Effects of cyclooxygenase inhibition on canine coronary artery blood flow and thrombosis

Ting-Ting Hong,† Jinbao Huang,† Terrance D. Barrett,‡ and Benedict R. Lucchesi†

†Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan; and ‡Johnson and Johnson Pharmaceutical Research and Development, San Diego, California

Submitted 5 June 2007; accepted in final form 30 September 2007

Hong TT, Huang J, Barrett TD, Lucchesi BR. Effects of cyclooxygenase inhibition on canine coronary artery blood flow and thrombosis. Am J Physiol Heart Circ Physiol 294:H145–H155, 2008. First published October 5, 2007; doi:10.1152/ajpheart.00646.2007.—This study was designed to determine the effect of inhibitors of cyclooxygenase (COX)-1, COX-2, and the nonselective COX inhibitor naproxen on coronary vasoactivity and thrombogenicity under baseline and lipopolysaccharide (LPS)-induced inflammatory conditions. We hypothesize that endothelial COX-1 is the primary COX isoform in the canine normal coronary artery, which mediates arachidonic acid (AA)-induced vasodilatation. However, COX-2 can be induced and overexpressed by inflammatory mediators and becomes the major local COX isoform responsible for the production of anti-thrombotic prostaglandins during systemic inflammation. The interventions included the selective COX-1 inhibitor SC-560 (0.3 mg/kg iv), the selective COX-2 inhibitor nimesulide (5 mg/kg iv), or the nonselective COX inhibitor naproxen (3 mg/kg iv). The selective prostacyclin (IP) receptor antagonist RO-3244794 (RO) was used as an investigational tool to delineate the role of prostacyclin (PGI2) in modulating vascular reactivity. AA-induced vasodilatation of the left circumflex coronary artery was suppressed to a similar extent by each of the COX inhibitors and RO. The data suggest that AA-induced vasodilatation in the normal coronary artery is mediated by a single COX isoform, the constitutive endothelial COX-1, which is reported to be susceptible to COX-2 inhibitors. The effect of the COX inhibitors on thrombus formation was evaluated in a model of carotid artery thrombosis secondary to electrolytic-induced vessel wall injury. Pretreatment with LPS (0.5 mg/kg iv) induced a systemic inflammatory response and prolonged the time-to-occlusive thrombus formation, which was reduced in the LPS-treated animals by the administration of nimesulide. In contrast, neither SC-560 nor naproxen influenced the time to thrombosis in the animals pretreated with LPS. The data are of significance in view of reported adverse cardiovascular events observed in clinical trials involving the use of selective COX-2 inhibitors, thereby suggesting that the endothelial constitutive COX-1 and the inducible vascular COX-2 serve important functions in maintaining vascular homeostasis.

inflammation; vascular reactivity

CONVENTIONAL NONSELECTIVE anti-inflammatory drugs (NSAIDs), such as naproxen, ibuprofen, and aspirin, inhibit both cyclooxygenase (COX)-1 and COX-2. COX-1, the COX isoform expressed constitutively in platelets, mediates the synthesis of thromboxane A2 (TXA2), a prostaglandin known to activate platelets and cause vasoconstriction. The second isoform of COX, COX-2, with ~50% sequence homology to COX-1, is an inducible form of COX during inflammation (30, 46).

Despite considerable study, the functionally important COX isoform or isoforms present in the normal vascular endothelium has been uncertain. On the basis of earlier studies that failed to demonstrate COX-2 expression in unstimulated endothelial cells, COX-1 was thought to be the COX isozyme responsible for vascular endothelial PGI2 synthesis (17, 52). A study by Gross and Moore (21) reported that COX-2 inhibition by SC-58236, a highly selective COX-2 inhibitor, had no effect on arachidonic acid (AA)-induced coronary artery vasodilatation. The authors (21) suggested that AA-induced coronary vasodilatation depends on the presence of COX-1 in the intact, noninjured canine coronary vasculature. On the contrary, our previous study in the canine coronary vascular bed indicated that selective COX-2 inhibition with celecoxib reduced the coronary vasodilator response to intracoronary AA and suppressed the antithrombotic benefits of aspirin in the prevention of electrolytic injury-induced arterial thrombosis (23). Furthermore, our previous data showed that other selective COX-2 inhibitors such as rofecoxib also reduced the coronary vasodilator response to AA and elicited prothrombogenic actions in the presence of vessel wall injury (24). These observations suggested a mechanistic link between the increased risks of adverse thromboembolic events (i.e., myocardial infarction and/or stroke) observed with selective COX-2 inhibitors in several clinical trials (6, 31, 33, 42, 44, 45).

The availability of a selective COX-1 inhibitor, SC-560, provides an opportunity to gain additional insight into the role of COX-1 in the formation of prostacyclin (PGI2) in the normal coronary circulation and its role in thrombogenesis in the absence and presence of systemic inflammation. The present study examines the respective effects of the selective COX-1 inhibitor SC-560, the selective COX-2 inhibitor nimesulide, and the nonselective NSAID naproxen on the vascular reactivity in the canine coronary circulation. Further studies with these compounds were conducted on arterial thrombosis in the presence of lipopolysaccharide (LPS)-induced systemic inflammation. LPS pretreatment was employed to increase the expression of endothelial-derived COX-2 and establish a simulated environment of an inflammatory state (36, 47) and its influence on arterial thrombus formation in response to vessel wall injury.

