Effect of the Combination of Anticoagulant and Thromboxane Synthetase Inhibitor (Y-20811) or Receptor Blockade (S-1452) on Preventing Thrombotic Cyclic Coronary Flow Reduction in Dogs with Coronary Stenosis

TAKASHI FUIJI, M.D., MASUNORI MATSUZAKI, M.D., TSUYOSHI ODA, M.D.
HISANORI SAKAI, M.D., NOBUAKI TANAKA, M.D., MASAFUMI YANO, M.D.
TOSHIRO MIURA, M.D., MICHIHIRO KOHNO, M.D., KAZUHIRO KATAYAMA, M.D.
AND REIZO KUSUKAWA, M.D.

We examined the hypothesis that combined actions of anticoagulant (heparin) and Y-20811, thromboxane A2 synthetase inhibitor (TXSI), or S-1452, receptor blockade (TXRB), can provide better antithrombotic protection than TXSI or TXRB alone. In 20 of 33 dogs instrumented, placement of a critical stenosis at a focus of coronary vascular injury initiated a reproducible cyclic coronary flow reduction (CCFR). TXSI (1 mg/kg, IV) perfectly inhibited CCFR in 6 of 10 dogs (60%), and was associated with a significant decrease in 11-dehydro-TXB₂ (85 ± 8% of control; p < 0.05) and an increase in 6-keto-PGF₁α (155 ± 58%; p < 0.05) in coronary sinus blood samples. In the remaining 4 dogs, additional administration of heparin (2000 IU) completely abolished CCFR. On the other hand, TXRB (1 mg/kg, IV) perfectly inhibited CCFR in 7 of 10 dogs (70%), and was accompanied by a significant increase in 6-keto-PGF₁α (214 ± 65%; p < 0.05) and unchanged TXB₂ level. In the remaining 3 dogs, additional administration of heparin (2000 IU) completely abolished CCFR. Thus, the combination of anticoagulant and TXSI or TXRB were more effective than TXSI or TXRB alone in abolishing thrombotic CCFR, suggesting that the combination might be effective for treating patients with impending myocardial infarction.

(Jpn Circ J 1992; 56: 1191–1197)

PLATELETS and platelet-derived factors may participate in the development of impending myocardial infarction! Recent studies1–5 suggest that platelets accumulate on the coronary artery wall at sites of endothelial injury and stenosis, and release various factors, some of which can further activate platelets, initiate intracoronary thrombosis, increase coronary artery vascular tone and severely reduced blood flow to the myocardium. Cyclic coronary flow reduction (CCFR) were initially described by Folts et al4,5 and were caused by spontaneous platelet aggregation and dislodgment on a stenotic coronary artery wall with endothelial injury in the canine model. Studies from other colleagues6,7 have shown that both thromboxane A₂ and prostaglandin I₂ are important mediators of CCFR. Both thromboxane A₂ synthetase inhibitor (TXSI) and thromboxane A₂-prostaglandin H₂ receptor blockade (TXRB) are effective in eliminating cyclic flow variation, but do not completely eliminate CCFR.8,9 Thus, we assessed the effects of Y-20811, a thromboxane A₂ synthetase inhibitor and S-1452, a

Key words:
Thromboxane A₂
Prostaglandin I₂
Impending myocardial infarction

The Second Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Japan.
Mailing address: Takashi Fujijs, M.D., The Second Department of Internal Medicine, Yamaguchi University School of Medicine, 1144 Kogushi, Ube, Yamaguchi 755, Japan

Japanese Circulation Journal Vol. 56, November 1992 1191
thromboxane A2/H2 receptor blockade, on CCFR in a canine model with coronary stenosis. We also examined the effect of the combination of heparin and thromboxane A2 synthetase inhibitor or receptor blockade in eliminating cyclic flow variations than either action alone.

