Inhibition of Cyclic Flow Variations in Stenosed Canine Coronary Arteries by Thromboxane A2/Prostaglandin H2 Receptor Antagonists


We tested the hypothesis that thromboxane A2 and thromboxane A2/PGH2 receptor occupation are important in mediating cyclic reductions in coronary blood flow (CFVs) in concentrically narrowed canine coronary arteries. Two potent and selective thromboxane A2/PGH2 receptor antagonists, SQ29,548 and SQ28,668 eliminated CFVs and restored a normal pattern of blood flow through the severely narrowed vessels in 77 and 75% of the dogs, respectively. CFVs were eliminated within several minutes of an intravenous bolus injection of SQ29,548 or SQ28,688. A continuous infusion of SQ29,548 (0.2 mg/kg-min) prevented the recurrence of CFVs throughout the duration of its infusion. Left atrial infusions of the thromboxanes A2 mimetic, U46619, restored CFVs in 5 of 8 SQ29,548-treated and in 5 of 7 SQ28,668-treated dogs. Circulating concentrations of the stable metabolites of TxA2 and PGH2, TxB2 and 6-keto-PGF1α, respectively, were unaffected by administration of SQ29,548. However, stenosed vascular segments of the left anterior descending coronary artery (LAD) of SQ29,548-treated dogs produced significantly less thromboxane A2 than comparable segments from untreated dogs. Morphologic studies showed that stenosed coronary arteries in which CFVs had been abolished by either SQ29,548 or SQ28,668 had relatively few adherent platelets, whereas comparable coronary segments removed from untreated animals had relatively large, platelet-rich mural thrombi. SQ29,548 did not alter the synthesis of TxA2 by platelets. 6-keto-PGF1α concentrations in the stenosed LAD and nonstenosed circumflex coronary arteries were not altered by SQ29,548 administration. These data suggest that the thromboxane A2/PGH2 receptor antagonists, SQ29,548 and SQ28,668, inhibit cyclic reductions in coronary blood flow in this model by preventing the accumulation of platelets at the site of a critical coronary arterial stenosis. The data also suggest that TxA2 is important in mediating the interaction between platelets and the constricted coronary artery that is responsible for the development and maintenance of CFVs in this experimental model. (Circulation Research 1986;59:568-578)

Evidence from recent studies suggests that an important relation exists between the release of platelet-derived vasoactive substances during platelet aggregation and the development of acute coronary heart disease syndromes, such as unstable angina pectoris. Thromboxane A2 (TxA2), synthesized and released from platelets during aggregation, is a potent vasoconstrictor and platelet aggregating substance. The transcardiac concentrations of thromboxane B2 (TxB2), the inactive metabolite of TxA2, are elevated in patients with active unstable angina pectoris. An in vivo canine model of transient coronary flow reductions in concentrically narrowed coronary arteries has been used to examine potential mechanisms responsible for the recurring pattern of spontaneous declines in coronary blood flow followed by sudden flow restorations (cyclic flow variations, CFVs). Previous studies have shown that CFVs result from platelet aggregation and dislodgement at the stenotic site. We and others have shown that inhibition of cyclooxygenase with aspirin or inhibition of thromboxane synthetase with dazoxiben, are effective in eliminating or greatly attenuating the occurrence of CFVs in this canine model. Thus, in this model, TxA2 appears to play a central role in maintaining CFVs. However, inhibition of TxA2 synthesis in vivo may result in 1) elevated levels of the cyclic endoperoxide prostaglandins (PG) PGG2 and PGH2, which are less potent but longer lived proaggregatory agents; 2) enhanced diversion of PGG2 and PGH2 degradation to other platelet active substances (PGI2, PGD2); and 3) enzymatic degradation of PGG2 by vessels to form PGI2.
the hypothesis that TxA<sub>2</sub> and TxA<sub>2</sub>/PGH<sub>2</sub> receptor occupation are important in mediating CFVs in stenosed canine coronary arteries. We used the selective TxA<sub>2</sub>/PGH<sub>2</sub> receptor antagonists, SQ29,548 and SQ28,668, to test this hypothesis. The pharmacological specificity and potency of these antagonists have been reported previously. We determined the effect of these antagonists on the frequency and severity of CFVs in a canine model of coronary artery narrowing. Similarly, the effect of TxA<sub>2</sub> receptor antagonism on the circulating concentrations of TxA<sub>2</sub> and 6-keto-PGF<sub>1α</sub>, arachidonate-stimulated platelet TxA<sub>2</sub> synthesis, and coronary arterial prostanooid production was determined. The results from this study demonstrate that TxA<sub>2</sub> and occupation of the TxA<sub>2</sub>/PGH<sub>2</sub> receptor are important in mediating the observed transient platelet aggregation that leads to CFVs since selective antagonism of these receptors rapidly and effectively abolishes CFVs in most animals. Further, administration of a TxA<sub>2</sub>-mimetic, U46619, restored CFVs in the majority of animals. The stenosed coronary arterial segments obtained from SQ29,548-treated dogs synthesized significantly less TxA<sub>2</sub> than identical untreated stenosed coronary arterial segments. Arachidonate-stimulated TxA<sub>2</sub> synthesis was not affected in platelets obtained from dogs treated with SQ29,548 and morphologic examination of the stenosed coronary arterial segments from SQ29,548-treated dogs demonstrated a marked reduction in the accumulation of platelets. Thus, in this model, SQ29,548 abolishes transient CFVs by inhibiting the accumulation of platelets at the site of endothelial damage at a severe coronary arterial stenosis.

