Cyclooxygenase-2 Blockade Does Not Impair Endothelial Vasodilator Function in Healthy Volunteers

Randomized Evaluation of Rofecoxib Versus Naproxen on Endothelium-Dependent Vasodilatation

Subodh Verma, MD, PhD; Satish R. Raj, MD, FRCP; Lana Shewchuk, BSc, RN; Kieren J. Mather, MD, FRCP; Todd J. Anderson, MD, FRCP

Background—From a cardiovascular standpoint, the safety of cyclooxygenase-2 (COX-2) blockers has been a topic of increasing concern. This concern stemmed from observations indicating that the COX-2 isoform is the major source of endothelium-derived prostacyclin and, hence, that selective blockade of this enzyme may impair endothelial health. To investigate this matter, we examined the effects of 7 days of treatment with rofecoxib versus naproxen on endothelial function in healthy volunteers.

Methods and Results—Thirty-five healthy volunteers were randomized to receive 7-day treatment with either rofecoxib (25 mg/d, n=18) or naproxen (750 mg/d, n=17). Vascular response measurements were conducted using forearm strain-gauge plethysmography. Changes in forearm blood flow in response to the endothelium-dependent vasodilator acetylcholine (3, 10, and 30 μg/min) and the endothelium-independent vasodilator sodium nitroprusside (1 and 10 μg/min) were assessed before and after treatment. Acetylcholine evoked a dose-dependent increase in forearm blood flow in all groups. Importantly, treatment resulted in no change in acetylcholine-mediated increases in forearm blood flow in either group (naproxen, P=0.27; rofecoxib, P=0.58). Similarly, there was no change in forearm blood flow in response to sodium nitroprusside (naproxen, P=0.55; rofecoxib, P=0.63).

Conclusions—We herein describe, for the first time, the effects of COX-2–selective inhibition on endothelium-dependent vasodilatation in healthy adults. COX-2 blockade, when used at the doses employed therapeutically (which are known to inhibit vascular prostacyclin production) did not result in significant changes in endothelial vasodilator responses in healthy volunteers. The effects of COX-2 inhibitors on vasodilator responses in patients with coronary artery disease remain to be determined. (Circulation. 2001;104:2879-2882.)

Key Words: endothelium-derived factors • patients • prostaglandins

From a cardiovascular standpoint, the safety of cyclooxygenase-2 (COX-2) inhibitors has been the topic of much discussion, debate, and anxiety. The concerns about the use of these agents stemmed from observations suggesting that the COX-2 isoform may be one of the major sources of endothelium-derived prostacyclin (PGI2) under physiological conditions. These observations raised the possibility that selective blockade of COX-2 may impair endothelial health and promote cardiovascular disease.

The present study was designed to determine the effects of selective versus nonselective COX blockade (with rofecoxib and naproxen, respectively) on endothelium-dependent and -independent vascular responses in healthy volunteers. We hypothesized that COX-2 blockade at doses known to inhibit endothelium-derived PGI2 would impair endothelial function in healthy volunteers.

Methods

Patients
Healthy volunteers were recruited with the following exclusion criteria: known atherosclerotic vascular disease, blood pressure >140/90 mm Hg, treatment with vasoactive medications or NSAIDs, hypercholesterolemia (total cholesterol >6.2 mmol/L), hypertriglyceridemia (triglycerides >2.3 mmol/L), smoking history, diabetes, microalbuminuria or macroalbuminuria, current participation in another clinical trial, and contraindications to NSAID therapy.

Protocol
Thirty-five subjects underwent vascular response measurements using forearm plethysmography, before and after randomization to 7 days of treatment with rofecoxib (25 mg once daily, n=18) or naproxen (750 mg sustained release once daily, n=17). Cuff blood pressure and body mass index were determined before the vascular study. Blood pressure was measured again after treatment.
Vascular Responses

Forearm blood flow (FBF) responses were determined using standard techniques of forearm strain-gauge plethysmography, which were described previously by our laboratory. Dose-response profiles were obtained for brachial arterial infusions of the endothelium-dependent vasodilator acetylcholine (Ach; at 3, 10, and 30 μg/min) and the endothelium-independent vasodilator sodium nitroprusside (SNP; at 1 and 10 μg/min). The sequence of Ach and SNP was randomized on the pretreatment day for each patient and repeated on the second study day. Each dose was infused for 6 minutes. Vasodilator infusions were separated from each other by infusions of normal saline for at least 18 minutes to allow flow to return to baseline, with flow measurements performed during the last 3 minutes of each infusion. Measurements were performed simultaneously in both the infused and contralateral arms. Flow data analysis was performed at a later date by a blinded investigator. Data were expressed as the percent increase in flow relative to the immediately preceding baseline measurement.

