Lack of Involvement of Thromboxane A\textsubscript{2} in Postischemic Recovery of Stunned Canine Myocardium

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Modification of the thromboxane: prostacyclin ratio alters the severity of reperfusion arrhythmias and postischemic damage in long-term, irreversibly injured myocardium. In this study, the effects of the thromboxane synthetase inhibitor dazmegrel and the thromboxane receptor antagonist BM 13.505 on myocardial postischemic functional recovery and preservation of tissue adenine nucleotides was examined after a 15-minute episode of ischemia followed by 3 hours of reperfusion (myocardial stunning). Dazmegrel (3 or 8 mg/kg) or BM 13.505 (10 mg/kg) was given 15 minutes before coronary occlusion and compared with a control group in barbital-anesthetized dogs. Regional segment shortening (percent segment shortening, sonomicrometry), regional myocardial blood flow (microspheres), and coronary venous eicosanoid and high-energy phosphate levels (biopsies after 3 hours of reperfusion) were measured. Areas at risk, regional myocardial blood flow, and regional segment shortening during coronary occlusion were similar in all groups. Dazmegrel (3 mg/kg) attenuated the decrease in endocardial and midmyocardial adenosine 5'-triphosphate, and both doses significantly improved regional segment shortening during reperfusion. Coronary venous thromboxane levels were significantly decreased throughout the experiment in both dazmegrel-treated groups, and thromboxane levels were significantly elevated in the control group 3 hours after reperfusion. Prostacyclin, measured in the form of its main metabolite, 6-keto-prostaglandin F\textsubscript{1alpha}, did not change significantly in the control group throughout the experiment, but it was markedly increased in dazmegrel groups throughout reperfusion, particularly in the dazmegrel group receiving 3 mg/kg. BM 13.505 exerted no beneficial effects on postischemic function or metabolism. In conclusion, after a reversible ischemic insult, postischemic recovery of function and metabolic status was not enhanced by preocclusion treatment with a thromboxane receptor blocker, and thus, the beneficial effects of thromboxane synthesis inhibition on postischemic abnormalities was not due to a reduction in thromboxane but was the result of endoperoxide shunting and a subsequent increase in prostacyclin. Therefore, thromboxane does not appear to be an important mediator of reversible ischemia-reperfusion damage. (*Circulation* 1988;78:450–461)

Since the discovery of a strongly vasospastic compound, rabbit aorta-contracting substance,\textsuperscript{1} that is released during anaphylaxis, and since the subsequent identification\textsuperscript{2} of the main component as the arachidonic acid metabolite thromboxane A\textsubscript{2}, there have been many studies suggesting a role for thromboxane as a pathophysiological mediator in thrombosis,\textsuperscript{3} vasospasm,\textsuperscript{4,5} arrhythmias,\textsuperscript{6} and in the progression of ischemic damage after coronary artery occlusion. Inhibition of thromboxane synthesis\textsuperscript{7} and blockade of thromboxane receptors\textsuperscript{8} have been shown to inhibit cyclic-flow variations in severely narrowed coronary arteries. Although thromboxane is a potent vasoconstrictor in vitro\textsuperscript{5} and in vivo,\textsuperscript{6} it may be premature to attribute clinical vasospasm to an increase in thromboxane because inhibition of thromboxane synthesis by OKY-046, a thromboxane synthetase inhibitor, has not resulted in a decrease in the number or length of ischemic episodes in patients with vasospastic angina.\textsuperscript{9} Several studies indicate that thromboxane may, in part, mediate...
arrhythmias observed during coronary artery occlusion and reperfusion, whereas others have provided evidence that thromboxane may not be involved in arrhythmias occurring during acute myocardial ischemia.

Previous studies with various thromboxane inhibitors demonstrated efficacious responses in reducing both myocardial creatine kinase and amino-nitrogen loss, as indexes of ischemic damage, in cats and rats after long-term ischemic insults. Also, the thromboxane synthetase inhibitor CV-4151 has been shown to decrease infarct size after 60 minutes of coronary occlusion and 60 minutes of reperfusion in dogs.

Because inhibition of thromboxane synthesis has been shown to have beneficial effects in many models of experimental ischemia, blockade of thromboxane receptors may offer a potential theoretical advantage in reducing ischemic damage because a receptor blocker would also antagonize prostaglandin endoperoxides and possibly 6-keto-prostaglandin F2α (6-keto-PGF2α), which are thought to have overlapping activity at the same receptors. Another potential advantage of thromboxane receptor blockers over synthetase inhibitors is that thromboxane receptor antagonists, in contrast to synthetase inhibitors, do not generate bisenic prostaglandins, which may be detrimental in certain situations. In studies of long-term coronary occlusion in which thromboxane appears to enhance the extension of ischemic damage, receptor blockers may be more efficacious than synthetase inhibitors. Although pharmacological agents that inhibit thromboxane synthetase or block thromboxane receptors have been shown to be beneficial in long-term ischemic damage, their effects on postschemic contractile dysfunction in reversibly injured myocardium have not been evaluated. Coronary artery occlusions of less than 20 minutes do not produce cell death or irreversible morphological changes, yet they result in prolonged abnormalities in regional contractile function, tissue blood flow, and high-energy phosphate levels. The causative mechanism of this phenomenon, termed the "stunned" myocardium, is currently unknown, as is thromboxane's role as a possible mediator of the prolonged dysfunction. Therefore, the purpose of this study was to both determine whether pretreatment with the thromboxane synthetase inhibitor dazmegrel or the thromboxane receptor blocker BM 13.505 was able to enhance postschemic contractile function after 15 minutes of coronary artery occlusion and after 3 hours of reperfusion and to delineate possible mechanisms by which these agents may be efficacious. A sham occlusion group was also used to examine the stability of regional function over time.