This study was designed to determine the effect of inhibitors of COX-1, COX-2, and the nonselective COX inhibitor naproxen on coronary vasodilator activity and thrombogenicity under baseline and LPS-induced inflammatory conditions. The highly selective prostacyclin (IP) receptor antagonist,
RO-324794 (RO) (4), served to delineate the role of prostacyclin (PGI₂) in modulating vascular reactivity. We hypothesize that J) in the in vivo, normal coronary artery, COX-1 mediates PGI₂-dependent vasodilation in response to locally administered AA; 2) the COX-1-mediated vasodilator response is attenuated by both COX-1/COX-2-selective inhibitors and by the nonselective NSAID naproxen; 3) platelet COX-1-dependent ex vivo platelet aggregation in response to AA is inhibited by both selective COX-1 antagonists and the nonselective NSAID naproxen but not by selective COX-2 inhibitors; and 4) the coronary artery vasodilator response to AA is mediated by PGI₂.

MATERIALS AND METHODS

Guidelines for the Use and Care of Experimental Animals

Procedures used were approved by and performed in accordance with the guidelines of the University of Michigan Committee on the Use and Care of the Animals and conform to the standards in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23, Revised 1996). The University of Michigan Unit for Laboratory Animal Medicine provided veterinary care.

Drugs and Reagents

SC-560 and nimesulide were obtained from Cayman Chemical (Ann Arbor, MI). Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). RO was provided by Mary Frances Jett from Roche Pharmaceuticals (Palo Alto, CA).

Surgical Preparations for Experimental Models

Purpose-bred beagle dogs, weighing 9–12 kg, were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, and ventilated with room air using a Harvard respirator (Harvard Apparatus, Holliston, MA) adjusted to deliver a tidal volume of 30 ml/kg at a frequency of 12 strokes/min. A catheter was inserted into the right femoral vein for drug administration. Blood pressure was recorded from the right femoral artery using a Millar blood pressure catheter transducer (Millar Instruments, Houston, TX). A standard limb lead II electrocardiogram was recorded continuously to monitor heart rate. All physiological parameters were recorded continuously on a Grass cardiograph was recorded continuously to monitor heart rate. All physiological parameters were recorded continuously on a Grass model 7 polygraph (Grass Instrument, Astro-Med, West Warwick, RI) interfaced to a data acquisition system and a computer for storage and subsequent data analysis.

Model for the Assessment of Coronary Artery Vasomotor Reactivity

The vasodilator responses to the intracoronary administration of AA or acetylcholine (ACh) were determined in the anesthetized canine. An intracoronary infusion cannula, consisting of a 5-mm distal model 7 polygraph (Grass Instrument, Astro-Med, West Warwick, RI) adjusted to deliver a tidal volume of 30 ml/kg at a frequency of 300 strokes/min. A catheter was inserted through the vessel wall and positioned in a manner that allowed the noninsulated segment of the electrode to remain in direct contact with the intimal surface of the vessel. Proper positioning of the electrodes and confirmation of a deep vessel wall injury in the respective vessels were obtained by visual inspection at the conclusion of each experiment.

The intravascular electrode was connected to the positive pole (anode) of a dual-channel stimulator (Grass S88 stimulator and Grass Constant Current Unit; Model CCU1A; Grass Instrument, Astro-Med). The cathode was placed at a distant subcutaneous site. The application of an anodal direct current (DC) to the intimal surface of the carotid artery resulted in a deep lesion in the vascular wall with exposure of the subendothelial components. The current delivered to the vessel was monitored continuously with an ammeter and maintained at 300 μA for a maximal period of 3 h, if thrombotic occlusion failed to develop, or was discontinued 30 min after a stable occlusive thrombus had formed.

Experimental Protocols

Protocol I: effect of SC-560, nimesulide, and naproxen on coronary artery vasomotor reactivity. Fourteen purpose-bred beagle dogs were randomized among three groups that received SC-560 (0.3 mg/kg iv; n = 5), nimesulide (5 mg/kg iv; n = 4), or the nonselective COX inhibitor naproxen (3 mg/kg iv; n = 5). The LCX was isolated and subjected to intracoronary injection of AA (3–300 μg) and ACh (10–300 ng). The dose-dependent vascular responses to each of the agonists were tested at baseline and repeated 1 h after the animals received intravenous administration of the respective COX inhibitors. The dose-related changes in total volume blood flow (in ml) in response to ACh or AA after administration of the respective COX inhibitors were compared with the respective baseline responses. The method for determining total volume blood flow, as described by Coffman and Gregg (13), is illustrated in Fig. 1.

The ex vivo platelet aggregation responses to AA (0.65 mM) and ADP (20 μM) were determined at baseline and 1 h after administration of the respective COX inhibitors. Venous blood samples were drawn at the same time points and prepared for determination of PGE₂ and thromboxane B₂ (TxB₂). Canine-clotted blood was used to test for the inhibition of COX-1 activity, and whole blood stimulated by LPS was used to test for the inhibition of COX-2 activity (51).

Protocol II: effect of SC-560, nimesulide, and naproxen on arterial thrombosis. Figure 2 illustrates the experimental protocol. Thirty purpose-bred beagle dogs were randomized among the following treatment groups: group 1 (n = 10), 0.9% NaCl solution (0.5 ml iv); group 2 (n = 5), LPS (0.5 mg/kg iv) administered 17 h before surgery; group 2A (n = 5), 0.9% NaCl solution (0.5 ml iv); group 2B (n = 5), SC-560 (0.3 mg/kg iv); group 2C (n = 5), nimesulide (5 mg/kg iv); and group 2D (n = 5), naproxen (3 mg/kg iv).