Materials and Methods

Surgical Preparation

Eighty-eight male mongrel dogs (18-33 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV) and artificially ventilated with room air. Catheters were placed in the common carotid artery and jugular vein for arterial pressure measurement and fluid administration, respectively. A thoracotomy was performed in the fifth left intercostal space, and the heart was suspended in a pericardial cradle. A segment of the left anterior descending coronary artery (LAD) was gently dissected free from surrounding tissue, and a pulsing Doppler flow probe was positioned around the LAD proximally but adjacent to where the coronary constrictor would be placed, as previously described. Arterial blood gases and body temperature were maintained within normal physiological limits.

Experimental Protocols

At the conclusion of surgery and instrumentation, dogs were allowed to stabilize for 30 minutes. Control hemodynamics, including systolic and diastolic blood pressures, heart rate, and mean and phasic coronary blood flow velocities, were recorded continuously on an 8-channel recorder (Hewlett-Packard, model 7758). A hard plastic, cylindrical constrictor was placed around the LAD with a lumen diameter that reduced control blood flow velocity to an extent required to eliminate reactive hyperemia after a 10-second temporary coronary artery occlusion. Ten (11%) of 88 dogs did not have cyclical declines in coronary blood flow, called cyclic flow variations (CFVs), following placement of a number of different constrictors. The protocols used in the 78 remaining dogs are described below.

Group I — Control Animals

Cyclic flow frequency, severity of CFVs (nadir of blood flow velocity), and hemodynamics were monitored for 3 hours in 28 dogs. These animals were subsequently sacrificed, and in 9 of these animals the LAD coronary artery segments under the constricted area and circumflex coronary artery segments were removed and evaluated morphologically or prostaglandin and TxB<sub>2</sub> production were measured as described below.

Three additional dogs had CFVs initiated and documented for a period identical to the total study time for Group II (1) and Group III (2) dogs, which had 30 minutes of CFVs, and had CFVs abolished with SQ29,548 or SQ28,668 for 30 minutes. The hearts from these 3 dogs were removed when coronary blood flow had declined about three fourths of the way to nadir flow (the lowest flow prior to restoration of flow) and were prepared for morphological studies as described below.

Group II — SQ29,548-Treated Animals

Cyclic flow variations were initiated in 35 additional dogs. CFV frequency, severity, and hemodynamics were monitored for 30 minutes following establishment of reproducible CFVs. SQ29,548 (E.R. Squibb and Sons, Inc., Princeton, N.J.) was dissolved in ethanol (10 mg/ml) and further diluted in 2 mM sodium carbonate to a 1-mg/ml solution. The 35 dogs were subdivided into three groups:

Group II-A: Six dogs received a 1 mg/kg IV injection of SQ29,548. The effect of this drug on systemic hemodynamics and coronary blood flow velocity was evaluated. The time to abolish or attenuate CFVs and the duration of effect of a single dose of the drug in abolishing CFVs were determined.

Group II-B: Nine dogs received SQ29,548 as an IV injection (0.2 mg/kg) followed by a continuous infusion (0.2 mg/kg-hr). These animals had 30 minutes of reproducible CFVs prior to the administration of SQ29,548. If the initial 0.2 mg/kg dose of SQ29,548 did not eliminate CFVs, additional SQ29,548 was injected in 0.2 mg/kg doses at 20-minutes intervals accompanied by a continuous infusion of 0.2 mg/kg-hr until CFVs disappeared. Systemic hemodynamics and coronary blood flow velocity were monitored for 2-4.5 hours following abolition of CFVs. Thereafter, the TxA<sub>2</sub> mimic, U46619, was administered in repeated 20-μg intraarterial injections at 10-minute intervals to restore CFVs in 8 dogs in whom CFVs had been abolished. Systemic hemodynamics and coronary blood flow velocity were observed until CFVs were
restored or until a total dose of 140 μg of U46619 was administered.