Materials and Methods

General Preparation

Adult mongrel dogs (18–28 kg) of either sex were anesthetized with barbital sodium (300 mg/kg i.v.) and sodium pentobarbital (15 mg/kg i.v.), ventilated at 10–15 breaths/min with a tidal volume of 15 ml/kg (Harvard Respirometer, Harvard Apparatus, South Natick, Massachusetts) and supplemented with 100% O2. An end-expiratory pressure of 5–7 cm H2O was maintained with a trap to prevent atelectasis. Body temperature was maintained at 38°C with a heating pad. Blood gas samples obtained from the right femoral artery were monitored (ABL2, Radiometer, Uppsala, Sweden) at various times throughout the experiment and maintained within a normal physiological range (pH 7.39–7.43, PCO2 = 30–35 mm Hg, PO2 = 100–136 mm Hg).

A double-tipped pressure transducer catheter (PC 771, Millar Instruments, Houston, Texas) was advanced into the aorta and left ventricle through the carotid artery to monitor aortic and left ventricular systolic and diastolic pressures. Left ventricular dP/dt was determined by electronically differentiating the left ventricular pressure pulse as a measure of global cardiac contractility. The right femoral vein was cannulated for administration of drug, vehicle, or subsequent anesthesia as needed.

A left thoracotomy was performed at the fifth intercostal space, the lungs were retracted, and the heart was suspended in a pericardial cradle. A segment (1–1.5 cm) of the left anterior descending coronary artery (LAD) was dissected free from surrounding tissue distal to the first diagonal branch, and a calibrated electromagnetic flow probe (Statham SP 7515, Gould-Statham, Cleveland, Ohio) placed around the vessel. LAD coronary blood flow was measured with a flowmeter (Statham 2202). A micrometer-driven mechanical occluder was placed distal to the flow probe so that there were no branches between the occluder and the flow probe. The occluder was used to calibrate the flow probe to zero and later to occlude the artery. Limb lead II from the electrocardiogram and a tachograph (model 7P4F, Grass Instruments, Quincy, Massachusetts) were used for monitoring heart rate. All hemodynamics were monitored on a Grass model 7 polygraph.

Myocardial Segment Shortening

Subendocardial segment function (percent segment shortening, %SS) was measured in the regions perfused by the LAD and the left circumflex (LCx) coronary arteries using two sets of piezoelectric crystals (5 MHz) inserted into the subendocardium (approximately 10–15 mm apart and 7–9 mm deep). The crystals were inserted parallel to subendocardial fiber orientation at approximately the same level (30 cm from left coronary bifurcation) of the heart in both LAD and LCx areas. Crystal depths were verified at the end of each experiment. The crystal leads were connected to an ultrasonic amplifier that transforms the crystal-transmitted sound pulse into an electrical signal proportional to the distance between them. The tracings were monitored with an oscilloscope (model 520 Soltec, Sun Valley, California). Changes in transmission time...
indicated the distance between the two crystals. Diastolic segment length (DL) was determined at the beginning of the rise phase of positive dP/dt (onset of isovolumic contraction) and systolic segment length (SL) determined at peak negative dP/dt. %SS was calculated by use of the equation

\[ \%SS = \left( \frac{DL - SL}{DL} \right) \times 100 \]

The segment-length data were normalized by a value of 10 for the control diastolic segment length.\(^{29}\)

**Myocardial Blood Flow**

Transmural myocardial tissue blood flow was determined by the radioactive microsphere technique as described previously.\(^{24}\) Briefly, the left atrial appendage was cannulated for the administration of radioactive microspheres, and the right femoral artery was cannulated for withdrawal of a reference blood-flow sample. Radioactive microspheres (15 ± 3 \(\mu\)m diameter) labeled with \(^{111}\)In, \(^{141}\)Ce, \(^{103}\)Ru, or \(^{95}\)Nb were suspended in isotonic saline with 0.01% Tween 80 and were administered after 5 minutes of ultrasonication and 5 minutes of vortexing. Approximately 2–4 \(\times\) 10\(^8\) microspheres were injected into the left atrium followed by a 6-ml saline flush. Before microsphere administration, reference blood flows from the right femoral artery were collected at the rate of 6.8 ml/min for 2–3 minutes.

At the completion of each blood-flow collection, the LAD was reoccluded, and 10 ml India ink was injected into the LAD distal to the occlusion site to delineate the area subjected to ischemia, and saline was simultaneously injected at a similar pressure (100 mm Hg) into the left atrium. The heart was then removed, stored overnight in 10% formalin, subsequently sectioned into epicardium, midmyocardium, and endocardium of both the normal (three pieces) and ischemic regions (five pieces), and the tissue samples were weighed. All samples were counted in a gamma counter (1195, Searle Analytic, Elk Grove, Illinois) to determine the activity of each isotope in each tissue and reference blood-flow sample. Myocardial blood flow was calculated with a preprogrammed computer to obtain the true activity of each isotope in individual samples, and tissue blood flow was determined with the equation:

\[ Q_m = Q_s \times C_s / C_t \]

where \(Q_m\) is myocardial blood flow (ml/min/g), \(Q_s\) is rate of withdrawal of the reference blood-flow sample (6.8 ml/min), \(C_s\) is activity of the reference blood-flow sample (cpm), and \(C_t\) is activity of the tissue sample (cpm/g). Transmural tissue blood flow was calculated as the weighted average of the three layers in each region. Myocardial blood-flow distribution (endocardial:epicardial blood-flow ratio) was calculated by dividing the average endocardial flow of each region by the average epicardial flow. The ischemic area transmural flow normalized to the nonischemic area was also calculated.