Animals in group 1 served as placebo-treated controls. The 20 animals in group 2 were pretreated with LPS, as indicated above, and 17 h later they were randomized among groups 2A–2D. The animals in group 1 and groups 2A–2D were subjected initially to electrolytic injury (300 μA, DC) of the right carotid artery (RCA), which served as the control vessel. The injury current was applied 30 min after commencing the administration of 0.9% NaCl solution for injection.
(vehicle-treated control vessel). Thirty minutes after the formation of an occlusive thrombus in the RCA, the animals were randomized into the respective subgroups (groups 2A–2D) and were administered the treatment regimen, as outlined above and illustrated in Fig. 2. The animals were administered the respective interventions 30 min before applying the electrolytic injury current to the intimal surface of the left carotid artery (LCA). Application of the anodal injury current continued for 30 min after thrombotic occlusion developed in the LCA, or in the absence of occlusion, the current was terminated 3 h from the time of its onset. In the animals in which the LCA remained patent at the end of the 3 h, the animal was monitored for an additional hour. At the conclusion of each experiment, the respective carotid arteries were removed and opened longitudinally. The vessel segments were inspected to confirm proper positioning of the anodal electrode and the presence of a deep vessel wall lesion. The experimental design allowed each dog to serve as its own control. In each experiment, the RCA served as the drug-treated vessel. The use of both carotid arteries served as the vehicle-treated control vessel, whereas the LCA was used for 30 min after thrombotic occlusion developed in the LCA, or in the absence of occlusion, the current was terminated 3 h from the time of its onset. In the animals in which the LCA remained patent at the end of the 3 h, the animal was monitored for an additional hour. At the conclusion of each experiment, the respective carotid arteries were removed and opened longitudinally. The vessel segments were inspected to confirm proper positioning of the anodal electrode and the presence of a deep vessel wall lesion. The experimental design allowed each dog to serve as its own control. In each experiment, the RCA served as the vehicle-treated control vessel, whereas the LCA served as the drug-treated vessel. The use of both carotid arteries made it possible to compare the effects of each intervention (groups 2A–2D) with those of the vehicle in the same animal, which had received LPS 17 h earlier. The end-point determination was time-to-occlusive thrombus formation with each animal serving as its own control.

Protocol III: IP receptor blockade and its effects on coronary artery vasodilator responses to ACh and AA. The IP receptor antagonist, RO (4), was used as a tool to assess the role of PGI2 as an endogenous mediator of vascular smooth muscle relaxation and alteration in coronary artery blood flow in response to intracoronary administration of AA. RO is a competitive antagonist, lacks agonistic activity at the IP receptor, and acts as a functional antagonist in multiple experimental models (4). The dose of RO required to effectively block the IP receptor was based on the data provided in the publication by Bley et al. (4). Pilot studies in three dogs demonstrated significant dose-related (1.0 and 3.0 mg/kg) inhibition of the vasodilator action of AA in the coronary vascular bed.

Healthy male or female purpose-bred beagle dogs (9–13 kg) were anesthetized, intubated, and ventilated with room air. Hearts were exposed via a left thoracotomy, and the pericardium was removed. The LCX was identified and instrumented with an infusion cannula and ultrasonic flow probe. LCX artery vascular responses to intracoronary injections of ACh (10, 30, and 100 ng) and AA (3, 10, 30, and 100 μg) were evaluated in five dogs before and 1 h after the intravenous administration of RO (3.0 mg/kg).

Ex Vivo Platelet Aggregation

Venous blood samples (anticoagulated with 3.8% sodium citrate) for platelet aggregation studies were obtained at baseline and after each treatment. Ex vivo platelet aggregation was assessed as described previously, in which AA (0.65 mM) and ADP (20 μM) were the agonists used for the induction of platelet aggregation (23).

Determination of COX-1 or COX-2 Inhibition

COX-1 activity was assessed by determining the amount of the platelet-derived TXA2 metabolite, TXB2, in the serum after blood was allowed to clot at 37°C for 1 h. Blood (4 ml) was withdrawn into a plastic syringe and divided into four glass tubes (each containing 1 ml of whole blood). After incubation in a 37°C water bath for 1 h, the clotted blood samples were centrifuged at 3,000 rpm for 10 min (4°C). The supernatant was collected and stored at −70°C for later analysis of the TXB2 concentration.

COX-2 activity was determined by LPS-induced PGE2 formation in whole blood (7). Venous blood (2 ml) was anticoagulated with heparin (10 IU/ml) and divided into two tubes (1 ml/tube). LPS (20 μl, final concentration 10 ng/ml) was added to the whole blood sample and incubated in a 37°C water bath for 24 h. After incubation, the blood was centrifuged at 3,000 rpm for 10 min (4°C), followed by the collection of the plasma supernatant. The plasma was frozen at −70°C for later analysis of PGE2. TXB2 and PGE2 were analyzed by enzyme
immunoassay using the respective kits obtained from Cayman Chemical.