Group II-C: Twenty dogs received SQ29,548 as in Group II-B dogs until CFVs were abolished. In 5 of these dogs, aortic blood samples were collected on ice from a carotid arterial catheter in 15-m1 vacutainer (Becton-Dickinson) tubes containing 2000 U of bovine heparin and 40 μg of indomethacin (Sigma). Blood was collected immediately following the placement of the constrictor, before CFVs occurred, during CFVs at high and low flow velocities, and 30 minutes following the abolition of CFVs with SQ29,548. Animals were sacrificed and the stenosed LAD coronary artery segments were dissected from the heart in 6 of these dogs and processed as described below. Blood samples were centrifuged at 2000g and 4°C. Plasma was separated and frozen for subsequent analysis of TxB2, and 6-keto-PGF1α by radioimmunoassay. The hearts from 3 of the 20 dogs were removed after 30 minutes of flow restoration following SQ29,548 administration and prepared for morphological studies described below.

Group III — SQ28,668-Treated Animals

Cyclic flow variations were observed for 30 minutes in 12 additional dogs. SQ28,668 (E.R. Squibb and Sons, Princeton, N.J.) was dissolved in ethanol (100 mg/ml) and diluted in sodium carbonate for administration to the animals. SQ28,668 was administered as an IV infusion beginning at a rate of 5 μg/kg-min and increased at 15-minute intervals at 12.5, 25, 50, and 125 μg/kg-min until CFVs were abolished. Systemic hemodynamics and mean and phasic coronary blood flow velocity were documented. The dose of SQ28,668 required to abolish CFVs was calculated by adding the duration of infusion at each infusion rate until CFVs were abolished. In seven of the dogs in whom CFVs were abolished with SQ28,668, U46619 was infused at a constant rate beginning at 2 μg/min and increased at 10 minute intervals to 5, 10, and 25 μg/min until CFVs were restored or a 625 μg total dose was administered. Two of the 12 dog hearts were removed following 30 minutes of flow restoration with SQ28,668 and processed for morphological studies described below.

Analysis of Prostacyclin and Thromboxane Production by Vessels and Platelets

A short segment of LAD surrounding the stenotic area and segment of circumflex coronary artery in 9 Group I (control) and 6 Group II-C dogs were dissected from the hearts and placed in calcium-free Medium 199 containing 3 mM EDTA, pH 7.4 (GIBCO, Grand Island, N.Y.) at room temperature. The arteries were cleaned of excess connective tissue and cut into 2 mm rings that were subsequently split longitudinally. One segment of coronary artery was placed in each incubation tube containing 1 ml of Medium 199 and 1.8 mM calcium. Incubation media for SQ29,548-treated dogs also contained 1 μM SQ29,548. Vessel segments were incubated with 10-8, 10-7, or 10-6 M arachidonic acid (Sigma Chemical Company, St. Louis, Mo.) or its vehicle. Incubations were performed at 37°C in a shaking water bath for 10 minutes, and the reaction was terminated by removal of the coronary artery tissue segment. The remaining media was quick-frozen in a dry ice–methanol bath and stored at -40°C until analyzed for 6-keto-PGF1α and TxB2. Coronary artery tissue segments were blotted dry and weighed.

Blood was collected in 0.1 volume of 3.5% sodium citrate, pH 7.4. Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 100g for 20 minutes. After removal of the PRP, the remaining red cell suspension was centrifuged at 1500g for 10 minutes to obtain platelet-poor plasma (PPP). The PRP was diluted with PPP to a final platelet concentration of 109/ml; 1 ml of PRP was incubated at 37°C with constant stirring at 1,000 rpm. Aggregation was stimulated by the addition of arachidonic acid (10-4 M). The PRP was sampled before the addition of arachidonic acid and at various times thereafter. A 50-μl aliquot of PRP was removed from the cuvette, immediately added to 450 μl of ice-cold phosphate-buffered saline containing indomethacin (1 μg/ml) and quick-frozen in a methanol-dry ice bath. These samples were kept frozen at -40°C until they were assayed for TxB2. The results were expressed as nanograms of TxB2/106 platelets.

Prostanoid Measurements

TxB2 and 6-keto-PGF1α were measured by the method of Dray et al20 as modified by Campbell et al.21 Plasma samples were extracted and purified by silicic acid chromatography while media or diluted platelet suspensions were analyzed directly. Using this procedure, 0.1 ml of the diluted sample (0.1 ml) was combined with 0.1 ml of 3H-TxB2 or 3H-6-keto-PGF1α, and 0.1 ml of specific antiserum. Following incubation overnight at 4°C, antibody-bound prostaglandins were separated from free prostaglandins with dextran-coated charcoal. SQ29,548 did not cross-react with either the TxB2 or 6-keto-PGF1α antibody. The bound radioactivity was counted using a Beckman liquid scintillation spectrometer. Results were expressed as picograms per milligram tissue weight.