**Prostanoid Measurements**

Blood samples (4 ml), obtained at various times from a local coronary vein draining the ischemic-reperfused area, were collected into ice-chilled plastic tubes containing 0.2 ml 2.8 mg/ml ethylenediaminetetraacetic acid as anticoagulant and 0.8 ml 0.1 mg/ml indomethacin to prevent ex vivo generation of prostanoids. Blood samples were centrifuged at 2,500 rpm for 10 minutes, and the resulting plasma was stored at \(-70^\circ\)C for later analyses. Thromboxane B\(_2\) and 6-keto-PGF\(_{1\alpha}\), the stable breakdown products of thromboxane A\(_2\) and prostacyclin, respectively, were measured with radioimmunoassay (New England Nuclear, Boston, Massachusetts).

**Metabolism Biopsy**

Immediately before killing the dogs at 3 hours postreperfusion, small areas in normal and postischemic regions were painted with methylene blue dye. Transmural tissue samples were obtained within the dye sites by the use of a cylindrical cutting tool mounted onto a hand drill. The sample was clamped immediately between two large aluminum blocks precooled in liquid nitrogen. The frozen biopsies were divided into three approximately equal transmural sections: epicardium (identified by the methylene blue dye), midmyocardium, and endocardium. The frozen sections were weighed and homogenized at 4° C in 6% perchloric acid with a Tekmar tissue homogenizer. Extracts were neutralized with 5 M K\(_2\)CO\(_3\), and the supernatant was used for biochemical analyses as performed previously.\(^{22,30}\) Analyses were performed at 340 nm on a Gilford 250 spectrophotometer. An aliquot of neutralized extract was used in a coupled enzymatic reaction to determine phosphocreatine and adenosine 5'-triphosphate (ATP), and in a separate coupled reaction, adenosine 5'-diphosphate (ADP) and adenosine 3,5'-monophosphate (AMP) were determined. Tissue slices from normal and postischemic regions were weighed and dried to constant weight at 95°C in preweighed vials. The total tissue water was expressed as milliliters of water per 100 grams of dry tissue. Tissue nucleotides were expressed as micromoles per gram of dry weight, and the total adenine nucleotide pool was calculated as the sum of ATP, ADP, and AMP in each layer.

**Experimental Protocol**

After surgical preparation and stabilization, pretreatment control measurements of hemodynamics, myocardial segment function, blood flow, blood gases, and prostanoids were obtained (Figure 1). Microspheres were administered before saline or drug intervention. Saline (control series), control; dazmegrel at 3 mg/kg, dazmegrel-3; dazmegrel at 8 mg/kg, dazmegrel-8; or BM 13.505 at 10 mg/kg were administered as a slow intravenous bolus in 30 ml saline into the right femoral vein 15 minutes
before LAD occlusion. Ten minutes after drug treatment, hemodynamics, myocardial segment function, and prostanoid levels were determined. The LAD was then occluded, and at 12 minutes of occlusion, myocardial segment shortening and myocardial blood flow were determined, and blood samples were obtained. At the end of 15 minutes of ischemia, the occlusion was slowly released, allowing LAD blood flow to return to pretreatment control values, that is, attenuating the reactive hyperemic response. Hemodynamics and myocardial function were determined at 5, 15, 30, 60, 120, and 180 minutes of reperfusion. Blood samples for prostanoid and blood gas determinations were obtained at 5, 30, and 180 minutes of reperfusion. Radioactive microspheres were administered at 30 and 180 minutes of reperfusion to determine regional myocardial blood flow. In a separate group, animals were surgically prepared, and myocardial wall function was measured at the same times as listed above; however, no coronary occlusion was performed. This group is referred to as the “sham group.”

In a separate series of three dogs, anesthetized and ventilated as described above, aortic systolic pressures were monitored through a pressure transducer catheter inserted into the aorta from the carotid artery. The thromboxane analogue U46619 was administered intravenously in increasing doses through a femoral vein cannula. Doses were increased when pressure had stabilized after the previous administration (approximately 10–15 minutes). After administration of the highest dose and a return of pressure to predrug levels, BM 13.505 was administered as an intravenous bolus at 1 mg/kg, and U46619 was again administered in increasing doses, and the aortic pressure changes were recorded. This protocol was repeated for BM 13.505 at 10 mg/kg.

Statistical Analyses

All values are mean ± SEM. Hemodynamics were obtained from a mean of 3–5 cardiac cycles. Groups were compared with a two-way analysis of variance with repeated measures design. Fisher’s least-significant difference was used to test for the significance of difference between two groups at specific time points isolated from the repeated measures analysis or when values were compared with the pretreatment control after a one-way repeated measures analysis of variance. A nonpaired t test was used to compare differences between adenine nucleotide and tissue water values in nonischemic and ischemic-reperfused tissue. Fisher’s exact test was used to test for differences in percent ventricular fibrillation between groups. Means were considered significantly different when p was less than 0.05.

