British Journal of Pharmacology (2000) 129, 1423–1430 © 2000 Macmillan Publishers Ltd All rights reserved 0007–1188/00 $15.00

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Selective cyclo-oxygenase-2 inhibition with celecoxib elevates blood pressure and promotes leukocyte adherence

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1 Selective inhibitors of cyclo-oxygenase-2 have been shown to be effective anti-inflammatory drugs with reduced gastrointestinal toxicity relative to conventional nonsteroidal anti-inflammatory drugs (NSAIDs). In the present study, we examined the possibility that selective COX-2 inhibition, by blocking prostacyclin synthesis, would increase blood pressure and cause leukocyte adherence and platelet aggregation.

2 Normal rats and rats with hypertension induced by chronic administration of N^{ω}-nitro-L-arginine methylester were given celecoxib (10 mg kg^{-1}) daily for 3 weeks. Celecoxib significantly elevated of blood pressure in both the normal and hypertensive rats (mean increase of >33 mm Hg after 3 weeks).

3 In normal rats, celecoxib had no effect on serum 6-keto prostaglandin (PG)$F_{1\alpha}$ levels. Hypertensive rats exhibited a significant increase (82%) in serum 6-keto $PGF_{1\alpha}$ levels, and this was reduced to the levels of normal rats by treatment with celecoxib.

4 Rats treated with celecoxib exhibited significant increases in weight gain (20%), plasma arginine-vasopressin levels (148%) and plasma urea (69%) relative to vehicle-treated controls. Plasma creatinine levels were unaffected by treatment with celecoxib, while plasma renin levels were significantly decreased (30%) relative to controls.

5 Superfusion of mesenteric venules with celecoxib (3 μM) *in vivo* resulted in significant increases in leukocyte adherence to the endothelium in both normal and hypertensive rats.

6 These studies suggest that suppression of COX-2 significantly influences vascular and/or renal function, leading to elevated blood pressure and leukocyte adherence.

British Journal of Pharmacology (2000) **129,** 1423–1430

Keywords: Hypertension; cyclo-oxygenase-2; neutrophil adherence; renal function; prostacyclin; endothelium

Abbreviations: COX, cyclo-oxygenase; L-NAME, N^{ω}-nitro-L-arginine methylester; mRNA, messenger ribonucleic acid; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; RT–PCR, reverse transcriptase polymerase chain reaction

Introduction

The existence of multiple forms of cyclo-oxygenase, the key enzyme in the biosynthesis of prostaglandins, was first suggested in 1972 (Flower & Vane, 1972). The molecular characterization of two distinct isoforms of this enzyme in 1991 (Kujubu *et al*., 1991; Xie *et al*., 1991) led to a burst of activity in the pharmaceutical industry aimed at the development of selective inhibitors of COX-2, the isoform found to be highly expressed at sites of inflammation (Vane *et al*., 1994; Seibert *et al*. 1997). Inhibition of COX-2, it was proposed, would result in anti-inflammatory and analgesic properties similar to what can be achieved with conventional nonsteroidal anti-inflammatory drugs (NSAIDs), which block both COX-1 and COX-2 activity. However, by sparing COX-1 activity, the selective COX-2 inhibitors would have greatly reduced toxicity, particularly in the gastrointestinal tract (Xie *et al*., 1992).

Selective COX-2 inhibitors have now reached the marketplace and data from clinical trials suggest that these agents are effective in treating the symptoms of arthritis while causing less gastrointestinal injury than conventional NSAIDs (Simon *et al*., 1998). Concerns have been raised, however, that selective COX-2 inhibitors will exacerbate inflammatory conditions in the gastrointestinal tract as well as delay ulcer healing (Reuter *et al*., 1996; Mizuno *et al*., 1997; Wallace, 1999). Moreover, there is emerging evidence for important physiological roles of COX-2 in many circumstances, raising the possibility that selective COX-2 inhibition will result in adverse effects that were not initially predicted. For example, McAdam *et al*. (1999) and Catella-Lawson *et al*. (1999) recently demonstrated that the selective COX-2 inhibitors, celecoxib and rofecoxib, markedly suppressed prostacyclin synthesis in healthy volunteers when administered at doses in the range presently used by patients with osteoarthritis or rheumatoid arthritis. Prostacyclin is produced mainly by the vascular endothelium and the kidney (Bunting *et al*., 1983). Its potent vasorelaxant properties contribute to the maintenance of normal vascular tone. Moreover, prostacyclin is a powerful inhibitor of leukocyte and platelet adherence and aggregation (Bunting *et al*., 1983). Counteracting the effects of endothelial-derived prostacyclin synthesis is the production of thromboxane, *via* COX-1, by the platelet. Thromboxane is a potent vasoconstrictor and stimulus for platelet aggregation (Bunting *et al*., 1983). Based on the results of McAdam *et al*. (1999) and Catella-Lawson *et al*. (1999), one might speculate that inhibition of endothelial prostacyclin synthesis (COX-2), while sparing platelet throm-

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boxane synthesis (COX-1), could lead to increased vascular tone and increased leukocyte and platelet aggregation.