Statistical Analysis

The comparison within each group before and after treatment was analyzed by either a paired t-test (2 time points) or one-way ANOVA followed by the Dunnett’s posttest (≥3 time points) to compare each posttreatment value with the baseline value. The difference across the groups was analyzed by one-way ANOVA followed by the Bonferroni’s posttest to compare between any two groups. The dose-dependent effect of AA- or ACh-induced vasodilatation was compared between the baseline and postdrug treatment by two-way ANOVA. The results were considered significant at a level of \( P < 0.05 \). The data are expressed as means ± SE.

RESULTS

Protocol I

Effect of SC-560, nimesulide, and naproxen on coronary artery vasomotor reactivity. Intracoronary administration of AA produced a dose-related increase in arterial volume blood flow characterized as a dose-dependent increase in peak flow, followed by a dose-related prolongation in the vasodilator response. Both the peak response to AA and the duration of the response were suppressed equally by SC-560, nimesulide, and naproxen. The changes in volume blood flow to graded doses of ACh, an endothelium-dependent vasodilator, were not altered after the administration of SC-560, nimesulide, or naproxen.

The LCX changes in coronary artery volume blood flow to ACh and AA in the respective groups are summarized in Figs. 3A, 4A, and 5A. The observation that each of the COX inhibitors tested produced a near maximal suppression of the vasodilator responses to AA suggests that a single COX isozyme mediates the vasodilator response.

Effect of SC-560, nimesulide, and naproxen on ex vivo platelet aggregation. The ex vivo platelet aggregation responses to AA and ADP were determined in platelet-rich plasma prepared from each animal before and after treatment with the respective COX inhibitors. ADP-induced platelet aggregation was not affected by the administration of SC-560, naproxen, or nimesulide. The ex vivo platelet aggregation responses to AA, however, were inhibited significantly by SC-560 (a COX-1 inhibitor) and naproxen (a COX-1/COX-2 inhibitor) but not by nimesulide (a COX-2 inhibitor) (Table 1).

Effect of SC-560, nimesulide, and naproxen on inhibition of COX-1/COX-2 in the canine whole blood assay. The whole blood assay for assessing inhibition of COX activity in whole blood is a standard and accepted method for assessing the selectivity of a compound (17). Canine whole blood was divided into two aliquots. One aliquot, without heparin, was
allowed to clot at room temperature and served for the determination of platelet COX-1-derived TxB2 formation, whereas the second aliquot, anticoagulated with heparin, was incubated at 37°C for 24 h with LPS and served for determining the formation of the COX-2-derived PGE2 primarily from monocytes.

The serum TxB2 concentration is an indicator of the COX-1 activity and is derived mostly from the blood platelets, which are stimulated during activation of the coagulation cascade and the clotting of whole blood. The serum TxB2 concentration was reduced significantly by both SC-560 and naproxen in blood samples obtained from dogs that had been pretreated with the respective compounds. The results are consistent with what is expected to occur with an inhibitor of the platelet COX-1 isoform. However, the treatment of the blood donor animal with nimesulide, a selective COX-2 inhibitor, resulted in an insignificant decrease in the generation of TxB2 ($P < 0.05$), a finding that agrees with the relatively greater selectivity of nimesulide for COX-2.

Fig. 3. A: volume flow change (in ml) in left circumflex coronary artery in response to the intracoronary injection of acetylcholine (ACh; 10, 30, 100, and 300 ng; left) and AA (10, 30, 100, and 300 μg; right) before and 1 h after the intravenous administration of 0.3 mg/kg SC-560 ($n = 5$ animals/group). *$P < 0.05$ when compared with the respective baseline response. B: the effect of vehicle or SC-560 (0.3 mg/kg iv) on serum thromboxane B2 (TxB2) concentration and LPS-induced PGE2 production in whole blood ($n = 5$ animals/group). The serum TxB2 concentration was reduced significantly after SC-560 treatment. The LPS-induced PGE2 production was not affected by SC-560 treatment ($**P < 0.01$ when compared with baseline).

Fig. 4. A: volume flow change (in ml) in left circumflex coronary artery in response to the intracoronary injection of Ach (10, 30, 100, and 300 ng; left) and AA (3, 10, 30, and 100 μg; right) before and 1 h after the intravenous administration of 5 mg/kg nimesulide ($n = 4$ animals/group). *$P < 0.05$ compared with the baseline response. B: the effect of nimesulide (5 mg/kg iv) on serum TxB2 concentration and LPS-induced PGE2 production in whole blood ($n = 4$ animals/group). The serum TxB2 concentration was reduced after nimesulide treatment, although the difference was not statistically significant. The LPS-induced PGE2 production was significantly inhibited by nimesulide treatment (*$P < 0.05$ when compared with baseline).
ity of nimesulide for the COX-2 isoform. On the other hand, nimesulide or naproxen, but not SC-560, significantly inhibited LPS-induced PGE2 (an indicator of COX-2 activity) formation derived primarily from monocytes in whole blood. The data are presented graphically in Figs. 3B, 4B, and 5B.

Protocol II

Effects of LPS administration on carotid artery thrombosis in response to vessel wall injury. LPS pretreatment for 17 h resulted in a significant increase in the circulating white blood cell count and a reduction in the platelet count (Table 3). The time to occlusion in the RCA was increased in the LPS-treated animals (108.2 ± 8.1 min) compared with the placebo-treated control group (71.4 ± 7.9 min) (Table 2 and Fig. 6).