Statistics

Data are expressed as mean±SD. Statistical analyses were performed by repeated-measures ANOVA, with significance set at P≤0.05.

Results

Demographics

Patient demographics are depicted in the Table. The groups were matched in terms of age, sex, blood pressure, body mass index, glucose, and cholesterol. Medication compliance was 100% by pill count. Follow-up was complete. One week of treatment, with either rofecoxib or naproxen, did not affect blood pressure (Table).

Plethysmography

Plethysmography results are presented as a ratio of the infused to the noninfused arm. Baseline FBF did not differ within or between study days in any group (n=0.89). Figure A depicts the effects of 7 days of treatment with rofecoxib and naproxen on endothelium-dependent vasorelaxation. Ach evoked a dose-dependent increase in FBF in both groups. Importantly, treatment resulted in no change in Ach-induced increases in FBF in either group (FBF before versus after treatment: naproxen, P=0.27; rofecoxib, P=0.58). Figure B depicts the percent increase in FBF in response to peak dose SNP (10 μg/min) before and after treatment with either rofecoxib or naproxen. There was no significant change in FBF responses at any dose studied (rofecoxib, P=0.63; naproxen, P=0.55).

Discussion

Key Observation

Short-term treatment with the COX-2 specific inhibitor rofecoxib and the nonselective COX antagonist naproxen does not impair endothelium-dependent or -independent vascular function in healthy volunteers. Given the increasing concern regarding the potential deleterious effects of COX-2 antagonists on cardiovascular health, these data have important clinical implications.

COX-Specific Isoforms and Vascular Health

COX activity has been associated with the presence of 2 distinct isoforms, COX-1 and COX-2. Results of early investigations suggested that the COX-1 isoform was constitutively expressed in a number of tissues and played an important role as a physiological homeostatic regulator. In contrast, COX-2 was thought to represent the inducible form of the enzyme that played an important role in pathological
states of inflammation. The basic tenet of this paradigm is that the effects of these isoforms were mutually exclusive: a concept that has now been demonstrated to be incorrect.

It has been conventionally held that COX-1 mediates the production of PGI2, an important endothelium-derived vasodilator and inhibitor of platelet aggregation. In addition, COX-1 catalyzes the production of platelet-derived thromboxane A2, a key mediator of platelet aggregation and thrombosis. However, the main action of COX-2 was thought to be the production of inflammatory prostaglandins, secondary to stimuli such as free radicals, cytokines, growth factors, hormones, and hypoxia. It was this concept that led to the development of COX-2–specific antagonists: selective anti-inflammatory and analgesic agents with gastrointestinal-sparing side effects. However, recent studies have demonstrated that up to 80% of PGI2 production is mediated through the COX-2 isomeron.4,6,7 In these studies, celecoxib and rofecoxib markedly suppressed PGI2 synthesis in healthy volunteers when administered at doses used therapeutically. These observations led to the hypothesis that the inhibition of endothelial PGI2 (with COX-2 antagonists), although sparing platelet thromboxane A2 production, may lead to endothelial dysfunction. Indeed, this hypothesis was confirmed in a recent study by Muscara et al,8 who treated rats with celecoxib and observed a sustained rise in blood pressure accompanied by a marked increase in leukocyte adherence. These recent reports have been the source of increasing concern, and a recent meta-analysis of coxibs has suggested that they increase cardiovascular risk.1

To examine the contribution of each isoform toward “net” vascular tone, we studied the effects of 7 days of treatment with rofecoxib versus naproxen on FBF in healthy volunteers. At the doses used in this study, rofecoxib has been demonstrated to markedly attenuate the production of vascular PGI2. Our study demonstrates that selective COX-2 blockade does not impair vascular responses to either Ach or SNP, which are endothelium-dependent and -independent vasodilators, respectively.