The aim of the present study was to test the hypothesis that chronic suppression of COX-2 activity through daily administration of celecoxib would lead to increased systemic blood pressure, increased leukocyte adherence and increased platelet aggregation. Studies were carried out in both normal and hypertensive rats. Hypertension was induced through the addition of a nitric oxide synthase inhibitor to the drinking water of the rats. As nitric oxide has several properties that are similar to those of prostacyclin, such as the ability to relax vascular smooth muscle, inhibit platelet aggregation and inhibit leukocyte adherence, it was possible that rats chronically treated with a nitric oxide synthase inhibitor would exhibit increased dependency on the vascular actions of prostacyclin.

Methods

Male, Wistar rats weighing 175–200 g were obtained from Charles River Breeding Farms (Montreal, PQ, Canada). All experimental procedures described below were approved by the Animal Care Committee of the University of Calgary.

Selection of dose of celecoxib

In order to determine a dose of celecoxib (Monsanto, St. Louis, MO, U.S.A) for use in the *in vivo* studies that exhibited selectivity for COX-2 over COX-1 and was capable of suppressing inflammatory prostaglandin synthesis, the following experiment was performed. An airpouch was induced as described in detail previously (Edwards *et al*., 1981; Sedgwick *et al*., 1985; Seibert *et al*., 1994). Carrageenan (2 ml of a 1% wt vol^{-1} solution in sterile saline) was injected into the airpouch. Six hours later, the rats were anaesthetized with 5% (vol vol^{-1}) halothane, the pouch opened by a small incision and the exudate was collected. PGE_2 concentrations in the exudate were measured using a specific enzyme-linked immunosorbent assay (Wallace *et al*., 1999). A sample of blood was drawn from the inferior vena cava for measurement of whole blood thromboxane synthesis, as an index of COX-1 activity (Wallace *et al*., 1999). Groups of five rats each were treated orally with vehicle or celecoxib (1–45 mg kg^{-1}) 1 h prior to the injection of carrageenan into the airpouch. We have previously demonstrated that virtually all of the PGE_2 produced in this model is derived from COX-2 (Wallace *et al*., 1999).

Effects of celecoxib in normotensive rats

Groups of five rats each were orally treated each day with celecoxib (10 mg kg^{-1}) or vehicle. Blood pressure measurements were performed using the tail cuff method (Muscará *et al*., 1998) prior to initiating treatment and at 1-week intervals for a total of 3 weeks. The rats were conscious during the measurement of blood pressure. Blood pressure measurements were always performed 16–20 h after administration of celecoxib or vehicle. At the end of the study period, 2 h after the final dose of celecoxib or vehicle, the rats were anaesthetized with sodium pentobarbital (65 mg kg^{-1} i.p.) and a blood sample was drawn from the inferior vena cava for measurement of thromboxane B_2 (as an index of COX-1 activity) and serum 6-keto $PGF_{1\alpha}$ levels using specific enzyme-linked immunosorbent assays (Wallace *et al*., 1999). Serum nitrite and nitrate concentrations were measured by high performance liquid chromatography (Muscará & de Nucci, 1996). Additional rats underwent exactly the same treatment, but blood samples were drawn by cardiac puncture and were used for measurement of plasma renin activity, and plasma levels of endothelin-1 and arginine-vasopressin. For plasma renin activity, blood samples (1 ml) were collected in pre-cooled tubes containing 0.8 mg of EDTA and the plasma was immediately separated by centrifugation ($2000 \times g$, 10 min, 4°C). Plasma renin activity was estimated by the generation of angiotensin I from endogenous angiotensinogen (pH 6.5, 37°C, over 4 h), and angiotensin I concentrations were measured by radioimmunoassay. Plasma endothelin-1 and arginine-vasopressin were also measured by radioimmunoassay (all kits from Peninsula Labs, San Diego, CA, U.S.A). Plasma creatinine and urea levels were measured spectrophotometrically (Sigma Chemical Co., St. Louis, MO, U.S.A.).

In vivo *responses to endothelin-1 and norepinephrine*

Six rats were anaesthetized with sodium pentobarbital (65 mg kg^{-1} i.p.) and a femoral vein and carotid artery were cannulated for administration of drugs and measurement of blood pressure, respectively. Each rat was given single bolus injections (i.v.) of endothelin-1 (50–20 pmol kg^{-1}) or norepinephrine (0.25–2.5 μg kg^{-1}). After the blood pressure had recovered to basal levels, another injection was performed. The rats were then given celecoxib (10 mg kg^{-1} i.p.) and 90 min later, the responses to endothelin-1 and norepinephrine were assessed. Thus, in each rat, the responses to a range of doses of each agent were assessed before and after administration of celecoxib. In control rats, which received vehicle in place of celecoxib, the responses to the second round of injections with endothelin-1 and norepinephrine were not significantly different from the responses to the initial injections.