Morphological Studies

Following removal of 3 Group I, 1 Group II, and 2 Group III hearts, a catheter was placed in the left coronary ostium and warm tissue culture media (Dulbecco's modified Eagle medium, GIBCO, Grand Island, N.Y.) was gently flushed through the LAD with the constrictor still in place to remove residual blood. Glutaraldehyde (3% in 0.1 M phosphate buffer) was perfused through the catheter at 100 mm Hg pressure for 5–10 minutes. The hearts were placed in 10% phosphate-buffered formalin prior to processing for scanning electron microscopy. The proximal LADs were carefully dissected from the heart and the constrictors removed. The LAD segments were bisected longitudinally and visualized under a dissecting microscope.

For scanning electron microscopy, specimens were dehydrated through a graded series of alcohols, criti-
cal-point dried, mounted on stubs using a colloidal carbon paste, coated with 30 mm of gold palladium alloy using a sputter-coater, placed in a scanning electron microscope, examined, and photographed. After this procedure, the same specimens were again placed in alcohol and embedded in glycol methacrylate. Plastic sections were cut, stained with hematoxylin and eosin, examined by light microscopy, and photographed. This approach permitted comparison of findings by scanning electron microscopy and light microscopy of the same specimens.

For the morphological studies, Group I animals having CFVs at the end of the experiment were sacrificed at a mean flow velocity near the nadir of coronary flow. Group II and Group III animals with stable coronary blood flows were sacrificed at the end of the 30-minute observation period following abolition of CFVs with SQ29,548 or SQ28,668.

### Statistical Analyses

All values are expressed as mean ± SEM. Comparisons between variables obtained at multiple times in each group of dogs were made by a two-way analysis of variance and Duncan's multiple range test. In all cases, a p value < 0.05 was used to define a significant difference between values.

### Results

Placement of the coronary artery constrictor in 78 dogs having CFVs in this study resulted in a reduction in peak phasic (diastolic) blood flow velocity of 50.1 ± 2.1% and a reduction in mean coronary blood flow velocity of 33.4 ± 2.8%.

### Hemodynamic Changes

Heart rate declined immediately after CFVs were initiated in 28 Group I dogs and remained lower than

### Table 1. Hemodynamic Effects of SQ29,548 and SQ28,668 in Dogs With Stenosed Coronary Arteries

<table>
<thead>
<tr>
<th></th>
<th>HR (BPM)</th>
<th>AOM (mm Hg)</th>
<th>PHF Peak (%)</th>
<th>PHF Nadir* (%)</th>
<th>MNF Peak (%)</th>
<th>MNF Nadir* (%)</th>
<th>CFVs (cycles/hour)</th>
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<td>No intervention</td>
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<td>142 ± 4</td>
<td>113 ± 2</td>
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<tr>
<td></td>
<td>Constricted</td>
<td>140 ± 4</td>
<td>112 ± 2</td>
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<td>62 ± 5†</td>
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<td></td>
<td>Hour 1</td>
<td>130 ± 4†</td>
<td>117 ± 2†</td>
<td>75 ± 5†</td>
<td>107 ± 9</td>
<td>7 ± 2†</td>
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<tr>
<td></td>
<td>Hour 2</td>
<td>121 ± 4†</td>
<td>115 ± 2</td>
<td>74 ± 5†</td>
<td>102 ± 9</td>
<td>8 ± 2†</td>
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<td></td>
<td>Hour 3</td>
<td>114 ± 4†</td>
<td>112 ± 2</td>
<td>69 ± 5†</td>
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<tr>
<td>Control</td>
<td>145 ± 3</td>
<td>115 ± 2</td>
<td>100 —</td>
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<tr>
<td>Constricted</td>
<td>143 ± 4</td>
<td>112 ± 2†</td>
<td>54 ± 3†</td>
<td>68 ± 3†</td>
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<td></td>
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<tr>
<td>Initial CFVs</td>
<td>138 ± 4†</td>
<td>111 ± 3†</td>
<td>85 ± 5†</td>
<td>10 ± 4†</td>
<td>116 ± 8†</td>
<td>13 ± 3†</td>
<td>11.2 ± 0.6</td>
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<tr>
<td>Post-SQ29,548</td>
<td>Abolished (27/35)</td>
<td>130 ± 5†</td>
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<tr>
<td></td>
<td>Not abolished (8/35)</td>
<td>128 ± 9</td>
<td>111 ± 5†</td>
<td>82 ± 9†</td>
<td>4 ± 2†</td>
<td>110 ± 11</td>
<td>4 ± 2†</td>
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<td>CFVs restored — U46619 (5/8) (II-B)</td>
<td>128 ± 20</td>
<td>129 ± 12</td>
<td>87 ± 7</td>
<td>20 ± 8†</td>
<td>108 ± 16</td>
<td>30 ± 11†</td>
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<tr>
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<td>SQ28,668</td>
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<tr>
<td>Control</td>
<td>136 ± 5</td>
<td>114 ± 3</td>
<td>100 —</td>
<td>100 —</td>
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<tr>
<td>Constricted</td>
<td>135 ± 6</td>
<td>114 ± 3</td>
<td>52 ± 5†</td>
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<td>Initial CFVs</td>
<td>131 ± 6</td>
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<td>96 ± 6</td>
<td>15 ± 3†</td>
<td>138 ± 10†</td>
<td>12 ± 3†</td>
<td>10.5 ± 0.5</td>
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<tr>
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<td>107 ± 6</td>
<td>81 ± 6</td>
<td>94 ± 8</td>
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<tr>
<td></td>
<td>Not abolished (3/12)</td>
<td>112 ± 15†</td>
<td>106 ± 6</td>
<td>95 ± 5</td>
<td>5 ± 3†</td>
<td>140 ± 10†</td>
<td>10 ± 6†</td>
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<tr>
<td>CFVs restored — U46619 (5/7)</td>
<td>129 ± 11</td>
<td>117 ± 9</td>
<td>104 ± 11</td>
<td>32 ± 7†</td>
<td>146 ± 14†</td>
<td>41 ± 8†</td>
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*Nadir is defined as the lowest flow velocity recorded (average 2 cycles) just prior to flow restoration and is expressed as a percent of control blood flow velocity.

