Mechanisms of Protection Afforded by Cyclooxygenase Inhibitors to Endothelial Function Against Ischemic Injury in Rat Isolated Hearts

[Articles]

Bouchard, Jean-François; Lamontagne, Daniel

Faculty of Pharmacy, University of Montreal, Montreal, Quebec, Canada

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Address correspondence and reprint requests to Dr. D. Lamontagne at Faculty of Pharmacy, University of Montreal, P.O. Box 6128, Station Centre-ville, Montreal (Quebec), Canada H3C 3J7. E-mail: lamontad@ere.umontreal.ca

Summary

The aim of this study was to assess whether cyclooxygenase (COX) inhibitors affect the endothelial function against the deleterious effect of ischemia and reperfusion. Isolated rat hearts perfused under constant-flow conditions were exposed to 30 min of partial ischemia (flow, 1 ml/min) followed by 20 min of reperfusion, after which coronary vessels were precontracted with U-46619, and the response to the endothelium-dependent vasodilator, serotonin (5-HT), was compared with that of the endothelium-independent vasodilator, sodium nitroprusside (SNP), in untreated hearts, ischemia diminished selectively 5-HT-induced vasodilation, compared with sham hearts (without ischemia). The vasodilation to SNP was unaffected in all groups. Pretreatment with 6-MNA, 30 µM (COX-1 and -2), or 1-(7-carboxyheptyl) imidazole, 10 µM (thromboxane (TX) synthase inhibitor) but not indomethacin, 10 µM (COX-1 inhibitor) preserved the vasodilation induced by 5-HT after ischemia. Enzyme immunoassays indicated that all COX inhibitors decreased the concentration of TXB2 and 6-keto-PGF1α (stable metabolites of TXA2 and prostacyclin (PGI2), respectively) in coronary effluent during ischemia. Furthermore, indomethacin was the only one to abolish the concentration of PGE2 during ischemia and early reperfusion. No clear trend on ventricular postischemic recovery could be observed between treated and untreated groups under our experimental protocols. These data suggest that, under our conditions, 6-MNA, diclofenac, and 1-7-CI, but not indomethacin, protect the endothelial function via a reduction in TX concentration. Disparities between COX inhibitors may be due to the complete abolition of PGE2 concentration during ischemia and reperfusion in the indomethacin group.

An increasing number of experimental studies attribute an important role to eicosanoids in myocardial ischemia and reperfusion injury (1). Phospholipase activation with the subsequent cleavage of membrane phospholipids and fatty acid accumulation within the ischemic tissue are initial events in myocardial ischemia. A large increase in arachidonic acid in ischemic myocardium has been reported (2). Cardiac tissue has the capacity to synthesize all the major types of eicosanoids (3). Although the coronary vasculature and endothelium were once considered to be the only important cardiac sites of prostaglandin synthesis, PGE2 and PGF2α also are produced in cardiac myocytes (4). In ischemia induced by coronary ligation, enhanced production of eicosanoids is known to occur (5,6). Their involvement in ischemia-reperfusion injury is extremely complex, primarily because both beneficial and deleterious effects of these compounds have been proposed (7,9). Prostacyclin (PGI2) is the major prostaglandin produced by the heart during ischemia and reperfusion, and its release is well documented in the literature (10,11). Some authors also reported enhanced release of PGI2 and PGE2 from cardiac tissue after coronary ligation (5,12). As vasodilators, PGE2 and PGI2 (13) may have protective effects during ischemia and reperfusion.

Furthermore, endogenous release of thromboxanes (TXA2) during ischemia and reperfusion has been reported to contribute to the progression of myocardial infarction (14,15) and the increased occurrence of arrhythmias after coronary artery occlusion (16). TXA2 is a potent vasoconstrictor and has enhanced membrane-permeability action (16).

Beneficial effects of TX-synthesizing inhibitors (17,18) and TX-receptor antagonists (19) have been reported in models of ischemia and reperfusion. Thus TXA2 could be potentially deleterious in myocardial ischemia, extending ischemic damage in the heart and leading to increased cell death (16).