Results

Of the 50 dogs initially instrumented, 11 died of ventricular fibrillation within 2 minutes after reperfusion; six of 16 in the control group, one of 11 in the dazmegrel-3 group, two of 12 in the dazmegrel-8 group, and two of 11 in the BM 13.505 group. Although the incidence of ventricular fibrillation after reperfusion after treatment with dazmegrel was less than that in the control group, this difference was not statistically significant. Analysis of data was performed for 10 control dogs and 10 dazmegrel-treated dogs at each of the two doses studied and for nine BM 13.505–treated dogs.

Hemodynamics

The hemodynamics of all groups during pretreatment control, at 10 minutes after drug treatment, at 12 minutes of occlusion and at 180 minutes after reperfusion are summarized in Table 1. There were no significant differences in any hemodynamic variable between control, dazmegrel-treated dogs, and BM 13.505–treated dogs at any time, and there were no differences within any group throughout the experiment, with the exception of dP/dt in the BM 13.505 group, which was significantly decreased during reperfusion compared with the control group (p < 0.05).

Intravenous infusion of the thromboxane agonist U46619 resulted in marked increases in aortic systolic pressure (10 and 50 μg U46619 lead to increases of 14 and 76 mm Hg, respectively). Pretreatment with 1 mg/kg BM 13.505 significantly attenuated these pressure increases, and 10 mg/kg BM 13.505 totally prevented the increase in aortic systolic pressure produced by doses of U46619 as high as 500 μg.

Area at Risk

The left ventricular weights were similar in all groups (Table 2). The percentage of the left ventricle at risk was also similar between saline, dazmegrel, and BM 13.505–treated dogs, demonstrating that equivalent perfusion bed areas were subjected to ischemia.
TABLE 1. Hemodynamics in Saline-, Dazmegrel-, and BM 13.505-Treated Dogs

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>HR (beats/min)</th>
<th>MAP (mm Hg)</th>
<th>LVSP (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>dP/dt (mm Hg/sec)</th>
<th>CBF (ml/min)</th>
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<td>Pretreatment</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>151 ± 5</td>
<td>98 ± 5</td>
<td>109 ± 5</td>
<td>2 ± 1</td>
<td>2,543 ± 175</td>
<td>27 ± 4</td>
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<td>Dazmegrel-3</td>
<td>150 ± 6</td>
<td>100 ± 4</td>
<td>114 ± 5</td>
<td>4 ± 1</td>
<td>2,458 ± 146</td>
<td>26 ± 2</td>
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<tr>
<td>Dazmegrel-8</td>
<td>149 ± 4</td>
<td>99 ± 4</td>
<td>111 ± 5</td>
<td>2 ± 1</td>
<td>2,430 ± 176</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>BM 13.505</td>
<td>135 ± 7</td>
<td>88 ± 5</td>
<td>96 ± 5</td>
<td>2 ± 1</td>
<td>2,092 ± 147</td>
<td>30 ± 3</td>
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<td>After drug</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>151 ± 5</td>
<td>96 ± 5</td>
<td>107 ± 5</td>
<td>2 ± 1</td>
<td>2,603 ± 170</td>
<td>28 ± 4</td>
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<td>Dazmegrel-3</td>
<td>145 ± 6</td>
<td>97 ± 5</td>
<td>111 ± 5</td>
<td>3 ± 1</td>
<td>2,406 ± 123</td>
<td>29 ± 2</td>
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<td>Dazmegrel-8</td>
<td>147 ± 4</td>
<td>100 ± 4</td>
<td>112 ± 4</td>
<td>3 ± 1</td>
<td>2,528 ± 184</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>BM 13.505</td>
<td>135 ± 8</td>
<td>91 ± 5</td>
<td>98 ± 6</td>
<td>2 ± 1</td>
<td>2,125 ± 161</td>
<td>30 ± 3</td>
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<td>Occlusion</td>
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<tr>
<td>Control</td>
<td>154 ± 5</td>
<td>93 ± 5</td>
<td>103 ± 5</td>
<td>4 ± 1</td>
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<td>Dazmegrel-3</td>
<td>149 ± 7</td>
<td>94 ± 5</td>
<td>109 ± 5</td>
<td>4 ± 1</td>
<td>2,372 ± 138</td>
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<td>Dazmegrel-8</td>
<td>153 ± 4</td>
<td>91 ± 5</td>
<td>104 ± 6</td>
<td>4 ± 1</td>
<td>2,295 ± 173</td>
<td>...</td>
</tr>
<tr>
<td>BM 13.505</td>
<td>135 ± 8</td>
<td>87 ± 5</td>
<td>92 ± 5</td>
<td>4 ± 1</td>
<td>1,950 ± 145</td>
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<td>Reperfusion (180 min)</td>
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<tr>
<td>Control</td>
<td>149 ± 5</td>
<td>107 ± 5</td>
<td>119 ± 4</td>
<td>3 ± 1</td>
<td>2,513 ± 109</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Dazmegrel-3</td>
<td>146 ± 8</td>
<td>91 ± 5</td>
<td>107 ± 5</td>
<td>5 ± 1</td>
<td>2,278 ± 185</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>Dazmegrel-8</td>
<td>154 ± 4</td>
<td>93 ± 7</td>
<td>105 ± 7</td>
<td>3 ± 1</td>
<td>2,390 ± 197</td>
<td>23 ± 3</td>
</tr>
<tr>
<td>BM 13.505</td>
<td>138 ± 8</td>
<td>90 ± 7</td>
<td>99 ± 7</td>
<td>2 ± 1</td>
<td>1,942 ± 170*</td>
<td>24 ± 3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n = 10, control and dazmegrel groups; n = 9, BM 13.505 group.
HR, heart rate; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; CBF, coronary blood flow; Dazmegrel, dazmegrel-treated group, 3 or 8 mg/kg; BM 13.505, 10 mg/kg.
*p<0.05 compared with the control group.