†Value is significantly different from original control value, p < .05.

Values are expressed as mean ± SEM. HR = heart rate; AOM = aortic mean pressure; PHF = phasic or peak diastolic coronary blood flow velocity; MNF = mean coronary blood flow velocity (both PHF and MNF are expressed as a percent of respective control blood flow before placement of the constrictor). CFVs/hour = frequency of cyclic flow variations per hour.
control for the duration of the study (Table 1). In the second and third hours of CFVs, heart rates were significantly lower than during the first hour of CFVs. This heart rate depression was also observed in all Group II dogs (SQ29,548-treated). Systemic mean arterial pressure was slightly but significantly elevated during the first hour of CFVs in the control dogs (Group I) as shown in Table 1. Mean arterial pressure was slightly but significantly lowered in Group II groups after CFVs began.

Cyclic Flow Variations — Frequency and Severity

CONTROL DOGS (GROUP I). In 28 dogs in which CFVs were initiated and continued for a 3-hour period with no pharmacological interventions, CFV frequency (cycles/hour) did not change significantly during the 3-hour interval (10.4 ± 0.6, first hour; 9.7 ± 0.5, second hour; 10.3 ± 0.5, third hour, Figure 1, Panel A). Similarly, CFV nadir, the lowest flow velocity recorded just prior to restoration of flow through the vessel, expressed as a percent of control flow velocity, was not significantly different during the three hour period (Figure 1, Panel B), with a mean flow velocity nadir of 7 ± 2% of control flow the first hour, followed by 8 ± 2% and 8 ± 2% in hours 2 and 3, respectively.

SQ29,548-TREATED DOGS (GROUP II). A representative recording of the coronary artery flow changes that occurred during an initial period of CFVs and following an injection of SQ29,548 is shown in Figure 2. The rapid effect of this drug on coronary blood flow and the absence of important hemodynamic effects of the thromboxane receptor antagonist is evident in this tracing.

A summary of CFV frequency per 30 minutes prior to and following administration of SQ29,548 to all Group II dogs combined is shown in Figure 3. In addition, changes in mean flow velocity during an initial control period, following placement of the con-
The initial group of 35 dogs had a CFV frequency of 11.2 ± 0.4 cycles per hour and a mean flow velocity nadir of 13 ± 3% of control velocity (Table 1). CFVs were abolished in 27 of 35 dogs (77%) regardless of the method of administration, returning mean and phasic coronary flow velocity to a level slightly higher than constricted blood flow (Table 1, Figure 4A).