METHODS

Animal preparation

Thirty three mongrel dogs weighing 9 to 17 kg were premedicated with morphine (5 mg/kg) 30 min prior to induction anesthesia with a-chloralose (50 mg/kg). The dogs were ventilated with a mixture of 40% oxygen in room air supplied by a ventilator (Havard). The heart was exposed through a left fifth intercostal thoracotomy and suspended in a pericardial cradle. Catheter-tip micromanometers (Millar Instruments) were inserted from the carotid and the femoral arteries to the ascending aorta and left ventricle to measure aortic and left ventricular pressures, respectively. The drift of the micromanometer was corrected by matching the pressure to simultaneously recorded fluid filled pressure (Statham P23Db). The zero pressure level was defined as the estimated level of right atrium. For the measurement of regional myocardial function, 1 pair of miniature ultrasonic crystals (5 MHz) was implanted in the left ventricular posterior wall to measure transmural wall thickening, as described elsewhere.10 Endocardial ECG was recorded from a crystal inserted in the subendocardium. A 20 mm section of the proximal left circumflex coronary artery was dissected free, and a pulsed Doppler flow probe (10 MHz) was placed around the coronary artery, proximal to the constricting plastic cylinder. A silastic catheter was inserted into the great cardiac vein for sampling coronary sinus blood to measure platelet count, fibrinogen and prostanoids. Data were recorded with a multichannel recorder (Electronics for Medicine VR12, USA) and on magnetic tape for subsequent playback. The first derivative of the left ventricular pressure (dp/dt) was obtained using an analog circuit (filter 100Hz).

STUDY PROTOCOL

When the instrumentation was completed, dogs were allowed to stabilize for approximately 30 min. After the control recordings without coronary stenosis, a hard cylindric plastic constrictor (3 mm in length) was then placed around the artery at a site of endothelial injury to reduce the flow velocity to approximately 50% of control values using the constrictor with an appropriate internal diameter. At the above degree of coronary stenosis the myocardial reactive hyperemic response after brief coronary occlusion was markedly eliminated. When the cyclic coronary flow reduction was present, careful observation was kept for confirming the reproducible occurrence of the cyclic flow reduction and reperfusion for at least 30 min.

Thromboxan A2 synthetase inhibitor (TXSI)

In the dogs with reproducible cyclic coronary flow reduction, Y-20811 (Yoshitomi & Co., Ltd., Osaka, Japan.), a thromboxane A2 synthetase inhibitor (1 mg/kg) was treated by intravenous bolus injection. If the cyclic flow variation was not abolished over 30 min after the injection, an additional intravenous bolus injection of heparin (2000 IU) was given.

Thromboxan A2 receptor blockade (TXRB)

Ten dogs were treated by intravenous bolus injection with S-1452 (Shionogi & Co., Ltd., Osaka, Japan.), a selective thromboxane A2-prostaglandin H2 receptor antagonist (1 mg/kg). If cyclic flow reductions were not abolished, heparin (2000 IU) was given intravenously as in thromboxan A2 synthetase inhibitor group.

Prostanoids measurement in coronary sinus blood

We measured prostanoids, 11-dehydrothromboxane B2 (11-DTXB2) and 6-keto-prostaglandin F1α (6-keto-PGF1α), i.e., stable degradation products of TXA2 and prostaglandin I2 (PGI2), respectively, in coronary sinus blood samples using a specific radioimmunoassay.11,12 Blood samples were taken before the placement of the constrictor, during CCFR at low flow velocity, and 30 min following the abolition

Japanese Circulation Journal Vol.56, November 1992
of CCFR with TXSI or TXRB injection. If TXSI or TXRB could not eliminate CCFR, heparin (2000 IU) was given intravenously and blood samples were taken 30 min after the abolition of CCFR.

STATISTICAL ANALYSIS

Statistical analysis of the differences of platelet count, fibrinogen, and prostanoids among at control rest, during CCFR and after treatments was carried out by analysis of variance. When an overall difference was detected, Student’s t-test was used to determine the significant means. Data are reported as a percent fraction of control value without coronary stenosis, and as mean ± SE.

RESULT

[1] Comparison of the blood samples between the cases with and without CCFR

In 20 of 33 dogs (61%), CCFR was induced about 5 to 30 min after partial coronary constriction. With coronary constriction LCX flow velocity reduced to approximately half of the control levels both in dogs with (52±3%) and without CCFR (49±5%). Platelets, fibrinogen and 6-keto-PGF₁α in coronary sinus blood were the same with and without CCFR. However, the 11-DTxB₂ level in dogs without CCFR tended to decrease compared with the dogs with CCFR (10.6±4.2 vs 14.1±6.8 ng/dl; NS, respectively).