Effects of celecoxib in hypertensive rats

Three groups of 6–7 rats each were studied. In two of the groups, hypertension was induced by addition of a nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine methylester (L-NAME), to the drinking water at a concentration of 400 mg l^{-1}. The third group received normal drinking water. Two weeks after starting the study, blood pressure was measured using the tail cuff method, as described above. The rats with L-NAME-induced hypertension continued to receive the supplemented drinking water for the following 3 weeks. During that time, one group was treated orally each day with celecoxib (10 mg kg^{-1}), while the other group was treated with vehicle. The group of rats that received normal drinking water also received daily oral treatments with vehicle. Blood pressure measurements were made, as described above, at the end of the second, third, fourth and fifth weeks of the study. These measurements were always made 16–20 h after the administration of celecoxib or vehicle. After the final (fifth week) measurement of blood pressure, the rats were given the final dose of celecoxib or vehicle, and 2 h later were anaesthetized with sodium pentobarbital (65 mg kg^{-1} i.p.). Blood samples were drawn from the inferior vena cava for measurement of serum thromboxane B_2, 6-keto $PGF_{1\alpha}$ and nitrate/nitrite, as described above. The descending aorta, right kidney were excised and processed as described previously (8) for determination of COX-2 mRNA expression by RT–PCR and COX-2 protein expression by Western blotting.

Additional groups of rats ($n=5$) received L-NAME-supplemented or normal drinking water for 2 weeks and were then sacrificed. Aortic rings were prepared from the thoracic

descending aorta and were mounted under 2 g of passive tension in 25 ml organ baths containing a physiological salt solution (37°C, bubbled with 95% O_2/5% CO_2). The salt solution consisted of (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 12.5 and glucose 11.1. Tissues (endothelium-intact preparations) were allowed to equilibrate for 1 h, then isometric tension generated in response to agonists was measured. Naproxen or celecoxib ($10^{-9}-10^{-5}$ M) was added cumulatively to the organ bath under different levels of phenylephrine-induced pre-contraction:(1) no phenylephrine; (2) 10–30 nM phenylephrine (10 and 20% of the maximal contractile response), 3) 1–10 μM phenylephrine (maximal contractile response).

Effects of celecoxib on leukocyte adherence

The effects of celecoxib on leukocyte adherence to the vascular endothelium in normal rats and in rats with L-NAME-induced hypertension (2 weeks of L-NAME administration) were examined using an intravital microscopy preparation, as described previously (Muscará *et al*., 1998). Rats ($n=5$ per group) were anaesthetized with sodium pentobarbital (65 mg kg^{-1} i.p.). Images of the mesenteric microcirculation were recorded for 5 min after a 15-min equilibration period. The mesentery was then superfused with bicarbonate-buffered saline containing 3 μM celecoxib or buffer alone and the images were recorded for 5 min beginning 15, 30, 45 and 60 min after the start of the superfusion. This concentration of celecoxib was selected because it is in the range of plasma concentrations required for anti-inflammatory effects in humans (McAdam *et al*., 1999), and is less than the concentration necessary for significant anti-inflammatory effects in the carrageenan-induced paw oedema model (Smith *et al*., 1998). Leukocyte adherence was quantified on video playback in a blind manner. A leukocyte was considered adherent to the endothelium if it remained stationary for 30 s or more.

Additional studies were performed to determine if hypertension altered the propensity of leukocytes to adhere the vascular endothelium in response to a different agonist (*N*-formyl-methionyl-leucine-phenylalanine, FMLP, 5 μM).

Effects of celecoxib on platelet aggregation

Normal rats and rats given L-NAME in the drinking water for 2 weeks were used. The rats were anaesthetized with Halothane and blood was drawn into a syringe from the inferior vena cava. Platelet-rich plasma (PRP) was prepared as described previously (Radomski & Moncada, 1983). Aliquots (0.4 ml) of the PRP were placed in the cuvette of a platelet aggregometer (Payton Instruments, Buffalo, NY, U.S.A.) and continuously stirred at 37°C. After 1 min, celecoxib (3 μM) or vehicle (10 μl) was added to the platelet suspension. One minute later, adenosine diphosphate (ADP; 2.5–40 μM) or thrombin (1–100 u ml^{-1}) was added to the cuvette and aggregation was monitored for the following 5 min.

Statistical analysis

All data are shown as mean±s.e.mean. Differences among groups were analysed using one-way analysis of variance followed by the Student-Neuman-Keuls test for multiple comparisons or, where appropriate, with a paired *t*-test. Values of probability less than 5% ($P<0.05$) were considered significant.