Moreover, in isolated rat hearts, structurally dissimilar nonsteroidal antiinflammatory drugs (NSAIDs) including aspirin, ibuprofen, indomethacin (20), naproxen (21), and flurbiprofen (22), as well as sulfinpyrazone (23), all of which inhibit formation of PGs and TX, were shown to enhance post-ischemic ventricular recovery (20,23) and reduce myocardial infarct size (21,22,24) after reperfusion. The protective role of indomethacin is controversial. Another study reported that a pretreatment with indomethacin exacerbates cardiac ischemia (25). For the moment, little is known about the effect of COX or TX synthase inhibitors on endothelial coronary function during ischemia.

The first aim of this study was therefore to evaluate whether COX inhibitors afford protection against ischemic injury to the endothelium of coronary vessels in the isolated rat heart. The second aim was to identify the mechanisms whereby these agents provide this protection. One mechanism postulated to explain this protective effect of COX was through inhibition of TX formation.

METHODS

Preparation of hearts

The investigation was performed in accordance with the Canadian Council on Animal Care. Male Sprague-Dawley rats (300-350 g) were narcotized with CO2 until complete loss of consciousness and promptly decapitated. The thorax was rapidly opened, and the heart excised and immersed in ice-cold heparinized buffer (10 IU/ml). It was immediately mounted on the experimental setup and perfused at constant flow by means of a digital roller pump. A 20 ml compliance chamber along the perfusion line ensured a continuous flow. The flow rate was adjusted at the beginning of the experiment to obtain a coronary perfusion pressure of 75-100 mm Hg. The flow rate was held constant, and the pressure was monitored to calculate coronary resistance. Flow rate was measured during all the experiments with an in-line ultrasonic flow probe and meter (model T106; Transonic Systems Inc., Ithaca, NY, U.S.A.). The normal perfusion solution consisted of a modified Krebs-Henseleit buffer containing (in mM) NaCl, 118; KCl, 4; CaCl2, 2.5; KH2PO4, 1.2; MgSO4, 1; NaHCO3, 2; d-glucose, 5; and pyruvate, 2. The perfusate was gassed with 95% O2/5% CO2 (pH 7.4) and kept at a constant temperature of 37°C. All drugs were freshly prepared before each experiment.

Preparation of hearts

The volume of the balloon was adjusted to obtain a diastolic pressure of between 5 and 10 mm Hg. Heart rate was derived from the left ventricular pressure trace by a tachograph. Coronary perfusion pressure was measured with a pressure transducer connected to a side arm of the aortic perfusion cannula. Isovolumetric left ventricular pressure and its first derivative (dP/dt) was measured with a fluid-filled latex balloon inserted into the left ventricle and connected to a second pressure transducer. The volume of the balloon was adjusted to obtain a diastolic pressure of between 5 and 10 mm Hg. Heart rate was derived from the left ventricular pressure trace by a tachograph. Data were recorded on a polygraph system (Grass model 79 polygraph, Astro-Med Inc., West Warwick, R.I.).

Experimental protocols

The animals were randomized into 10 groups, which were exposed to two different experimental protocols (Fig. 1). The hearts in all groups were subjected to a 20-min stabilization period. Drug or vehicle infusion was then started, followed by an additional 30-min perfusion period. The ischemic groups were subjected to a partial ischemia (flow rate, 1 ml/min) before a 20 min reperfusion period. The sham groups were not exposed to ischemia/reperfusion, but to a time-matched normal perfusion. After these periods, coronary arteries were precontracted with 0.1 µM U-46619 administered throughout the end of the experiment. Fifteen minutes after the beginning of U-46619 infusion, the endothelial function was evaluated by the vasodilation produced by 10 µM serotonin (5-HT), whereas coronary smooth muscle function was evaluated with 3 µM sodium nitroprusside (SNP). These infusions were maintained for 10 min, which was long enough to reach a steady state. A washout period of 10 min was allowed between each infusion. Vasodilation was evaluated by computing percentage changes in coronary resistance (coronary perfusion pressure divided by coronary flow), measured immediately before each drug infusion, and after a new steady state.