The duration of action of a single IV injection (1 mg/kg) of SQ29,548 was determined in 6 Group II-A dogs. This dose abolished CFVs within 1–3 minutes in 4 dogs, and CFVs returned again within 2.90 ± 0.8 hours in 3 of these 4 dogs. One dog of the 6 had CFVs abolished with 2 mg/kg of SQ29,548 but they returned within 33 minutes. An additional dog was resistant to the drug. In this dog, CFVs were markedly attenuated following 1 mg/kg SQ29,548, and an additional 3 mg/kg did not change the small, frequent reductions in flow followed by spontaneous flowbacks. However, severity of CFVs was markedly reduced in this dog.

In the remaining 29 Group II dogs (Groups II-B and C), CFVs were abolished in 21 dogs with an average IV dose of 0.38 ± 0.6 mg/kg in 10.1 ± 3.9 min (range 1–85 min). Eight of the 29 dogs did not have CFVs abolished after administration of an average dose of 1.63 ± 0.20 mg/kg. Frequency of CFVs in this group of dogs was significantly reduced by administration of SQ29,548 (initial CFV frequency = 12.3 ± 1.6 cycles/hr, following SQ29,548 = 10.5 ± 1.4 cycles/hr, p<0.01). However, the severity of coronary blood flow reduction was unaffected by SQ29,548 since the flow velocity at the nadir of CFVs was not significantly different from that of the control CFV nadir (Table 1).

After CFVs were abolished in 8 of 9 Group II-B dogs (dose = 0.38 ± 0.7 mg/kg), SQ29,548 (0.2 mg/kg/hr) was infused continuously and prevented the reoccurrence of CFVs for the duration of the infusion (3 ± 0.3 hours). After this infusion period, the thromboxane A_2 mimetic, U46619, was injected in bolus doses in the left atrium while infusion of SQ29,548 continued. U46619 restored CFVs in 5 of the 8 dogs after 70 ± 20 μg had been injected (range 17–140 μg). The frequency of restored CFVs was significantly less than the initial CFVs generated in these 5 dogs (initial CFVs = 12.2 ± 0.8 cycles/hr; U46619 restored CFVs = 9.8 ± 0.8 cycles/hr, p<0.03) (Table 1). Nadir of coronary blood flow in U46619-restored CFVs was as low as the nadir of the initial CFVs (initial CFV nadir = 10.8 ± 5% of control blood flow; U46619-restored CFV nadir = 20.2 ± 7.6% of control blood flow). The remaining 3 dogs were given an average dose of 117 ± 12 μg U46619 intraatrially, and CFVs were not restored.

SQ28,668-TREATED DOGS (GROUP III). Twelve dogs in Group III had an initial CFV frequency of 10.2 ± 0.5 cycles/hr (Table 1). CFVs were eliminated in 9 of 12 dogs (75%) at an average dose of SQ28,668 of 3.1 ± 1.3 mg/kg (range 0.09 to 10 mg/kg). The 3 dogs of whom SQ28,668 was ineffective received an average dose of 6.0 ± 2.0 mg/kg, but CFV frequency was not significantly decreased (10.7 ± 0.4 before SQ28,668 versus 8.9 ± 1.7 cycles/hr after the highest dose of SQ28,668). Combined CFV frequency in all 12 dogs before and after SQ28,668 is shown graphically in Figure 3. Blood flow velocity changes throughout the protocol are shown in Figure 4, Panel B. Administration of a continuous infusion of U46619 (2 to 5 μg/min) restored CFVs in 5 of 7 dogs in whom CFVs were abolished. U46619-restored CFV frequency was

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**Figure 3.** Combined CFV frequency for a 30-minute period for all Group II (n = 35) and Group III (n = 12) dogs for the initial control CFVs and following administration of either SQ29,548 or SQ28,668. *p<0.05, CFV frequency following SQ treatment compared to CFV frequency during the control period.

**Figure 4.** Mean flow velocity changes through the study protocol for Group II (SQ29,548-treated, Panel A, n = 35) and group III (SQ28,668-treated, Panel B, n = 12) dogs. *p<0.05, blood flow velocity is significantly different from control, un-constricted blood flow velocity. tp<0.05, blood flow velocity is significantly different from constricted blood flow velocity prior to initiation of CFVs.
not significantly different from control frequency (10.6 ± 1.1 cycles/hr during the control period compared to 8.9 ± 0.7 cycles/hr for U46619-restored CFVs). The nadir of flow in U46619-induced CFVs was also not significantly different from control CFVs in the same dogs. Two additional dog hearts from Group III were processed for light and electron microscopy and did not receive U46619.

