of GFR. Given the known renal effects of NSAID, it seems likely that Cox-2 inhibition causes acute sodium retention, whereas the decline in GFR is attributable to the blockade of Cox-1.

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References

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