[2] Hemodynamic alterations during CCFR
Fig. 3. Effects of TXSI and heparin on platelet (Plt), fibrinogen (Fbg), 11-dehydro-thromboxane B₂ (11-DTXB₂), and 6-keto-prostaglandin F₁α (6-keto-PGF₁α) in coronary sinus blood sample. Data are shown as a percent fraction of control level. Panel A: effects of TXSI in cases with abolished CCFR. Panel B: effects of TXSI and heparin in cases with prolonged CCFR interval.

Fig. 4. Effects of TXRB and heparin on platelet (Plt), fibrinogen (Fbg), and 11-dehydro-thromboxane B₂ (11-DTXB₂), and 6-keto-prostaglandin F₁α (6-keto-PGF₁α). Panel A: effects of TXRB in cases with abolished CCFR. Panel B: effects of TXRB and heparin in cases with prolonged CCFR interval.

The mean duration of 1 CCFR was approximately 7.0±1.6 min in all 20 dogs with CCFR. Representative tracings of left ventricular pressure, LV dP/dt, posterior wall thickening, phasic coronary flow velocity and mean coronary flow velocity during CCFR and after treatment are shown in Fig. 1 and 2. In all cases of CCFR, the circumflex coronary artery blood was gradually reduced and finally disappeared, indicating total occlusion by clot formation. Following a period of a few minutes of coronary occlusion, abrupt reperfusion spontaneously appeared (Fig. 1, 2). During total coronary occlusion heart rate and LV systolic pressure did not significantly change, however, LV enddiastolic pressure was significantly ele-
vated (3.9±0.3 → 6.4±0.3 mmHg; p<0.01) and peak (+) dP/dt was reduced (1886±104 → 1500±104 mmHg/sec; p<0.05). During CCFR, wall thickening in the ischemic area gradually decreased and finally took place to systolic vulging, and the ST segment in subendocardial ECG of ischemic zone was markedly elevated (Fig. 1, 2).


A representative recording of hemodynamic variables and CCFR with and without TXSI is shown in Fig. 1A. In 10 dogs with CCFR, mean duration of CCFR before TXSI injection was 6.2±1.4 min. TXSI (1 mg/kg, IV) perfectly inhibited CCFR in 6 of 10 dogs (60%), associated with significant

\[\text{Japanese Circulation Journal Vol. 56, November 1992}\]
decrease in 11-DTXB₂ (85±8% of control; p<0.05) and increase in 6-keto-PGF₁α (155±38%; p<0.05) in coronary sinus blood (Fig. 3A). Fibrinogen levels were not changed after TXSI infusion, however, the platelet count was significantly reduced when compared with control level. In the remaining 4 dogs, administration of heparin (2000 IU) completely abolished CCFR (Fig. 1B). Platelet, fibrinogen, 6-keto-PGF₁α and 11-DTXB₂ were unchanged after the infusion of heparin, compared with control level (Fig. 3B).


A representative recording of hemodynamic variables and CCFR with or without TXRB is shown in Fig. 2A. TXRB (1 mg/kg, IV) perfectly inhibited CCFR in 7 of 10 dogs (70%), associated with significant increase in 6-keto-PGF₁α (214±65%; p<0.05) and unchanged TXB₂ level. Platelet number and fibrinogen concentration were not changed after TXRB infusion, compared with the control level (Fig. 4A). In the remaining 3 dogs, administration of heparin (2000 IU) completely abolished CCFR (Fig. 2B). Platelet did not change significantly, fibrinogen, 6-keto-PGF₁α and 11-DTXB₂ concentration after the infusion of heparin compared with the control level without CCFR (Fig. 4B).

DISCUSSION

Cyclic coronary flow reduction

Cyclical coronary flow reductions are based on critical coronary stenosis and endothelial injury. The situation mimics the pathologic basis of acute coronary heart disease syndrome, such as impending myocardial infarction. Morphologic studies have revealed that thrombus may exist in the endothelially injured and severely stenosed coronary artery when blood flow is reduced and that platelets are often the main components of the thrombus. Folts et al demonstrated that aggregation and deaggregation of platelet plugs might be mainly responsible for the disorders. Aggregability of platelet is modulated by several substances such as adenosine diphosphate, epinephrine, serotonin and thromboxane A₂. Ashton et al have shown that TXB₂ concentration; the inactive metabolite of TXA₂, in the distal coronary artery blood and at the site of a coronary constriction are elevated during CCFR. They have shown that inhibition of cyclooxygenase with aspirin is effective in attenuating the occurrence of CCFR in this canine model. Thus, in this model, TXA₂ appears to play a central role in maintaining CCFR.