![Graph](http://www.jcvp.com/graphic.jpg)

FIG. 1. Diagram showing the different experimental protocols. Each experiment started with a 20-min stabilization period, followed by infusion of either diclofenac (diclo, 1 µM), indomethacin (indo, 10 µM), flurbiprofen (22), 6-methoxy-2-naphthyl acetic acid (6-MNA, 30 µM), or vehicle. Hearts in the ischemia protocol underwent 30 min of low-flow (1 ml/min) ischemia and 20 min of reperfusion. Flow and smooth muscle function was evaluated after the 20-min reperfusion period. Hearts in the sham protocol were used as time-matched controls. Coronary arteries were precontracted by a continuous infusion of 0.1 µM U-46619. After 15 min, infusion of 5-HT (10 µM) was started for 10 min. A washout...
period of 10 min was allowed between 5-H and SNP (3 µM, 10 min) infusions. Effluent samples were collected after stabilization period (T-30), before ischemia (T0), at the end of ischemia (T29), and 30 s after the beginning of reperfusion (T30.5). The horizontal rule at the bottom of the figure represents time in minutes. Numbers between parenthesis indicate the numbers of rats in each group.

Sham and ischemic hearts were treated with either 1 µM diclofenac, a COX-1 and COX-2 inhibitor (24); 10 µM indomethacin, a COX-1 inhibitor (24); 30 µM 6-mercapto-2-naphthyl acetic acid (6-MNA), a somewhat more selective inhibitor for COX-2 than for COX-1 (27); 15 µM 1-T(carboxyethyl)imidazoline (1-T-CE), a TX synthase inhibitor (28); or vehicles, starting after the 20-min stabilization period and lasting throughout the ischemic period.

TXB2, 6-keto PGI2 (6-kPG), and PGE2 determination in coronary effluent

Coronary effluent samples were collected 4 times for each protocol (after the 20-min stabilization period, just before ischemic period, 29 min after the beginning of ischemic period, and 30 s after the beginning of reperfusion (Fig. 1).

TXB2, 6-keto PGF2a (stable hydrolysis products of TXA2 and PGI2, respectively), and PGE2 in coronary effluent samples were evaluated by enzyme immunoassays (EIA) (Boehringer Mannheim, Life Science, Buckinghamshire, U.K.).

Statistical analysis

Values represent the mean ± SD. Statistical significance of differences between means was evaluated by a two-way analysis of variance with Scheffe post hoc test. In the presence of an interaction between the different groups, one-way analyses of variance were used for each group. A commercially available software program (Systat for Windows) was used. Only probability values (p) < 0.05 were considered to be statistically significant.

Drugs

6-MNA was a kind gift of SmithKline Beecham Pharma. 1-7-CHI was bought from Tocris (Ballwin, MO, U.S.A.). All other drugs were obtained from Sigma-Aldrich (Mississauga, Ontario, Canada). Indomethacin (1 mM) was prepared in 600 µg 100% dimethylsulfoxide (DMSO) and 30 µl of Krebs-Henseleit buffer. 6- MNA, 3 mM, was dissolved in 300 µl 10 mM NaOH, this solution being added directly to 30 ml H2O. U-46619 (28.3 mM) was dissolved in 100% ethanol and diluted with Krebs-Henseleit buffer to obtain the desired final concentration. DMSO (0.02%), and NaOH (0.1 mM), at the concentrations obtained in the final dilutions, had no effect on any of the hemodynamic variables studied and on the dilator responses to 5-H and SNP. All the other drugs were dissolved in Krebs-Henseleit buffer.

RESULTS

Vascular function

Untreated groups. Coronary resistance measured just before 0.1 µM U-46619 perfusion (n = 18) was 5.94 ± 0.29 mm Hg · min/ml for a coronary flow rate of 6.72 ± 0.22 ml/min/g (mean heart weight of 1.90 ± 0.05 g). Infusion of U-46619 Induced a significant (p < 0.05) vasoconstriction in all groups of hearts (coronary resistance of 10.10 ± 0.82 and 11.35 ± 0.75 mm Hg · min/ml in sham and ischemia groups, respectively). Perfusion of 10 µM 5-HT produced a diminution in coronary resistance of 25.2 ± 1.88 in the sham group, and 30 min of partial ischemia significantly diminished the 5-HT-induced vasodilation by more than half (Fig. 2A). Endothelium-independent vasodilation to 3 µM SNP was not affected by ischemia and was found to be comparable in the two groups of hearts (sham and ischemic; Fig. 2B).