**Effect of SQ29,548 on Vascular and Platelet Production and Plasma Concentration of TxA2 and Prostacyclin**

Thromboxane B₂ and 6-keto-PGF₁α were measured in plasma samples collected from 5 group II-C dogs at five time periods in the protocol: control, following constriction of the LAD, during both peak and nadir of initial CFVs, and following CFV abolition with SQ29,548. TxB₂ concentrations ranged from 192 ± 38 pg/ml to 317 ± 43 pg/ml, and there was no significant difference in these values at any time point by a one-way ANOVA. Plasma 6-keto-PGF₁α concentrations were also not significantly different throughout the protocol, with values ranging from 118 ± 34 pg/ml to 185 ± 40 pg/ml at the five time periods.

Platelets were isolated from 5 dogs before and 1 hour after the abolition of CFVs with SQ29,548. Platelets obtained before and after SQ29,548 administration produced significant amounts of TxB₂ in response to exogenously added arachidonic acid. The production of TxB₂ by platelets was not significantly affected by the administration of SQ29,548 (Figure 5).

Segments of LAD coronary arteries immediately adjacent to the constrictor and circumflex coronary arteries were removed from 6 animals in whom CFVs were abolished with SQ29,548 for 30 minutes and from 9 dogs in whom CFVs had occurred without drug intervention for an equivalent period of time. These segments were incubated in the presence of increasing concentrations of arachidonic acid or its vehicle and the production of TxB₂ and 6-keto-PGF₁α measured (Figures 6A, B, stenosed LAD). Segments of LAD coronary arteries produced significant amounts of TxB₂; however, arteries obtained from the SQ29,548-treated animals synthesized significantly less TxB₂ than those segments obtained from control dogs (Figure 6A). In contrast, LAD coronary artery segments obtained from both SQ29,548-treated and untreated dogs synthesized comparable amounts of 6-keto-PGF₁α (Figure 6B). Circumflex coronary artery segments obtained from both SQ29,548-treated and untreated dogs produced comparable amounts of both TxB₂ and 6-keto-PGF₁α (Figures 6A and B).

**Morphological Findings**

The three untreated dogs had uninterrupted CFVs for 83, 110, and 114 minutes to match the total study duration for the 1 Group II and 2 Group III dogs. CFV frequency during the initial 30 minutes was comparable in the two groups: 6.2 ± 1.3 cycles/30 minutes in the untreated dogs and 4.2 ± 0.3 cycles/30 minutes in the SQ-treated animals.

LAD segments from the three control, untreated dogs had large obstructive thrombi at the constrictor sites, as shown by scanning electron microscopy (Figures 7A, B). Numerous platelets and some leukocytes were present on the luminal surface adjacent to the thrombus (Figure 7C). Away from the constrictor site, the LAD was lined by normal endothelium (Figure 7D). Light microscopy confirmed the presence of a
large, platelet-rich thrombus at the constrictor site (Figures 8A and B). The LAD segments from SQ29,548-treated and SQ28,668-treated dogs (1 Group II, 2 Group III) were denuded of endothelium at the constrictor sites and had some platelets attached to the subendothelial tissue (Figures 9A and B); however, the vessels were free of obstructive thrombi. The vessels had a normal lining of endothelium away from the constrictor site. The absence of obstructive thrombus in those vessels was also confirmed by light microscopy (Figure 8C).

Discussion

Preliminary results from our laboratory showed that the specific and potent TxA2/PGH2 receptor antagonist, SQ29,548, was effective in abolishing CFVs in 83% of dogs and markedly attenuating CFVs in the remaining dogs. The results of the present study demonstrate that TxA2/PGH2 receptor antagonism by SQ29,548 or SQ28,668 rapidly and effectively abolishes transient flow alterations through stenotic coronary arteries in 75–77% of dogs studied. This inhibition occurs without altering systemic hemodynamics in this canine model of coronary thrombosis. In most animals, CFVs were abolished throughout the duration of a continuous intravenous infusion of SQ29,548. Once cyclic flow variations were abolished with SQ29,548, the TxA2-mimetic, U46619,18 restored CFVs in 5 of 8 dogs, providing further evidence that the thromboxane/PGH2 receptor is important in mediating cyclic flow variations in stenosed canine coronary arteries. Data obtained in this study also demonstrate that the administration of a TxA2 receptor antagonist did not affect systemic concentrations of TxB2 or 6-keto-PGF1α. This finding suggests that SQ29,548 does not appreciably affect cyclic endoper-
oxide metabolism to prostacyclin or TxA₂, which could potentially influence the occurrence of CFVs in this model. Thus, our data suggest that TxA₂ per se and TxA₂ receptor activation are important in mediating CFVs in this model. Fitzgerald et al. have recently confirmed that TxA₂/PGH₂ receptor antagonism with SQ29,548 prolongs the time to thrombotic vascular occlusion in electrically-induced coronary thrombosis in dogs.