Effects of Y-20811 on cyclic coronary flow variations

The agent Y-20811, Sodium 4-[α-hydroxy-5-(1-imidazolyl)-2-methylbenzyl]-3,5-dimethylbenzoate dihydrate, is a newly developed antiplatelet agent that significantly inhibits thromboxane synthetase in platelet. Preliminary studies have shown that this drug inhibits human platelet aggregation induced by arachidonic acid without affecting the activation of cyclooxygenase, prostacyclin synthetase and 5, 12, 15-lipoxygenase. This effect was stronger than that of aspirin and imidazole. Thromboxane A₂ synthetase inhibitors eliminated cyclic flow variations in approximately 70% of treated dogs in previous reports. In the present study, cyclic flow variations were abolished in 60% of dogs treated with Y-20811. These results are consistent with other previous studies. When thromboxane A₂ synthesis is blocked, 11-DTXB₂, the stable metabolite of thromboxane A₂, in coronary sinus blood is expected to decrease. Accordingly, cyclic endoperoxide, the precursors of both thromboxane A₂ and prostaglandin I₂, accumulates and may be redirected toward the synthesis of prostacyclin and platelet inhibitor prostaglandin D₂. This suggests that the beneficial effect of Y-20811 is associated with increased prostacyclin production, however, aspirin blocks the synthesis of the precursor of prostacyclin, cyclic endoperoxides, resulting in the decrease of the CCFR elimination rate.

Effects of S-1452 on cyclic coronary flow variations

The agent S-1452, calcium 5 (Z)-1R, 2S, 3S, 4S-7-[3-phenylsulfo-ylaminobicyclo [2.2.1] hept-2-yl]-5-heptenoate hydrate, is a newly selective and potent TXA₂/PGH₂ receptor antagonist. It does not alter the activ-
ities of TX synthetase, cyclooxygenase or adenylate cyclase, and it does not posses TXA₂ agonist property. S-1452 was more effective in inhibiting U-46619 (5 nmol/kg)-induced canine aggregation than SQ29548 and dazoxiben. In the previous reports, thromboxane receptor antagonist, SQ29548, eliminated cyclic flow variation in approximately 70% of treated dogs. In the present study, CCFR were abolished in 70% (7/10) of dogs treated with S-1452, associated with significantly increased 6-keto-PGF₁α and unchanged 11-DTXB₂ levels. This suggested that S-1452 does not appreciably affect cyclic endoperoxide metabolism to TXA₂, which could potentially influence the occurrence of CCFR in this model. 6-keto-PGF₁α was significantly increased with S-1452 in the present study, suggesting that activated platelets might stimulate the normal endothelial cells and induce an increase in PGI₂ in the distal coronary artery.

Effect of the combination of anticoagulant (heparin) and TXSI or TXRB

Telford and Theroux et al reported that heparin treatment was associated with a reduction of the incidence of myocardial infarction and refractory angina during in the acute phase of unstable angina. Heparin is effective against fibrin clot formation, but not platelet aggregation. Folts et al suggested that platelet thrombi were the primary causes of cyclic flow reductions, so that heparin was not effective in this model. However, in this study the combinations of heparin and TXSI or TXRB perfectly inhibited CCFR. This suggested that platelet adhesion and aggregation were inhibited by TXSI or TXRB and fibrin clot formation was also inhibited by heparin treatment. Therefore, this indicated that cyclic coronary flow reduction in dogs was caused by both platelet thrombus formation and fibrin clot formation.

Our results indicate that the combined actions of anticoagulant and TXSI or TXRB are more effective than TXSI or TXRB alone in abolishing thrombotic CCFR. These findings of significant abolishment of CCFR may provide an objective basis for the use of these agents in combination clinically for the treatment of patients with impeding myocardial infarction.

REFERENCES


Japanese Circulation Journal Vol.56, November 1992