6-MNA-treated groups. Inhibition of COX-1 and COX-2 with 6-MNA (10 µM) was accompanied by a small but nonsignificant increase in coronary resistance when measured just before 0.1 µM U-46619 perfusion (6.77 ± 0.29 vs. 5.94 ± 0.29 mm Hg · min/ml in untreated hearts; p = 0.05). The perfusion rate was 5.72 ± 0.24 ml/min/g (mean heart weight, 1.98 ± 0.03 g). Infusion of U-46619 (60 µM, n = 17) induced a significant (p < 0.05) vasoconstriction in all 6-MNA-treated groups (coronary resistance, 11.73 ± 0.08 and 11.6 ± 0.44 mm Hg · min/ml in sham (n = 8) and ischemic (n = 9) groups, respectively). Vasodilation produced by 10 µM 5-HT (30.2 ± 6.2% in sham hearts, n = 6) was almost totally preserved in the ischemic group (n = 9; Fig. 2C). Vasodilation to 3 µM SNP was comparable in the two 6-MNA-treated groups (sham and ischemic; Fig. 2D).

Indomethacin-treated groups. Inhibition of COX-1 with indomethacin (10 µM) produced no significant increase in coronary resistance when measured just before 0.1 µM U-46619 perfusion (6.04 ± 0.36 mm Hg · min/ml, n = 7, 5.94 ± 0.29 mm Hg · min/ml in untreated hearts; p = 0.05). The perfusion rate was 5.39 ± 0.21 ml/min/g (mean heart weight, 2.28 ± 0.07 g). Infusion of U-46619 (0.1 µM, n = 15) induced a significant (p < 0.05) vasoconstriction in all indomethacin-treated groups (coronary resistance, 10.37 ± 0.67 and 10.75 ± 0.89 mm Hg · min/ml in sham (n = 7) and ischemic (n = 8) groups, respectively). Vasodilation produced by 10 µM 5-HT (31.7 ± 2.7% in sham hearts, n = 7) was reduced by more than half in the ischemic hearts (Fig. 2E). Vasodilation to 3 µM SNP was comparable in the two indomethacin-treated groups (sham and ischemic; Fig. 2F).

1-7-CHI-treated groups. The somewhat more selective inhibition of COX-2 than COX-1 with 1-7-CHI (30 µM) was accompanied by a small but nonsignificant increase in coronary resistance when measured just before 0.1 µM U-46619 perfusion (7.02 ± 0.52 vs. 5.94 ± 0.29 mm Hg · min/ml in untreated hearts; p = 0.05). The perfusion rate was 5.52 ± 0.29 ml/min/g (mean heart weight, 2.03 ± 0.10 g). Infusion of U-46619 (0.1 µM, n = 10) induced a significant (p < 0.05) vasoconstriction in all 1-7-CHI-treated groups (coronary resistance, 11.04 ± 1.56 and 11.39 ± 0.88 mm Hg · min/ml in sham (n = 6) and ischemic (n = 6) groups, respectively). Vasodilation produced by 10 µM 5-HT (-24.1 ± 8.2% in sham hearts, n = 4) was almost totally preserved in the ischemic group (n = 6; Fig. 2G). Vasodilation to 3 µM SNP was comparable in the two 1-7-CHI-treated groups (sham and ischemic; Fig. 2H).

Myocardial function

The isometric contractions of hearts pretreated with diclofenac, indomethacin, 6-MNA, and 1-7-CHI were comparable to those of untreated hearts: dp/dtmax values measured before the 30-min low-flow ischemia were 1,980 ± 449 (n = 9), 1,808 ± 190 (n = 9), 1,775 ± 119 (n = 8), 1,508 ± 47 (n = 6), and 1,625 ± 73 mm Hg (n = 6) for untreated, and hearts pretreated with diclofenac, indomethacin, 6-MNA, and 1-7-CHI, respectively. Low-flow ischemia was accompanied by a severe reduction in dp/dtmax (Fig. 3) in all groups. Indomethacin and 6-MNA pretreatment improved early postischemic dp/dtmax (Fig. 3B and C), but the other treatments had no effect (Fig. 3A).