Results

Selection of dose of celecoxib

Celecoxib did not significantly affect COX-1 activity, as measured by whole blood thromboxane synthesis, at any of the doses tested (1–45 mg kg^{-1}). However, celecoxib dose-dependently inhibited COX-2 activity (inflammatory PGE_2 synthesis). At a doses of 5 and 15 mg kg^{-1}, celecoxib inhibited COX-2 activity by 64±10% and 88±3% ($P<0.05$), respectively. Thus, a dose of 10 mg kg^{-1} was selected for subsequent experiments.

Blood pressure studies in normotensive rats

Daily oral administration of celecoxib resulted in a significant and progressive increase in blood pressure in normal rats. The mean increases in blood pressure 1, 2 and 3 weeks after beginning daily treatment with celecoxib were 9, 14 and 30 mmHg, respectively (Figure 1). In contrast, blood pressure did not change significantly in the group treated with vehicle over the 3-week study period.

Daily treatment with celecoxib for 3 weeks had no significant effect on serum thromboxane B_2 or 6-keto $PGF_{1\alpha}$ levels (Figure 2). Also, celecoxib treatment did not significantly affect serum nitrate (31.0±2.0 μM vs 27.7±2.1μM in vehicle-treated) or serum nitrite (1.78±0.07 μM vs 1.66±0.14 μM in vehicle-treated) levels.

Rats treated daily with celecoxib gained weight at a significantly greater rate than the vehicle-treated controls. At the end of 3 weeks of treatment with celecoxib, the mean increase in body weight was 145.6±6.8 g, approximately 20% more than that in the vehicle-treated group (121.5±11.3 g; $P<0.05$). Plasma renin activity was significantly reduced in the celecoxib-treated rats (9.5±1.5 ng ml^{-1} h^{-1} vs 13.6±1.4 ng ml^{-1} h^{-1} in vehicle-treated; $P<0.05$). On the other hand, plasma levels of arginine-vasopressin were increased in the celecoxib-treated rats (24.1±5.9 pg ml^{-1} vs 9.7±1.4 pg ml^{-1} in vehicle-treated; $P<0.05$). Plasma endothelin-1 levels did not differ between the celecoxib- and vehicle-treated groups (19.7±8.6 pg ml^{-1} in celecoxib vs

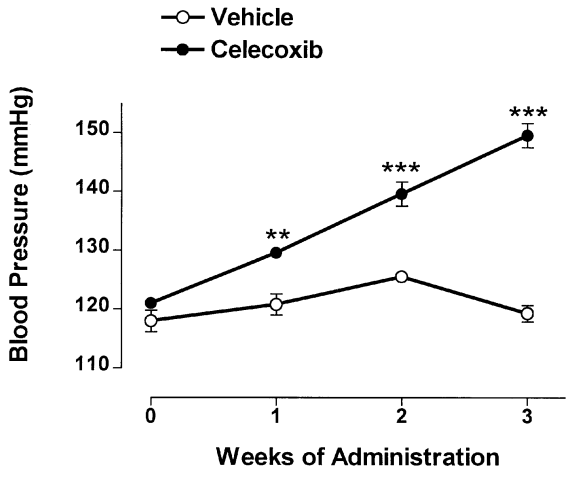

Figure 1 Systemic blood pressure in conscious rats over a 3-week period of daily oral treatment with celecoxib (10 mg kg^{-1}) or vehicle (mean±s.e.mean, $n=5$ per group). **$P<0.01$, ***$P<0.001$ vs the corresponding vehicle-treated group.

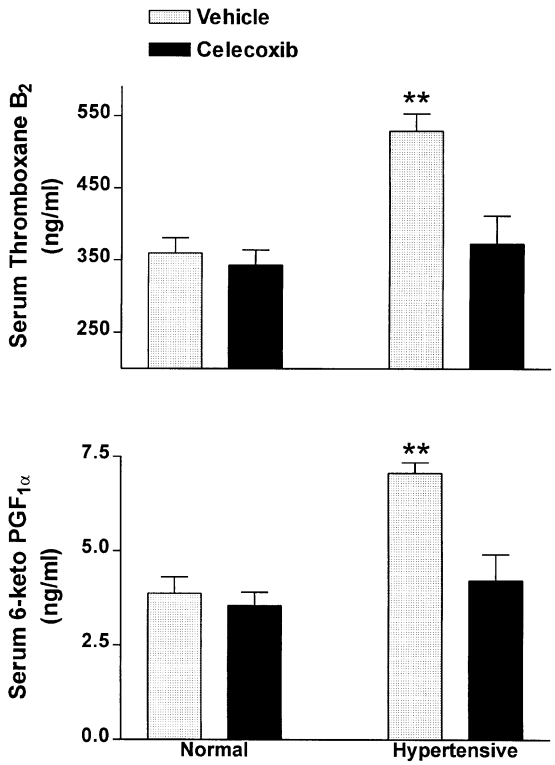

Figure 2 Serum levels of 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 in normal and hypertensive rats following 3 weeks of daily oral treatment with celecoxib (10 mg kg^{-1}) or vehicle (mean ± s.e.mean, $n = 5-7$ per group). Hypertension was induced through addition of L-NAME to the drinking water. Blood was collected 2 h following the final dose of celecoxib or vehicle. $^{**}P < 0.01$ vs all other groups.