Regional Myocardial Blood Flow

Tissue blood flow within the nonischemic, LCx-perfused bed was not different between the control, dazmegrel- and BM 13.505-treated dogs before or during occlusion or at 30 minutes after reperfusion (mean ± SEM, transmural pretreatment control flow: control group, 1.09 ± 0.14; dazmegrel-3 group, 1.26 ± 0.16; dazmegrel-8 group, 1.05 ± 0.12; BM 13.505 group, 0.93 ± 0.06 ml/min/g). However, by 3 hours after reperfusion, transmural tissue flow in the nonischemic area of the dazmegrel-8 group had significantly decreased to 0.69 ± 0.08 ml/min/g, whereas there were no significant changes in flow within the normal areas in the control, dazmegrel-3, or BM 13.505 groups. Although flow in the BM 13.505-treated animals tended to be lower in the ischemic-reperfused region, no significant differences in blood flow, endocardium:epicardium ratio, and ischemic-area transmural flow normalized to the nonischemic region were present before and during occlusion (Table 2), thus demonstrating that all groups were subjected to comparable degrees of ischemia. There were no differences present in tissue blood flow between the groups during reperfusion.

Prostanoid Measurements

Coronary venous thromboxane levels in control and dazmegrel groups are shown in Figure 2. Although thromboxane levels in the control group increased significantly by 3 hours after reperfusion

TABLE 2. Ischemic Bed Size and Regional Myocardial Blood Flow

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dazmegrel</th>
<th>BM 13.505</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 mg/kg</td>
<td>8 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Left ventricular weight (g)</td>
<td>92.8 ± 3.6</td>
<td>99.3 ± 3.5</td>
<td>105.4 ± 4.7</td>
</tr>
<tr>
<td>Percent left ventricle at risk</td>
<td>26.8 ± 1.5</td>
<td>26.7 ± 2.0</td>
<td>25.3 ± 1.7</td>
</tr>
<tr>
<td>Transmural collateral blood flow (ml/min/g)</td>
<td>0.20 ± 0.04</td>
<td>0.22 ± 0.05</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>Ischemic area (Endocardium/epicardium)</td>
<td>0.59 ± 0.08</td>
<td>0.43 ± 0.10</td>
<td>0.59 ± 0.08</td>
</tr>
<tr>
<td>Ischemic:nonischemic blood flow ratio in occlusion</td>
<td>0.19 ± 0.03</td>
<td>0.28 ± 0.10</td>
<td>0.21 ± 0.03</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n = 10, control and dazmegrel groups; n = 9, BM 13.505 group.
compared with pretreatment values (413 ± 52 vs. 274 ± 15 pg/ml, respectively), both doses of dazmegrel significantly decreased thromboxane levels throughout the experiment. The high dose appeared to provide a greater and more sustained suppression (peak thromboxane B₂ decreased with dazmegrel-3 by 20 ± 7% and with dazmegrel-8 by 9 ± 4% of pretreatment control).

The main metabolite of PGI₁, 6-keto-PGF₁α, did not change significantly in the control group during the 3 hours of reperfusion (pretreatment control, 313 ± 53; 3 hours of reperfusion, 438 ± 57 pg/ml). However, there was a significant increase in 6-keto-PGF₁α at both 30 minutes and 3 hours of reperfusion in animals treated with dazmegrel-8 and during occlusion and all three collection times during reperfusion in animals treated with dazmegrel-3 (dazmegrel-3, 268 ± 54%; dazmegrel-8, 211 ± 43% of pretreatment control at 3 hours; Figure 3). These values were significantly different from the control group during occlusion and throughout reperfusion in the dazmegrel-3 group and at 3 hours of reperfusion in the dazmegrel-8 group (Figure 3). In the BM 13.505 group, 6-keto-PGF₁α was not significantly different from values observed in the control group (pretreatment control, 310 ± 33; 3 hours of reperfusion, 457 ± 79).

The thromboxane B₂:6-keto-PGF₁α ratio did not change in the control group (Figure 4); however, this ratio was markedly decreased in dazmegrel-treated dogs before and during occlusion and during reperfusion. There were no differences between dazmegrel-3 and dazmegrel-8 groups in the thromboxane B₂:6-keto-PGF₁α ratio.

Myocardial Segment Function

There were no significant differences in subendocardial wall function in the LCx-perfused, nonischemic area between any group throughout the experiment. Segment shortening in this area remained relatively constant throughout 3 hours of reperfusion.