Effect of nimesulide, naproxen, and SC-560 on carotid artery thrombosis in LPS-pretreated canines. In the LPS-pretreated animals, the subsequent administration of nimesulide, naproxen, or SC-560 differentially altered the time-to-occlusive arterial thrombus development in the LCA. Selective COX-2 inhibition by nimesulide significantly decreased the time to occlusion in the LCA, whereas SC-560 and naproxen were associated with an increase in time-to-occlusive thrombus formation (Table 3 and Fig. 6).

Protocol III

Prostacyclin receptor (IP) blockade and its effects on coronary artery vasodilator responses to ACh and AA. The administration of the IP receptor antagonist RO significantly decreased the coronary artery vasodilator responses to the intracoronary administration of AA in a dose-dependent manner (Fig. 7). This result is consistent with the known mechanism by which AA is converted by endothelial COX to the vasodilator PGI2. Furthermore, the vasodilator response to the intracoronary administration of ACh was not altered by pretreatment with RO. The inability of RO to influence the vascular response to ACh is in agreement with its known pharmacological action. ACh is an endothelium-dependent vasodilator that mediates the vascular response through endothelial-derived nitric oxide (NO) synthesis. Thus RO is a

Table 1. The effect of SC-560, nimesulide, or naproxen on percent ex vivo platelet aggregation

<table>
<thead>
<tr>
<th></th>
<th>AA, 0.65 mM</th>
<th>ADP, 20 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Baseline</td>
</tr>
<tr>
<td>SC-560</td>
<td>5</td>
<td>80.0±2.9</td>
</tr>
<tr>
<td>Nimesulide</td>
<td>4</td>
<td>73.8±4.5</td>
</tr>
<tr>
<td>Naproxen</td>
<td>5</td>
<td>85.0±2.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals/group. *P < 0.01 when compared with baseline from each group. AA, arachidonic acid.

Table 2. The effect of SC-560, nimesulide, or naproxen on carotid artery: time to occlusion in LPS-pretreated canines

<table>
<thead>
<tr>
<th></th>
<th>RCA Vehicle, min</th>
<th>LCA Treated, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC-560</td>
<td>95.6±16.1</td>
<td>118.6±18.3</td>
</tr>
<tr>
<td>Naproxen</td>
<td>99.0±23.7</td>
<td>128.6±27.6</td>
</tr>
<tr>
<td>Nimesulide</td>
<td>116.0±13.6</td>
<td>153.0±27.4</td>
</tr>
<tr>
<td>Naproxen</td>
<td>122.2±11.5</td>
<td>57.0±11.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 animals/group. *P < 0.01 when time to occlusion in the right carotid artery (RCA) and left carotid artery (LCA) were compared within each group by a paired t-test.
selective inhibitor of the IP receptor in the coronary vascular bed in the canine heart and provides evidence favoring PGI2 as the mediator for the observed vasodilator response in the canine normal coronary artery.

DISCUSSION

The present study compares the actions of a nonselective NSAID naproxen with selective inhibitors of COX-1 and COX-2 for their respective effects on coronary artery blood flow responses to ACh and AA. In addition, the selective COX inhibitors and naproxen were compared for their antithrombotic efficacy in the absence and presence of a simulated systemic inflammatory state 17 h after the intravenous administration of LPS (39). Each of the COX inhibitors tested suppressed AA-induced increases in coronary artery blood flow to a similar extent irrespective of their reported selectivity for the COX isoforms. In a separate series of studies, nimesulide, a selective COX-2 inhibitor (49, 50), significantly reduced the time-to-carotid artery thrombotic occlusion, whereas nei-

ther the nonselective NSAID naproxen nor the selective COX-1 inhibitor SC-560 had a significant effect in modifying the development of thrombus formation in response to vessel wall injury.

The selectivity of the pharmacological interventions was assessed with the use of standard assays conducted on whole blood for COX inhibition (7, 50) and suppression of ex vivo platelet aggregation in response to AA (12). The formation of TxB2 in clotted whole blood was used to assess COX-1 inhibition, and PGE2 production in LPS-treated heparinized whole blood was used to assess COX-2 inhibition. The COX-1 selective inhibitor SC-560 and the nonspecific NSAID naproxen suppressed TxB2 formation, whereas nimesulide, a selective COX-2 inhibitor, was associated with a nonsignificant reduction in the formation of the TxA2 metabolite when compared with the control value (Fig. 4). In keeping with the observations in the whole blood assay, SC-560 and naproxen inhibited AA-induced platelet aggregation, whereas nimesulide was without significant effect. Platelet aggregation responses to ADP were not inhibited by any of the treatments, thereby demonstrating the selectivity of the response for the AA cascade leading to the formation of platelet COX-1-derived TxA2.

The data obtained in the whole blood assay are in keeping with the selectivity described in the literature for the respective compounds (28, 43, 48, 50). It is significant to note that COX-2 inhibition was not observed with SC-560, whereas the selective COX-2 inhibitor nimesulide inhibited the COX-2 isoform. Brenneis and coworkers (5) questioned the selectivity of SC-560 for COX-1 over COX-2. The authors suggested that SC-560 does not behave as a selective COX-1 inhibitor in some cell types in vitro and inhibits COX-2 under some conditions. Warner et al. (53) reported that the potency of NSAIDs for inhibition of COX-1 could be influenced by the presence or absence of plasma proteins. On the other hand, the efficacy of SC-560 for COX-1 was not influenced significantly by the presence or absence of plasma proteins. The current studies were conducted in intact animals where the concentration of plasma proteins is fixed and cell integrity is not compromised. Therefore, it is reasonable to assume that SC-560 acts as a selective COX-1 inhibitor at the dose used and under the in vivo experimental conditions in which physiological responses were in accord with the known pharmacodynamic properties of the respective interventions.