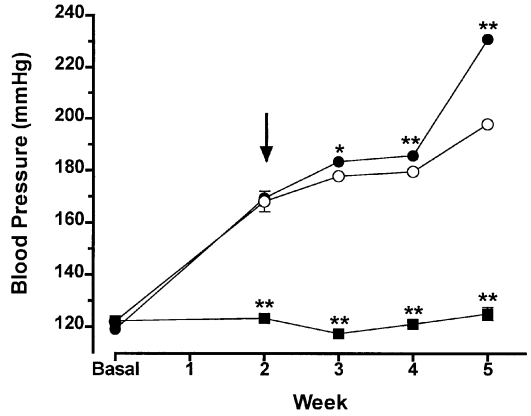

Figure 3 Systemic blood pressure in hypertensive rats over a 3-week period of daily oral treatment with celecoxib (10 mg kg^{-1}, filled circles) or vehicle (open circles) (mean ± s.e.mean, $n = 6-7$ per group). Hypertension was induced through addition of L-NAME to the drinking water. Two weeks after beginning treatment with L-NAME (arrow), the rats started receiving celecoxib or vehicle. A control group received normal drinking water (no L-NAME; filled squares). $^{*}P < 0.05$, $^{**}P < 0.01$, vs the L-NAME + vehicle group.

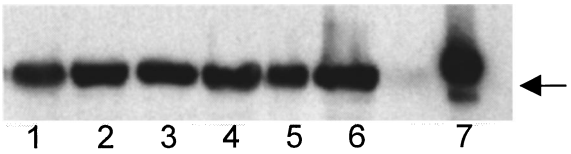

Figure 4 Western blot showing expression of COX-2 in dorsal aorta of normal rats (lanes 1–3) and rats that received L-NAME in the drinking water for 3 weeks (lanes 4–6). Lane 7 is a positive control (COX-2 from a mouse macrophage cell line; RAW 264.7). The monoclonal antibody used was a mouse anti-rat COX-2 from Transduction Labs (Lexington, KY, U.S.A.). Arrow indicates 70 kDa.

11.4 ± 1.2 pg ml^{-1} in vehicle). Plasma urea levels were significantly elevated in the celecoxib-treated rats (12.2 ± 1.1 mg dl^{-1} vs 7.2 ± 0.9 mg dl^{-1} in controls; $P < 0.05$), while plasma creatinine levels were not different in the celecoxib group (197 ± 86 mg dl^{-1}) in comparison to controls (115 ± 12 mg dl^{-1}). None of the rats in this experiment exhibited gastric damage at the end of the 3-week period of treatment with either celecoxib or vehicle.

Blood pressure studies in hypertensive rats

Addition of L-NAME to the drinking water resulted in a progressive increase in blood pressure. By 2 weeks after beginning this treatment, the mean blood pressure had increased from 122 ± 2 to 168 ± 4 mmHg (Figure 3). In rats treated each day with vehicle for the subsequent 3 weeks, the blood pressure continued to increase, reaching a mean of 198 ± 2 mmHg by the end of the fifth week. Daily treatment with celecoxib resulted in a significant increase in blood pressure over that in the vehicle-treated group at all three time-points, with the greatest increase being seen at the end of the fifth week of the study (mean increase of 33 ± 2 mmHg; $P < 0.01$).

Addition of L-NAME to the drinking water resulted in significant increases in serum levels of both 6-keto $PGF_{1\alpha}$ (~82%) and thromboxane B_2 (~47%) compared to the group receiving normal drinking water Figure 4). Daily treatment of hypertensive rats with celecoxib resulted in a significant decrease in both 6-keto $PGF_{1\alpha}$ and thromboxane B_2 levels, such that they were not different from those in normotensive controls. Gastric damage was not observed in any of the hypertensive rats (vehicle- or celecoxib-treated).

COX-2 mRNA and protein was constitutively expressed in the descending aorta and kidney of normal rats. Addition of L-NAME to the drinking water did not significantly change the expression of COX-2 mRNA or protein in these tissues relative to the control group (Figure 4).

In vitro *contractility of descending aorta*

Neither naproxen nor celecoxib ($10^{-9}-10^{-5}$ M) had any direct effect on the contractility of aortic rings prepared from either normotensive or hypertensive rats. The lack of effect of the two drugs was seen across the full range of pre-contraction with phenylephrine.