In the LAD-perfused area, %SS in the sham group (Figure 5) remained stable throughout the experiment (sham group %SS of pretreatment control at 3 hours of reperfusion, 103 ± 6%). In the groups subjected to coronary occlusion, %SS was similar between control, dazmegrel-, and BM 13.505-treated animals before and during LAD occlusion. Dazmegrel or BM 13.505 did not significantly influence %SS during the initial 15 minutes before coronary occlusion. In the ischemic-reperfused region, occlusion resulted in paradoxical systolic bulging as indicated by negative %SS values. The decrease in %SS was not different between groups (Figures 5 and 6), which suggests that dazmegrel or BM 13.505 pretreatment did not alter the severity of the ischemic insult. There were no significant differences in functional recovery between the control and dazmegrel-3 groups during early reperfusion (control group %SS of pretreatment control at 5 minutes of reperfusion, 31 ± 15%; dazmegrel-3 group,
FIGURE 4. Plot of plasma thromboxane B₂:6-keto-prostaglandin F₁α (TXB₂/6-Keto-PGF₁α) ratio (stable metabolites of thromboxane A₂ and prostacyclin (prostaglandin I₂), respectively, measured at pretreatment control (PTC), 10 minutes after dazmegrel or saline administration, 12 minutes of occlusion (Occ), and at various times after reperfusion from a local coronary vein draining the ischemic-reperfused region. *p<0.05 vs. PTC within a group based on absolute values.

FIGURE 5. Plot of percent segment shortening of pretreatment control (PTC) in the ischemic-reperfused region at PTC, 10 minutes after drug administration (Drug), 12 minutes of occlusion (Occ), and at various times after reperfusion. Time-course curves for sham (n=8), control (n=10), and dazmegrel-treated groups (n=10, each group) are shown. Each point is mean±SEM. PTC percent segment shortening were 18.8±2.0% (sham group), 19.9±2.1% (control group), 20.0±1.9% (dazmegrel-3), and 18.8±2.0% (dazmegrel-8). *p<0.05 vs. control group.

FIGURE 6. Plot of percent segment shortening of pretreatment control (PTC) in the ischemic-reperfused region at PTC, 10 minutes after drug administration (Drug), 12 minutes of occlusion (Occ), and at various times after reperfusion. Time-course curves for control (n=10) and BM 13.505-treated groups (n=9) are shown. Each point is mean±SEM. PTC percent segment shortening were 19.9±2.1% (control group) and 21.4±1.0% (BM 13.505 group).

64±8%); however, %SS in the dazmegrel-3 group was significantly greater than that in the control group from 30 minutes to 3 hours of reperfusion (%SS at 3 hours: control group, 2±14%; dazmegrel-3 group, 66±8%). %SS in the dazmegrel-8 group was significantly greater than that in the control group at all times after reperfusion (%SS at 5 minutes and 3 hours: dazmegrel-8, 83±6%, and 49±9% vs. control group, 31±15% and 2±14%, respectively). After 1 hour of reperfusion, %SS in the dazmegrel-3 group tended to recover to a greater extent than that in the dazmegrel-8 group although this difference was not statistically significant (Figure 5). There were no significant differences in functional recovery between control and BM 13.505 groups at any time during reperfusion (control group %SS of pretreatment control at 30 minutes reperfusion, 19±15%; BM 13.505 group at 3 hours, 32±13%; control group, 2±14%; BM 13.505 group, 26±11%) (Figure 6).

High-Energy Phosphates

Regional tissue levels of ATP, total adenine nucleotide pool, and phosphocreatine at 3 hours after reperfusion are shown in Table 3. In general, ATP was decreased and phosphocreatine increased in the ischemic-reperfused area of all groups; the greatest changes occurred in the endocardium. The total adenine nucleotide pool in the ischemic-
reperfused area was also decreased. In contrast, there was a reduction in the loss of ATP and total adenine nucleotides compared with the control group (p<0.05) in the endocardial area of the reperfused group in the dazmegrel-3 group. In contrast to the rebound increase in phosphocreatine observed in the epicardium, midmyocardium, and endocardium in the ischemic-reperfused region of the control group, no rebound increase in phosphocreatine was seen in the epicardium or midmyocardium of dogs treated with dazmegrel-3 or dazmegrel-8 (Table 3). There were no differences between control and BM 13.505 groups in myocardial content of adenine nucleotides in any layer. Similar to the reperfusion-induced rebound increase in phosphocreatine observed in the control group, a significant increase was also seen in the BM 13.505 group (Table 3).

Total tissue water in ischemic-reperfused regions was increased in all layers at 3 hours after reperfusion. Dazmegrel pretreatment (both groups) appeared to decrease the edema in endocardial layers; however, differences were not significant, whereas tissue edema was not affected by BM 13.505 treatment (endocardial tissue water of the nonischemic region subtracted from endocardial tissue water of the reperfused region: control group, 29.1±8.4; dazmegrel-3 group, 23.7±10.2; dazmegrel-8 group, 19.2±8.7; and BM 13.505 group, 35.0±8.5 ml H2O/100 g dry wt).