Characterization of the COX isoforms in normal human blood vessels shows only the expression of COX-1 with no detectable expression of COX-2 (29, 30). Similar results were found in the canine coronary artery by Stanfield et al. (46). Kawka et al. (26) performed double-label immunofluorescence microscopy studies to evaluate the extent of colocalization of prostacyclin synthase (PGIS) and thromboxane synthase (TXS) with the selectivity described in the literature for the respective interventions.
provide evidence for a functionally larger contribution of COX-1 to the synthesis of prostacyclin and thromboxane in aortic tissue. The data presented by Kawka et al. (26) demonstrate a significant role for COX-1 in the formation of both prostacyclin and thromboxane in normal arterial tissue. The results illustrate significant tissue differences in the coexpression patterns of these two enzymes, which might contribute to the confusion that has existed in the literature regarding the physiological role of endothelial COX-1.

An additional insight into the functional significance of endothelial COX-1 is provided by Mitchell et al. (28, 29), who express the view that endothelial COX-1 is more susceptible to inhibition by NSAIDs than platelet COX-1 and that selective COX-2 inhibitors are effective inhibitors of endothelial cell COX-1 while not altering the activity of the platelet COX-1 isoform. The results of the present study indicate that the inhibition of COX-1 by SC-560 as well as the nonselective and selective COX-2 inhibitors resulted in a comparable reduction in the AA-induced increases in coronary artery flow in the canine heart. If both COX isozymes had functional roles in the response to intracoronary administration of AA, then submaximal responses to selective COX-1 and COX-2 inhibition would have been anticipated. The loss of selectivity for COX inhibition as observed under cell-free conditions, as described in previous publications (5, 29, 30, 53), could not explain this, since cellular integrity and plasma protein conditions of this test system have not been perturbed. The selective IP receptor antagonist RO effectively negated the vasodilator responses to the intracoronary artery administration of increasing doses of AA, thereby further substantiating the role of PGI2 as the mediator of the AA-induced vasodilator response in the normal coronary artery.

We previously asserted that the selective COX-2 inhibitor celecoxib suppressed the vasodilator responses to intracoronary injection of AA via the selective inhibition of COX-2 (23). When the findings of Kawka et al. (26) and Mitchell et al. (28, 29) are taken into account, this would suggest that in the normal coronary vascular bed, celecoxib was insufficiently selective for the COX-2 over the constitutive endothelial cell COX-1, which was the source for PGI2 and is inhibited by coxibs.

As with the previously reported (23) suppression of AA-induced vasodilatation by celecoxib, it is most likely the result of endothelial COX-1 inhibition without affecting platelet COX-1, since the inducible COX-2 is not present in the canine normal endothelium and is induced by inflammatory stimuli such as bacterial endotoxin, cytokines (9, 10), and shear stress (34, 39). Our earlier study results as well as the present findings are in agreement with the observations published by Mitchell et al. (29) and Kawka et al. (26). The authors demonstrated that

Table 3. Hematologic parameters in the LPS-treated dogs

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>RBC, K/μl</th>
<th>WBC, K/μl</th>
<th>PLT, K/μl</th>
<th>AA</th>
<th>ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2A, LPS + vehicle</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>5.7±0.4</td>
<td>11.6±1.6</td>
<td>346.6±59.0</td>
<td>89.0±4.2</td>
<td>83.6±2.7</td>
</tr>
<tr>
<td>17 h LPS, 0.5 mg/kg</td>
<td></td>
<td>6.3±0.3</td>
<td>18.8±1.9*</td>
<td>260.8±56.4</td>
<td>81.8±5.4</td>
<td>73.4±7.3</td>
</tr>
<tr>
<td>LPS + vehicle</td>
<td></td>
<td>6.4±0.5</td>
<td>17.6±3.8*</td>
<td>280.8±32.2</td>
<td>74.0±10.8</td>
<td>61.8±7.8</td>
</tr>
<tr>
<td>Group 2B, LPS + SC-560</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>6.1±0.2</td>
<td>8.2±1.0</td>
<td>444.8±53.8</td>
<td>87.6±3.4</td>
<td>90.6±2.5</td>
</tr>
<tr>
<td>17 h LPS, 0.5 mg/kg</td>
<td></td>
<td>6.8±0.2</td>
<td>18.4±1.1*</td>
<td>280.0±34.7</td>
<td>83.0±3.0</td>
<td>78.0±2.5</td>
</tr>
<tr>
<td>LPS + SC-560</td>
<td></td>
<td>6.7±0.3</td>
<td>14.4±1.2*</td>
<td>290.3±84.3</td>
<td>13.8±3.9*</td>
<td>58.2±4.6</td>
</tr>
<tr>
<td>Group 2D, LPS + naproxen</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>6.4±0.5</td>
<td>4.9±1.3</td>
<td>279.3±28.7</td>
<td>92.6±2.6</td>
<td>78.8±12.5</td>
</tr>
<tr>
<td>17 h LPS, 0.5 mg/kg</td>
<td></td>
<td>7.2±0.2</td>
<td>15.5±3.5*</td>
<td>220.0±25.6</td>
<td>79.2±2.6</td>
<td>54.8±11.4</td>
</tr>
<tr>
<td>LPS + naproxen</td>
<td></td>
<td>6.5±0.2</td>
<td>10.4±3.4*</td>
<td>227.5±43.2</td>
<td>9.8±2.7†</td>
<td>43.6±10.8</td>
</tr>
<tr>
<td>Group 2C, LPS + nimesulide</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>6.5±0.2</td>
<td>6.8±0.8</td>
<td>334.0±30.0</td>
<td>91.4±3.9</td>
<td>86.8±4.0</td>
</tr>
<tr>
<td>17 h LPS, 0.5 mg/kg</td>
<td></td>
<td>6.7±0.2</td>
<td>17.1±1.5*</td>
<td>154.8±10.1</td>
<td>79.4±2.5</td>
<td>81.4±2.4</td>
</tr>
<tr>
<td>LPS + nimesulide</td>
<td></td>
<td>6.0±0.7</td>
<td>13.8±1.7*</td>
<td>215.0±51.3</td>
<td>64.8±11.0</td>
<td>63.6±7.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals/group. *P < 0.05 when compared with baseline from each group; †P < 0.01. RBC, red blood count; WBC, white blood count; PLT, platelets.
despite normal human vessels and normal endothelial cells containing COX-1 without any detectable COX-2, nonselective NSAIDs and COX-2-selective drugs more readily inhibit endothelial COX-1 than COX-1 in platelets.