In vivo *responses to endothelin and norepinephrine*

Endothelin administration causes an early hypotensive response, followed by a more pronounced and long-lasting hypertensive response. Pretreatment with celecoxib resulted in a significant attenuationtion of the hypotensive response to endothelin. For example, in vehicle-treated rats, endothelin-1 at doses of 100 and 250 pmol kg^{-1} caused decreases in mean arterial pressure of 12 ± 3 and 26 ± 5%, respectively. In rats pretreated with celecoxib, these responses were significantly reduced to 5 ± 4 and 14 ± 4%, respectively ($P < 0.01$). Pretreatment with celecoxib also significantly amplified the hyperten-

sive response to endothelin. For example, at a dose of 50 pmol kg^{-1}, mean arterial pressure increased by $28 \pm 4\%$ in vehicle-treated rats, significantly ($P<0.05$) greater than the $11 \pm 3\%$ increase in celecoxib-treated rats.

In contrast to the altered responsiveness to endothelin, pretreatment with celecoxib did not significantly affect the hypertensive responses observed following administration of norepinephrine. For example, the increases of mean arterial pressure in vehicle-treated rats produced with 0.25 and 2.5 $\mu g\ kg^{-1}$ doses of norepinephrine were 42 ± 6 and $69 \pm 9\%$, respectively. In celecoxib-treated rats, the responses to these doses of norepinephrine were 34 ± 5 and $73 \pm 13\%$, respectively.

Leukocyte adherence

In normal rats under basal conditions, there were typically two to four adherent leukocytes per 100 μm length of mesenteric venule (Figure 5). During a 60-min superfusion with celecoxib (3 μM), there was a significant increase in the number of adherent leukocytes. The peak level of leukocyte adherence was observed after 45 min of superfusion with celecoxib, with a mean increase of approximately 6 fold over basal levels.

In hypertensive rats, basal levels of leukocyte adherence were similar to those observed in the normal rats. Also in similarity to the studies of normal rats, superfusion with celecoxib resulted in a marked increase in leukocyte adherence (Figure 5). A significant increase in leukocyte adherence was detected as early as 15 min after exposure to celecoxib. In hypertensive rats, flow through the superfused vessel ceased in two of the five rats prior to completion of the experiment (Figure 5). This contrasted to the situation in normal rats, where cessation of blood flow was not observed in any of the five rats.

The diameter of the vessels examined by intravital microscopy did not change in normotensive or hypertensive rats during the superfusion with buffer or celecoxib. For example, in normal rats in which celecoxib was superfused over the mesenteric venules, the diameter at the end of the 60-min superfusion was $106 \pm 10\%$ of the initial diameter. In the case of hypertensive rats, the final diameter in the three rats in which flow did not stop was $102 \pm 2\%$ of the initial diameter. Even in the two rats in which flow stopped during the celecoxib superfusion, there was no significant change in vessel diameter.

Superfusion of mesenteric venules with FLMP also caused a significant increase in leukocyte adherence (Figure 6). The magnitude of the increase in leukocyte adherence in hypertensive rats was significantly greater than that observed in normotensive rats. Blood flow through the mesenteric venules did not stop in any of the normotensive or hypertensive rats during the 60-min superfusion with FMLP.

Platelet aggregation

ADP and thrombin caused concentration-dependent aggregation of rat platelets *in vitro*. The responses of platelets from rats that had received L-NAME in the drinking water for 2 weeks did not differ from the responses observed with platelets from normal rats. Pre-incubation of the platelets with celecoxib (3 μM) for 1 min prior to stimulation with ADP or thrombin did not significantly affect the magnitude of aggregatory responses.

Discussion

Selective inhibition of COX-2 has been shown to result in markedly less gastrointestinal ulceration than is seen with conventional NSAIDs (Boyce *et al*., 1994; Vane, 1994; Simon

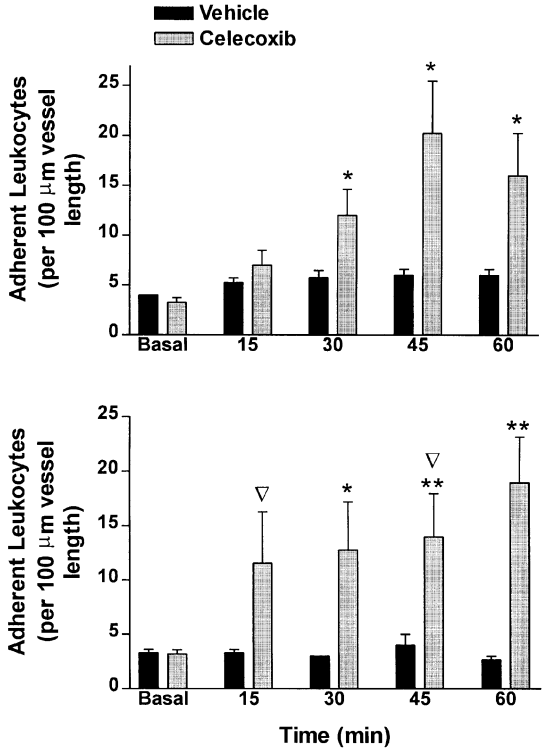

Figure 5 Leukocyte adherence to post-capillary mesenteric venules before and during superfusion of the vessels with celecoxib (3 μM) or vehicle in normotensive (top panel) and L-NAME-induced hypertensive (bottom panel) rats. The data are expressed as mean ± s.e.mean ($n=5-6$ per group). $^*P<0.05$, $^{**}P<0.01$ vs the corresponding vehicle-treated group. ▽ At the time point indicated by this symbol, blood flow through the vessel stopped in one of the rats in the group treated with celecoxib.