Arachidonic acid cascade products in coronary effluent

Thromboxane B2. Before COX or TX synthase inhibitor pretreatment (T-30), TXB2 in the coronary effluent amounted to 74.7 ± 6.4 femtomoles (fmol), n = 21 (Fig. 4). Treatment with diclofenac, 1-7-CHI, or indomethacin produced a nonsignificant decrease in TXB2 just before the ischemic period (T0). At the end of the low-flow ischemia (T29), levels of TXB2 were significantly increased in the ischemic nontreated group (n = 3) versus the sham untreated group (n = 3; p < 0.05). This increase in TXB2 levels was partially but significantly blocked by all COX (n = 4, 3, 3) and TX (n = 3) inhibitors (p < 0.05). After 30 s of reperfusion (T30.5) no statistically significant difference between treatments was found.
6-keto PGF$_{1\alpha}$ Before COX or TX synthase inhibitor pretreatment (T-30), 6-keto PGF$_{1\alpha}$ measured in the coronary effluent was 654 ± 56 nM, n = 21 (Fig. 5). Treatment with 6-MNA, diclofenac, and indomethacin, but not 1-7-CHI, produced a significant decrease in 6-keto PGF$_{1\alpha}$ levels just before the ischemic period (T0) (p < 0.05). At the end of the low-flow ischemia (T29), levels of 6-keto PGF$_{1\alpha}$ were significantly increased in the ischemic nontreated group (n = 3) versus the sham untreated group (n = 3; p < 0.05). This increase in 6-keto PGF$_{1\alpha}$ levels was partially but significantly blocked by all COX (n = 4, 3, 3) inhibitors at times T29 and T30.5 (p < 0.05).

FIG. 5. Coronary effluent concentration of 6-keto PGF$_{1\alpha}$ after stabilization period (T-30), before ischemia (T0), and at the end of the reperfusion (T30.5). *p < 0.05 compared with the indicated group(s).

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protective effects of PGEs. In support of that, treatment of isolated rat hearts with chelerythrine prevents the endothelial protective effect of exogenous PGEs. J-F. Bouchard and D. Lamontagne, unpublished data). PGEs can also act on EP2 receptors, stimulating the production of cyclic adenosine 3’5’-monophosphate (AMP) and inducing vasodilatation (45).

Effect of COX and TX synthase inhibitors on myocardial function

In our study, $dP/dt_{max}$ and $dP/dt_{time}$, which represent the capacity of the ventricle to contract during systole and its ability to relax during diastole, were used to evaluate the contractile function of the hearts. These variables recovered rapidly and completely within the 20-min reperfusion period. Furthermore, indomethacin and 6-MNA improved the $dP/dt_{max}$ only during early reperfusion, whereas all the others failed to improve ischemic or postischemic ventricular recovery. The inability of these COX and TX synthase inhibitors to improve ventricular recovery is most probably due to the fact that our ischemic conditions are too mild to depress the contractile function severely, leaving little room for improvement.

The advent of therapeutic capability for restoring blood flow to ischemic myocardium has stimulated clinical and experimental interest regarding cardiac effects of ischemia and reperfusion. Preservation of myocardial and endothelial function during ischemia and reperfusion is one of the major priorities of modern medicine. The future of the described drugs promises to be fruitful, not only to unlock the mechanism of important humoral mediators such as PGs, but also to provide the care giver with new therapeutic interventions in acute, life-threatening disease states, such as myocardial infarction, reperfusion injury, coronary spasm, and angina pectoris.

In conclusion, these data suggest that 6-MNA, diclofenac, but not indomethacin perfusions, before and during ischemia, afford protection to the endothelial function against ischemia in resistance coronary arteries. The similar protective effect of 1-7-CHI, a specific inhibitor of TX synthase, and the decreased concentration of TXs during perfusion of COX and TX synthase inhibitors, suggest the implication of TXs in the detrimental effect of ischemia. In our conditions, the protective lack of effect of indomethacin may be due not to an incapacity to inhibit the synthesis of TXs, but to its important reduction in PG concentration during ischemia and early reperfusion.

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