A study by Gross and Moore (21) yielded results that are in disagreement with the suggestion that celecoxib-induced COX-2 inhibition prevents AA-induced increases in blood flow in normal coronary arteries. Using an experimental protocol similar to that published by Hennan et al. (23) as well as that in the present study, Gross and Moore (21) compared the nonselective COX-1/COX-2 inhibitor naproxen with the COX-2-selective inhibitor SC-58236 on coronary vasodilator responses in the anesthetized dog. Coronary vasodilation was induced by direct intracoronary administration of ACh or AA in control animals and in animals pretreated with either naproxen or SC-58236. As expected, naproxen attenuated the AA-induced vasodilatation (PGI2 dependent) and was without effect on ACh-induced vasodilatation (NO dependent). Surprisingly, SC-58236 failed to attenuate the AA-induced vasodilator response. The latter observation and conclusions are in disagreement with our previously published findings (23, 24) as well as those in the present study and in recent literature (26, 29, 30). A possible reason for the discrepancy might be due to the manner in which the changes in coronary blood flow were calculated. In our previous studies (23, 24), as well as in the present study, blood flow changes were based on total volume blood flow (area under the total flow response), as described by Coffman and Gregg (13). An examination of the blood flow pattern in response to increasing doses of AA indicates that the volume blood flow, its duration, and peak flow values increase with increasing doses of AA. Therefore, the use of only the peak blood flow values, as presented by Gross and Moore (21), would lead to an underestimation of the total changes in blood flow, as illustrated in Fig. 1. Another possible explanation for the discrepancy (in addition to the difference in how the vasodilatation results are calculated) could be that the compound used by Gross and Moore (21) could have a unique mechanism of action that renders it ineffective against endothelial cell COX-1.

Caughey et al. (11) examined the synthesis of prostanooids derived from either COX-1 or COX-2 by human umbilical vein endothelial cells. They observed that TxA2 is the predominant COX-1 product, whereas the upregulation of COX-2 by IL-1 is associated with a greater increase in the synthesis of PGI2 and PGE2 than of TxA2. Both the PGI synthase and PGE synthase, but not TXS, were upregulated by IL-1. Additionally, an examination of the substrate concentration dependencies of PGI3, PGE2, and TxA2 synthases suggests that different kinetic parameters of the terminal synthases are a major determinant of the dominance of PGI2 and PGE2 production. Similar associations have been observed in rat peritoneal macrophages in which COX-1 was linked with TxA2 production, whereas the induction of COX-2 by LPS shifted prostanooid synthesis to favor PGE2 (8, 32) and PGI2 synthesis (8). In the present study, time-to-occlusive thrombus formation in the LPS-treated animals was prolonged significantly compared with controls. This finding is consistent with the view that the LPS-mediated induction of endothelial COX-2 and PGIS along with an increase in the synthesis of PGI2 would serve to counter platelet reactivity at the site of vessel wall injury.

Under conditions of low total COX activity, as when only COX-1 is present, TxA2 production predominates due to a higher rate constant of TXS. Upon endothelial cell stimulation after systemic administration of LPS, total COX activity would be increased due to the induction of COX-2. TXS becomes saturated with prostaglandin H2, whereas PGI2 and PGE2 synthases respond to the increased COX activity with increased synthesis of PGI2 and PGE2. The major determinant of increased PGI2 and PGE2 production over TxA2 by LPS treatment is increased total COX activity in combination with different kinetic characteristics of the terminal synthases. The selective increase in PGI synthase and PGE synthase activity further augments the increase in PGI2 and PGE2 synthases over that of TxA2 (11).