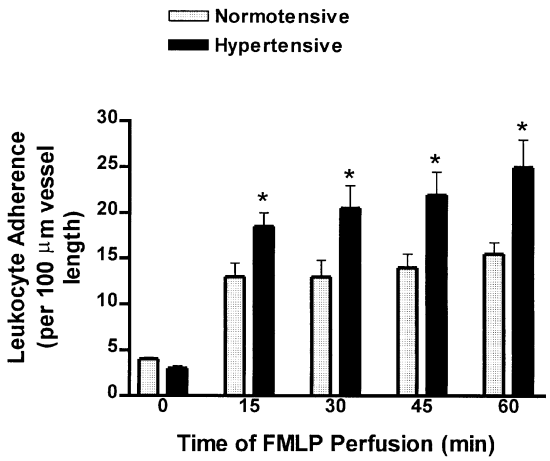

Figure 6 Leukocyte adherence to post-capillary mesenteric venules of normal and L-NAME-induced hypertensive rats before and during superfusion of the vessels with *N*-formyl-methionine-leucine-phenylalanine (FMLP; 5 μM) or vehicle. In both groups, the levels of leukocyte adherence were significantly elevated ($P<0.05$) over basal levels at all times after starting the superfusion with FMLP. Results are expressed as the mean ± s.e.mean ($n=5-6$ per group). $^*P<0.05$ vs the corresponding data from normotensive rats.

et al., 1998). Originally, selective COX-2 inhibition was also proposed to represent a renal-sparing approach to anti-inflammatory therapy (Vane, 1994). However, a number of studies have now documented constitutive expression of COX-2 in the kidney of humans and several other species, as well as the importance of COX-2 in regulating renal function (Harris *et al*., 1994; Harding *et al*., 1997; Komhoff *et al*., 1997). Moreover, two recent reports highlighted the possibility that selective inhibition of COX-2 may, through inhibition of prostacyclin synthesis, promote thrombogenesis (McAdam *et al*., 1999; Catella-Lawson *et al*., 1999). In the present study, celecoxib at an anti-inflammatory dose that is selective for COX-2 significantly increased systemic blood pressure in healthy rats and in rats with pre-existing hypertension. This was not a transient effect of celecoxib, since blood pressure measurements were performed 16–20 h after the final dose of the drug. Celecoxib also significantly increased leukocyte adherence to post-capillary mesenteric venules when superfused over those vessels at a clinically relevant concentration. Thus, the results of the present study suggest that COX-2 can play an important role in terms of modulating blood pressure and adhesive interactions between the vascular endothelium and circulating leukocytes.

Our initial hypothesis was that celecoxib would increase blood pressure by virtue of suppression of vascular prostacyclin synthesis. McAdam *et al*. (1999) found that doses of celecoxib in the range used for treatment of rheumatoid arthritis were capable of suppressing urinary excretion of prostacyclin metabolites in healthy volunteers by more than 80%. In the present study, however, celecoxib did not significantly affect serum levels of 6-keto $PGF_{1\alpha}$ yet did markedly elevate systemic blood pressure. In rats with L-NAME-induced hypertension, serum levels of 6-keto $PGF_{1\alpha}$ were significantly elevated above those of controls. The finding that celecoxib treatment reduced serum 6-keto $PGF_{1\alpha}$ levels to those of normotensive controls indicates that the increased production of this mediator occurred *via* COX-2. We could not detect a significant change in COX-2 mRNA or protein expression in descending aorta or kidney following administration of L-NAME for 3 weeks, but we cannot rule out the possibility that such an induction occurred in the smaller, resistance vessels. On the other hand, the elevated production of prostacyclin could have occurred as a consequence of increased COX-2 activity or increased substrate availability, rather than being due to a change in COX-2 expression. Suppression of prostacyclin synthesis by celecoxib may have contributed to the increased blood pressure in the L-NAME-treated rats. We have previously found that a conventional NSAID, naproxen, could significantly elevate blood pressure in normal rats and in rats receiving L-NAME in the drinking water, and in both cases, was associated with significantly reduced prostacyclin synthesis (Muscará *et al*., 1998). Thus, chronic suppression of nitric oxide synthesis may result in a greater dependence on prostacyclin synthesis in terms of maintenance of vascular tone.