Discussion

The major findings in this study were that the thromboxane synthetase inhibitor dazmegrel improved functional and metabolic recovery in the stunned myocardium, whereas the thromboxane receptor antagonist BM 13.505 did not. Dazmegrel-mediated enhancement of posts ischemic function occurred in the absence of increases in regional

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**Table 3. Myocardial High-Energy Phosphates After 3 Hours of Reperfusion**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>Dazmegrel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 mg/kg</td>
<td>8 mg/kg</td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardium Reperfused</td>
<td>19.2±0.9*</td>
<td>20.2±1.1</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>24.9±1.4</td>
<td>23.1±1.0</td>
</tr>
<tr>
<td><strong>Midmyocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>19.2±0.4†</td>
<td>19.9±1.1</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>26.2±0.9</td>
<td>22.9±0.9</td>
</tr>
<tr>
<td><strong>Endocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>19.8±0.8†</td>
<td>20.6±1.3</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>25.6±0.6</td>
<td>22.5±1.2</td>
</tr>
<tr>
<td><strong>Total adenine nucleotides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardium Reperfused</td>
<td>24.0±1.2</td>
<td>23.8±1.2</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>25.8±1.1</td>
<td>27.4±1.3</td>
</tr>
<tr>
<td><strong>Midmyocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>23.0±0.6†</td>
<td>23.9±1.3</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>31.2±1.1</td>
<td>26.9±1.1</td>
</tr>
<tr>
<td><strong>Endocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>23.9±0.9†</td>
<td>25.3±1.6</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>30.2±0.7</td>
<td>27.4±1.3</td>
</tr>
<tr>
<td><strong>Phosphocreatine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardium Reperfused</td>
<td>51.5±4.4*</td>
<td>40.8±1.8</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>43.6±3.7</td>
<td>39.1±2.9</td>
</tr>
<tr>
<td><strong>Midmyocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>60.2±4.1†</td>
<td>39.7±4.5</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>40.9±2.2</td>
<td>36.7±2.7</td>
</tr>
<tr>
<td><strong>Endocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reperfused</td>
<td>69.6±5.0†</td>
<td>44.4±5.1*</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>39.3±3.2</td>
<td>30.5±2.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM in units of micromoles per gram of dry weight. n=10, control and dazmegrel groups; n=9, BM 13.505 group.
Samples were obtained 3 hours after reperfusion.
*p<0.05, †p<0.001, ‡p<0.01 compared with corresponding value in nonischemic zone of same layer.
blood flow or a redistribution to subendocardial layers without a decrease in myocardial oxygen demand and without attenuating the loss of systolic wall function that occurs during ischemia. In this
and other studies,17,26 an increase in thromboxane from the ischemic-reperfused region, particularly during the reperfusion phase, was observed in untreated animals. Preocclusion treatment with dazmegrel completely attenuated the increase in thromboxane release during reperfusion and markedly decreased baseline levels. During the inhibition of thromboxane synthesis, a marked increase in prostacyclin synthesis occurred.

Thromboxane A2, produced mainly by the platelet and vessel wall, has recently been synthesized31 and characterized in vivo.8 Local injections of synthetic thromboxane A2 into the coronary vasculature resulted in significant reductions in coronary blood flow and in reductions in platelet count in coronary sinus blood.8 With the use of thromboxane synthesis inhibitors6,13,17,18 and receptor blockers,8,16 thromboxane has been implicated as a mediator of ischemia-reperfusion arrhythmias8,10,17 and of the progression of myocardial ischemic damage.13–17 Several studies suggest that thromboxane is arrhythmogenic during acute myocardial ischemia, particularly during reperfusion.5,10 However, in those studies, regional myocardial blood flow was not measured, and the possibility exists that the beneficial influence on arrhythmias after synthesis inhibition was secondary and was due to a decrease in ischemic damage. Results of the present study did not demonstrate a significant decrease in reperfusion-induced ventricular fibrillation in dogs pretreated with dazmegrel or BM 13.505. The reasons for the difference between these results and those of previous studies are unknown but may be related to the length of the occlusion period, species differences, or simply may be due to sample size.

In addition to antiarrhythmic properties, various thromboxane inhibitors have been shown to attenuate the loss of myocardial creatine kinase and nitrogenous compounds from the ischemic myocardium, to attenuate the rise in plasma creatine kinase activity, and to decrease the ischemia-induced ST segment elevation after permanent coronary occlusions.13–16,18 Also, the thromboxane synthetase inhibitor CV-4151 has been shown to decrease infarct size in a 1 hour occlusion–1 hour reperfusion model.17 Note that in these previous studies there were no observed drug effects on hemodynamics, myocardial oxygen demand, or myocardial oxygen supply. Cardioprotective actions were attributed to an attenuation of catecholamine release, an endoperoxide shunting to potentially beneficial prostanooids after the use of synthesis inhibitors, or an inhibition of thromboxane-mediated vasoconstriction, platelet aggregation, or cellular lysis.13–17,32

All previous studies except one,33 that have investigated the pathophysiological role of thromboxane in ischemic and postischemic myocardial dysfunc-

13-17
ondary to an increase in myocardial function. In addition, although beneficial results of dazmegrel were observed on metabolism, it appears that thromboxane inhibition may not be mediating this effect because thromboxane receptor blockade did not result in similar responses.

Postischemic dysfunction in the stunned myocardium has also been related to the presence of oxygen-derived free radicals. Potential sources of free radicals during ischemia and reperfusion include arachidonic acid metabolism, catecholamine autoxidation, mitochondrial electron transport disruption, xanthine oxidase, and activated neutrophils. Neutrophils, which may be a major cause of myocardial stunning, are also sites for thromboxane synthesis, and thus, inhibition of neutrophil thromboxane release may mediate some of the beneficial actions of dazmegrel. This hypothesis seems unlikely, however, for if this were true, pretreatment with BM 13.505 should have shown similar results. Therefore, if dazmegrel has effects on neutrophils, they are most likely not related directly to thromboxane inhibition.