An important question that requires discussion is whether Ach-mediated vasodilatation is an adequate measure of endothelial health. Ach is thought to release nitric oxide, endothelium-derived hyperpolarizing factor, and PGI2. Hence, it is possible that if PGI2 activity is depressed (secondary to COX-2 blockade), upregulation of nitric oxide and/or endothelium-derived hyperpolarizing factor may result in preserved Ach-mediated vasorelaxation.

We chose Ach-mediated relaxation as the endothelium-dependent stimulus for a number of reasons. First, Ach is the most widely studied and reproducible stimuli available. Its day-to-day variability is somewhat less than flow-mediated vasodilatation, exercise-induced vasodilatation, and reactive hyperemia (in our laboratory). Second, Ach-mediated vasodilatation has been shown to correlate with measures of platelet function.9 Third, Ach resistance vessel function (albeit in the coronary circulation) has been shown to correlate with measures of ischemia and clinical outcomes. Finally, we recently demonstrated improved FBF to pharmacological intervention with metformin using this methodology.5 Notwithstanding the above discussion, Duffy et al10 reported that Ach-induced vasodilatation was unaffected by local intra-arterial infusions of aspirin in the human forearm at doses that were sufficient to reduce resting FBF and forearm prostacyclin production. Hence, it is possible that the Ach–nitric oxide pathway may not be impaired, while other, more physiological mechanisms, such as responses to shear stress, are altered after COX-2 blockade.

**Perspective and Limitations**

The patient population studied in the present study was healthy and free of known cardiovascular disease risk factors. We are uncertain as to what the outcome of COX-2 antagonism would be in the face of atherosclerosis. Patients with atherosclerosis may be exquisitely sensitive to the effects of COX-2–mediated inhibition of PGI2 production. Studies performed by Duffy et al11 have defined the relative roles of vasodilator prostaglandins in patients with atherosclerosis. In these studies, vasodilatory prostaglandins were demonstrated to play an important role in the maintenance of resting conduit and resistance vessel tone in patients with atherosclerosis. In addition, vasodilator prostaglandins were demonstrated to mediate metabolic vasodilatation and flow-mediated vasodilatation in response to rapid cardiac pacing in patients with atherosclerosis.11

At the other extreme is the possibility that COX-2 antagonists may serve to improve vascular health and retard atherosclerosis. COX-2 is induced by many pro-inflammatory mediators of atherosclerosis. COX-2 has been demonstrated in atherosclerotic plaques and surrounding the lipid core. COX-2 is also found abundantly in macrophages, where it may facilitate the production of PGE2. PGE2 is a potent inducer of matrix metalloproteinase expression, and COX-2, through this mechanism, may lead to plaque disruption/rupture. Studies have shown that COX-2 is widely expressed in atherosclerotic lesions and may colocalize with inducible nitric oxide synthase and nitrosylarginine within macrophages.12

The data presented in this study raise the question as to whether Ach-mediated responses could be preserved in the face of alterations in a more physiologically relevant endothelium end point. Although this is possible, studies from the coronary circulation suggest that Ach-mediated vasodilatation is altered very early in the disease process.13 Studies conducted by Duffy et al14 have demonstrated an attenuation of pacing-mediated responses to intracoronary aspirin. In these studies, Ach was not given and, hence, it remains unknown whether drug-induced changes in endothelial function affect agonist-mediated and physiologically mediated responses together.

Our study addresses only a single dimension of endothelial health and would have benefited from an assessment of other physiologically relevant endothelium-dependent end points in addition to the measurement of prostacyclin production/metabolism.

In summary, this study compared the effects of COX-2 specific blockade (with rofecoxib, 25 mg daily) versus nonselective COX inhibition (with naproxen, 750 mg sustained release daily) on endothelium-dependent and -independent changes in FBF in healthy adult volunteers. Rofecoxib, when used at doses demonstrated to inhibit
vascular PGI₂ production, did not result in changes in endothelium-dependent vasodilatation or an increase in blood pressure. The effects of COX-2 blockade in patients with coronary artery disease require further investigation.

Acknowledgments
Supported by the Alberta Heart and Stroke Foundation (Dr Anderson) and the Medical Research Council of Canada (Drs Verma, Anderson, and Raj).

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Circulation. 2001;104:2879-2882
doi: 10.1161/hc4901.101350
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/24/2879

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