While we did not detect any effect on celecoxib on serum levels of 6-keto $PGF_{1\alpha}$, celecoxib did alter vascular reactivity. Endothelin-1 administration results in an early hypotensive response, which is mediated *via* the release of prostacyclin (De Nucci *et al*. 1988), followed by a pronounced pressor response. Acute celecoxib administration significantly attenuated the hypotensive response to endothelin-1, while significantly enhancing the pressor response. A similar effect can be produced through acute administration of a conventional NSAID, naproxen (unpublished observation). Thus, it is likely that suppression of endothelial prostacyclin synthesis by celecoxib accounts for the effects of this drug on vascular responiveness to endothelin-1. Celecoxib did not directly cause vascular contractile responses *in vitro*, nor did it alter the pressor response to norepinephrine *in vivo*.

The hypertensive effects of celecoxib may have been due to effects of this drug on the kidney. COX-2 is constitutively expressed in the macula densa and thick ascending limb of the rat (Wang *et al*., 1999), and also constitutively expressed in the human kidney (Komhoff *et al*., 1997). COX-2 has been suggested to play a role in regulating renin release (Harris *et al*., 1994; Harding *et al*., 1997) and, as confirmed in the present study, selective inhibition of COX-2 in the rat results in a reduction in plasma renin activity (Wang *et al*., 1999). It is interesting that the rats treated once-daily with celecoxib for 3 weeks exhibited significantly increased weight gain, consistent with fluid retention. Moreover, celecoxib treatment caused a 2.5 fold increase in plasma levels of arginine-vasopressin, which would contribute to fluid retention. The significant elevation of plasma urea together with unchanged plasma levels of creatinine suggest the celecoxib interfered with the normal excretion of urea, independent of generalized effects on renal blood flow or glomerular filtration rate.

Leukocyte adhesion is normally down-regulated by the release from the endothelium of nitric oxide and prostaglandins; thus, inhibition of nitric oxide synthesis or prostaglandin synthesis results in a significant increase in leukocyte adherence (Kubes *et al*., 1991; Wallace *et al*., 1993). In the present study, superfusion of mesenteric venules with celecoxib resulted in a marked increase in the numbers of leukocytes adhering to the vascular endothelium, similar to what has previously been observed with conventional NSAIDs (Asako *et al*., 1992; Wallace *et al*., 1993). The increase in leukocyte adherence observed following NSAID administration has been suggested to be due, at least in part, to a rapid up-regulation of ICAM-1 expression on the vascular endothelium (Wallace *et al*., 1993). Interestingly, COX-2 has been shown to down-regulate ICAM-1 (and VCAM-1) expression on cultured human vascular smooth muscle cells, and has been suggested to play 'a protective role in cardiovascular and inflammatory processes' (Bishop-Bailey *et al*., 1998). In rats that had received L-NAME in the drinking water for the previous 2 weeks, the increase in leukocyte adherence during superfusion with celecoxib occurred more rapidly than in normal rats, and was accompanied in some rats by cessation of flow through the vessel being studied. Enhanced leukocyte adherence in the L-NAME treated rats was also seen when FMLP was used as an agonist, rather than celecoxib. However, cessation of blood flow was not observed when FMLP was used, even though a greater level of leukocyte adherence was observed than in the celecoxib experiments. Despite causing cessation of blood flow in some rats, celecoxib did not affect vessel diameter. Thus, it would appear that in a setting of diminished nitric oxide synthesis, celecoxib produced changes in the vasculature, independent of alterations in vessel diameter that led to impairment of flow. One possibility was that celecoxib increased the propensity of platelets to aggregate. However, the *in vitro* studies of platelet aggregation suggested that platelet reactivity was not significantly affected by prior exposure to celecoxib, consistent with previous reports that this drug does not alter platelet aggregation (McAdam *et al*., 1999).

In summary, a major limitation to the use of conventional NSAIDs is their interference with the effectiveness of anti-hypertensive therapy. NSAIDs are used primarily by the elderly, the same group that is most likely to be using anti-hypertensive therapy. Selective COX-2 inhibitors are an

attractive alternative to conventional NSAIDs because of their reduced toxicity in the gastrointestinal tract. However, the results of the present study suggest that effects of selective COX-2 inhibitors on prostacyclin synthesis and on renal function could result in detrimental effects on blood pressure regulation similar to what is seen with conventional NSAIDs. Moreover, selective COX-2 inhibitors, while not affecting platelet aggregation, can promote the adherence of leukocytes to the vascular endothelium. Taken together, these results suggest that studies of these potential adverse effects of selective COX-2 inhibitors in humans are warranted and that, until such studies are performed, caution should be exercised in using these drugs in patients pre-disposed to hypertension and thrombosis.

This work was supported by a grants from the Heart and Stroke Foundation of Canada and the MRC of Canada. Dr M.N. Muscará is supported by a Merck Pharmacology Fellowship. Dr J.K. Wallace is supported by a MRC Senior Scientist award, and by an Alberta Heritage Foundation for Medical Research Senior Scientist award.

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(Received November 1, 1999
Revised January 5, 2000
Accepted January 11, 2000)