SQ29,548 {1S-[1α,2β(5Z),3β,4α]}-7-[3-{(2-[(phenylamino) carbonyl]hydrazino)methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid is the most selective and potent antagonist of the TxA₂/PGH₂ receptor in the family of compounds with the 7-oxabicyclo[2.2.1]heptane nucleus activity. Unlike previously synthesized, related compounds that stimulate platelet adenylate cyclase and inhibit TxA₂ synthesis, SQ29,548 has no intrinsic TxA₂ agonist activity and does not alter cyclooxygenase, thromboxane synthetase, prostacyclin synthetase, or adenylate cyclase activity. Data obtained from in vitro studies indicate that SQ29,548 significantly increases the release of TxA₂ from arachidonate-perfused guinea pig lungs but inhibits the constrictor effect of TxA₂ on superfused strips of rat aorta and bovine coronary artery. On the other hand, the thromboxane synthetase inhibitor, dazoxiben, significantly inhibits arachidonate-induced release of TxA₂ from guinea pig lungs but enhances prostacyclin synthesis, resulting in a diminution of the vasoconstrictor effects of thromboxane.

The related compound, SQ28,668 {1S-[1α,2β(5Z),3β,4α]}-7-[3-(3-hydroxy-4-pentenyl)-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid, is also a TxA₂/PGH₂ antagonist that has antithrombotic activity in vivo. SQ28,668 is somewhat less potent than SQ29,548, but it is being developed for use in man. Thus, we evaluated both SQ29,548 and SQ28,668 in this study.

Previous results reported from our laboratory indicated that 6-keto-PGF₁α synthesis in constricted canine coronary arteries with CFVs in vivo was decreased, but TxB₂ synthesis was considerably elevated above that occurring in normal, unconstricted canine coronary arteries. We also demonstrated that cells capable of thromboxane synthesis, such as platelets and mononuclear inflammatory cells, accumulate at the site of a coronary artery constriction. In the present study, stenotic LAD segments in which CFVs were abolished with SQ29,548, exhibited a significantly reduced capacity to synthesize TxA₂ but an unchanged capacity to synthesize 6-keto-PGF₁α when compared to LAD stenotic segments from untreated dogs. The synthesis of 6-keto-PGF₁α was similar in segments from treated and untreated dogs. Similarly, the synthesis of TxA₂ in canine platelets was unaffected by SQ29,548 treatment.
strated that SQ29,548 and SQ28,668-treated LAD segments had considerably fewer adherent platelets after CFVs were abolished compared to control vessel segments. Thus, the reduced TxB$_2$ synthesis in the vessel segments obtained from SQ29,548-treated dogs is most likely due to a substantial reduction in the number of platelets at the site of the coronary arterial stenosis rather than a direct effect of SQ29,548 on TxA$_2$ synthesis.

TxA$_2$ is produced by activated platelets. It induces irreversible platelet aggregation and contraction of vascular smooth muscle. Its effects are normally opposed by the vascular production of prostacyclin, a potent vasodilator and anti-platelet aggregating agent. The local balance between prostacyclin and thromboxane influences vascular homeostasis. We and others have shown that vascular injury at the site of a coronary stenosis reduces the release of prostacyclin and allows the effects of TxA$_2$ to occur relatively unopposed. We have previously reported that the administration of a selective thromboxane synthetase inhibitor, dazoxiben (UK37,248), eliminates or markedly attenuates the occurrence of CFVs in the animal model used in this study. The efficacy of the thromboxane synthetase inhibitor in abolishing CFVs in this model was similar to that described in the present study with TxA$_2$/PGH$_2$ receptor antagonists. These combined findings indicate that TxA$_2$ is an important mediator of CFVs. Several studies indicate that thromboxane synthetase inhibition can redirect cyclic endoperoxide metabolism to PGD$_2$ or PGE$_2$ in the platelet. Additionally, platelet endoperoxides may be released and used by endothelial cells to synthesize PGI$_2$. These mechanisms have been proposed as being partially responsible for elimination of CFVs after thromboxane synthesis inhibition in this model. However, previous studies from our laboratory indicate that there is a reduced capacity of damaged vessels to synthesize PGI$_2$. As a result, the damaged vessel is relatively unable to produce PGI$_2$ from platelet-derived endoperoxides.

The effectiveness of the selective TxA$_2$ receptor antagonist, SQ29,548, in eliminating or attenuating CFVs in most dogs without altering circulating TxA$_2$ or prostacyclin concentrations implicates TxA$_2$ as an important factor in the generation of CFVs in this experimental model. Elucidation of the relative importance of the platelet and the vascular TxA$_2$ receptor in mediating CFVs will require further study.

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**References**


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