The results of this study and those of previous experiments in our laboratory and others have demonstrated an increase in the release of thromboxane during ischemia and reperfusion. The physiopathological importance of this increase in thromboxane in mediating prolonged postischemic dysfunction in the stunned myocardium, although suggested by the study with dazmegrel, does not definitively elucidate the role of thromboxane in this model as exhibited by the lack of beneficial metabolic and functional effects after thromboxane receptor blockade. Herein represents a methodological problem in using only thromboxane synthetase inhibitors to delineate the role of thromboxane in any given experimental or clinical study. Thromboxane receptor antagonists must also be used.

The mechanism by which BM 13.505 produces vascular smooth muscle relaxation has recently been characterized in isolated, perfused cat coronary arteries and in rat aortic rings. The vasodilatory action of BM 13.505 was found neither to be endothelium dependent nor influenced by the guanylate cyclase inhibitor, methylene blue. Also, BM 13.505 neither produced relaxation in rings precontracted with norepinephrine or angiotensin II, nor did it scavenge oxygen free radicals. These authors concluded that the main action of BM 13.505 on inhibiting thromboxane-induced vasoconstriction in vascular smooth muscle was as a competitive receptor antagonist of thromboxane A2. Although many thromboxane receptor blockers exhibit different ratios of effectiveness in antagonizing thromboxane agonist-induced platelet aggregation and vasoconstriction, BM 13.505 appears to inhibit platelet and vascular receptors equally. BM 13.505 has also been shown to protect against thromboxane agonist–mediated membrane destabilization in human red blood cells. Although the thromboxane-receptor antagonist BM 13.177 has been shown to retard the progression of myocardial ischemic damage in long-term occlusion studies, the structurally related compound BM 13.505 has been shown to be seven to nine times more potent and longer lasting. Thus, if thromboxane were involved in myocardial stunning, pretreatment with BM 13.505 would be expected to have blocked all thromboxane-mediated effects and to have enhanced functional and metabolic recovery.

The increase in prostacyclin observed in our study after thromboxane synthesis inhibition is well documented and appears to be a likely mechanism for the beneficial effects of dazmegrel in the stunned myocardium. Also, enhanced production of PGD₂, PGF₂α, and PGE₂ after inhibition of thromboxane synthesis has also been suggested. An increase in the production of these prostanoids, as well as prostacyclin, would be possible in endothelium or leukocytes by conversion of endoperoxides released from platelets or from arachidonic acid released from tissues as a result of membrane damage. Prostacyclin could potentially inhibit many thromboxane-mediated events, such as vasoconstriction, platelet aggregation, cell membrane destabilization, catecholamine release, and profibrillatory action. By altering the ratio of thromboxane to prostacyclin in favor of the vasodilatory prostacyclin, myocardial perfusion at the microcirculatory level may also be enhanced.

We have previously demonstrated that infusion of the stable prostacyclin mimetic, iloprost, was able to significantly enhance postischemic function in the stunned myocardium when the drug was administered either before occlusion or during reperfusion. In addition to mechanisms previously mentioned, potential benefit from PG₁, in the stunned myocardium could be through the inhibition of neutrophil-derived superoxide anion production.

Treatment with the high dose of dazmegrel (8 mg/kg) resulted in less preservation of adenine nucleotides, slightly less functional recovery by 3 hours of reperfusion, and less of an increase in 6-keto-PGF₁α levels compared with the lower dose (3 mg/kg). Although the reasons for these observations are unknown, they may be related to either a nonspecific inhibition of prostacyclin synthase or cyclooxygenase by the higher dose or an endoperoxide reorientation to prostanoids other than prostacyclin. Because other prostanoids were not measured in the present study, evaluation of these hypotheses is not possible. Also, the effects of PGF₂α, PGD₂, and PGE₂ on the functional recovery of the stunned myocardium are unknown. However, the greater endoperoxide shunting to prostacyclin by the lower dose of dazmegrel is in accordance with the finding that receptor blockade shared none of the beneficial effects of synthetase inhibition and the hypothesis that rather than thromboxane being involved as a mediator of myocardial stunning, synthesis inhibition is beneficial because of the
increase in prostacyclin. Thus, one would predict that the greater increase in prostacyclin with the lower dose of dazmegrel would lead to greater postischemic recovery.

In conclusion, inhibition of thromboxane synthetase by dazmegrel, but not thromboxane receptor blockade by BM 13.505, significantly enhanced the recovery of regional myocardial function (%SS) in the stunned myocardium. This cardioprotective effect was achieved in the absence of changes in myocardial oxygen supply-demand variables and without preventing the loss of systolic wall function that occurs during ischemia. Dazmegrel, but not BM 13.505, resulted in the preservation of myocardial adenine nucleotides within the ischemic-reperfused myocardium. Thus, although blockade of thromboxane actions by antagonism of receptors does not enhance postischemic function or metabolism, inhibition of thromboxane synthesis and subsequent prostanooid redirection to prostacyclin augments postischemic recovery after a reversible ischemic insult and demonstrates a lack of pathophysiological significance for thromboxane as a mediator of the stunning phenomenon.

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