Pretreatment (17 h) of the animals with LPS resulted in a significant increase in the time-to-thrombotic occlusion in response to electrolytic induction of vessel wall injury. The dose of LPS was effective in inducing a systemic inflammatory response, as demonstrated by the elevation in the white blood cell count and body temperature consistent with observations reported in the literature (22, 25, 27, 35, 38). LPS was administered with the intent of maximizing the expression of inducible COX-2-derived prostaglandins (PGI2) in the endothelium and circulating inflammatory cells (monocytes) and tissue macrophages. Analysis of the time to occlusion in vehicle-treated control animals versus the LPS-treated animals indicated an increase in the time-to-thrombotic occlusion in the latter group. The data are interpreted as suggesting an LPS-mediated antithrombogenic effect, as suggested by Tseng et al. (51). The purported LPS-induced antithrombotic effect was annulled by pretreatment with the COX-2-selective inhibitor nimesulide. We interpret this as an indication that the inhibition of COX-2-derived PGI2 removes a protective constraint on thrombogenesis that is otherwise enhanced in the presence of LPS-induced endotoxemia (38). This suggestion is supported by the observation that nimesulide reduced the time-to-thrombotic occlusion, whereas naproxen and SC-560 both exhibited a trend toward prolonging the time-to-occlusive thrombus formation supposedly resulting from the inhibition of platelet COX-1-derived TxA2 synthesis. Similar results of a reduction in time-to-thrombotic occlusion in response to deep vessel wall injury were obtained previously with the selective COX-2 inhibitors celecoxib (23) and rofecoxib (24).

LPS was administered 17 h before initiating the procedure to elicit thrombus formation in the carotid artery at a time when endotoxemia had been established. Acute inflammatory reactions, in contrast to chronic inflammatory reactions, are usually self-limiting and undergo resolution, which is reported to be dependent on COX-2 (14, 19, 37, 41, 54). The inflammatory response, often viewed as being detrimental, might actually serve to protect against irreversible injury but can itself become dysregulated with serious consequences to the host. There is a convincing body of published reports supporting the concept that the initial phase of inflammation is followed by a second phase that initiates resolution of the inflammatory response and in which the induction of COX-2 and the synthesis of PGI2 have a prominent anti-inflammatory action (14, 15, 18–20, 54). Thus the late phase of LPS-induced endotoxemia might be associated with the induction of COX-2 and PGI2 synthesis in endothelial cells and monocytes that suppress platelet adhesion.
to the injured vessel wall and aggregation, thereby prolonging the time-to-occlusive thrombus formation. Inhibition of COX-2 and the decline in PGI2 synthesis would favor the development of an occlusive platelet-dependent thrombus, as was observed when nimesulide was administered to the animals in the late phase of LPS-induced endotoxemia.

Two aspects of the pharmacological action of the selective COX-2 inhibitors in the dog thrombosis model are worthy of note. The first is the effect on coronary blood flow in which the selective COX-2 inhibitors favored thrombus formation (decreased time to occlusion), and the second is that the selective COX-2 inhibitors did not suppress ex vivo platelet reactivity in response to AA. The apparent neutral effect of both SC-560 and naproxen on time to thrombosis illustrates the importance of platelet COX-1-derived TxA2 in thrombus formation. The data also suggest that in the presence of a systemic inflammatory response and/or vascular inflammation, a COX-1-selective inhibitor such as SC-560, which inhibits platelet COX-1 but not the inducible COX-2, in the vascular endothelium and inflammatory cells (monocytes and macrophages) would have antithrombotic actions through the sparing of COX-2-derived PGI2.

The results presented in this study emphasize the important role of endothelial COX-2 induction in vascular homeostasis by favorably altering the ratio of prostanoids from a prothrombotic (high TxA2/PGI2) to an antithrombotic (high PGI2/TxA2) combination. The in vivo study results suggest that the induction of COX-2 in the endothelium results in increased synthesis of PGI2, but not TxA2, thereby favoring an antithrombogenic state. The use of selective COX-2 inhibitors, although relatively free of thrombotic consequences in most healthy individuals, could result in an exacerbation of the potential for thrombotic complications, particularly in patients with inflammatory conditions such as vasculitis (16). Because COX-2 inhibitors do not suppress platelet COX-1-derived TxA2 synthesis, unlike conventional NSAIDs, they would further alter the TxA2/PGI2 balance toward a prothrombotic state. COX-2 is not detected in healthy arterial and venous tissues but is highly expressed in atherosclerotic lesions (1–3, 40). The cardiovascular consequences of COX-2 inhibition are further emphasized by clinical trials that reported a marked increase in myocardial infarction in patients using a selective COX-2 inhibitor (6, 33).

The results of this study provide evidence that AA-induced vasodilation in the normal coronary vasculature is dependent on COX-1 formation of PGI2, which can be prevented by selective COX-2 inhibitors as well as by traditional NSAIDs. In addition, the data from the thrombosis studies suggest a mechanism by which the anticipated prothrombotic vascular response to electrolytic injury becomes self-limiting in animals pretreated with LPS, due to the induction of COX-2 and the increased production of PGI2. The data suggest that the shift in total cellular COX activity and a selective induction of PGIS, but not TXS, mediates an antithrombogenic response that can be abrogated by subsequent administration of a selective COX-2 inhibitor. The data are of significance when viewed with reported adverse cardiovascular events observed in clinical trials involving the use of selective COX-2 inhibitors, thereby suggesting that the inducible vascular COX-2 serves an important function in maintaining vascular homeostasis.

ACKNOWLEDGMENTS

We thank Edward M. Driscoll for technical assistance. We thank Erin A. Booth for the critical review of the manuscript.

GRANTS

The Cardiovascular Research Fund of the University of Michigan Medical School, Department of Pharmacology, supported this study.

REFERENCES

Effect of COX-1/COX-2 inhibitors on coronary flow and thrombosis.


