Effects of the selective thromboxane synthetase inhibitor dazoxiben on variations in cyclic blood flow in stenosed canine coronary arteries

Larry R. Bush, Ph.D., William B. Campbell, Ph.D., L. Maximilian Buja, M.D., Gregory D. Tilton, M.D., and James T. Willerson, M.D.

ABSTRACT Recent studies suggest that platelet activation and subsequent thromboxane (TX) A2 release play important roles in certain coronary syndromes. To further test this possibility, we examined the ability of a selective TXA2-synthetase inhibitor, dazoxiben (UK-37-248), to abolish cyclic flow reductions (CFRs) that occur in experimentally stenosed canine coronary arteries. CFRs, which are characterized by progressive declines in coronary blood flow and interrupted by sudden and usually spontaneous restorations of flow, were produced by placing hard plastic cylindrical constrictors (5 mm long × 4.5 mm outer diameter) on the proximal left anterior descending or circumflex coronary artery in open-chest, anesthetized dogs. Coronary blood flow was measured with pulsed Doppler flow probes placed proximal to the constrictors and regional myocardial blood flow with 15 μm radiolabeled microspheres. CFRs were observed for 1 hr, during which coronary blood flow was monitored continuously. Regional myocardial blood flow was measured before constriction, when coronary blood flow appeared to be at its nadir, and after spontaneous restorations of flow. After 1 hr dazoxiben (2.5 mg/kg iv) or an equal volume of saline was given and coronary blood flow was monitored for another hour. Dazoxiben abolished CFRs completely in 18 of 28 dogs and significantly reduced their frequency in the dogs receiving the drug (10.1 ± 0.8 vs 3.2 ± 1.0 per hour [± SE]; p < .001, n = 28). The frequency and magnitude of variations in cyclic blood flow were unchanged after saline (8.8 ± 0.8 vs 9.0 ± 1.0 per hour; p = NS, n = 13). The lowest levels of coronary blood flow before and after dazoxiben were 8.6 ± 2.2% and 48.8 ± 5.4% of control, respectively (p < .001, n = 28), whereas this parameter remained unchanged after saline (18.7 ± 5.7% vs 13.4 ± 4.1%, respectively; n = 13).

The levels of TXB2 and 6-keto-prostaglandin (PG) F1α (stable breakdown products of TXA2 and prostacyclin, respectively) were measured in blood collected from aortic and distal coronary arterial catheters before coronary constriction (control), during CFRs, and after administration of dazoxiben. TXB2 levels measured distal to the stenosis were increased fivefold during CFRs (352 ± 126 vs 71 ± 18 pg/ml plasma; p < .03) and were reduced to preconstriction (control) levels by dazoxiben (57 ± 12 pg/ml). Aortic TXB2 levels almost doubled with CFRs and also returned to control levels after dazoxiben. Distal coronary arterial 6-keto-PGF1α levels also increased significantly during CFRs (133 ± 22 to 344 ± 41 pg/ml plasma), but remained elevated (343 ± 82 pg/ml plasma) after dazoxiben treatment. Systemically administered dazoxiben (2.5 mg/kg iv) suppressed arachidonic acid–induced production of TXB2 (but not PGE2) by platelets. Dazoxiben (1 μM) did not affect PGI2 synthesis by canine coronary arterial rings in vitro. Thus, dazoxiben eliminates or markedly attenuates cyclic flow reductions in stenotic canine coronary arteries and selectively inhibits TXA2 synthesis. The data obtained in this study suggest that increases in coronary arterial TXA2 concentration play a role in the genesis of cyclic flow reductions in this experimental model.


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THERE IS increasing evidence that platelets are important in certain occlusive coronary syndromes. The interaction between platelets and coronary vascular endothelium is thought to depend on the balance between the production of the proaggregatory thromboxane (thromboxane A2, TXA2) by platelets and the antiaggregatory prostacyclin (prostaglandin I2, PGI2) by endothelium. Nonsteroidal anti-inflammatory drugs block PG synthesis nonselectively, and their re-
ported beneficial effects in myocardial ischemic syndromes have been interpreted as evidence that TXA₂ production is more prominent in this setting. However, it is unclear whether these agents truly exert a beneficial influence in myocardial ischemic syndromes since they possess the ability to block both PGI₂ and TXA₂ synthesis. A more desirable approach would be to inhibit TXA₂ synthesis selectively, without affecting PGI₂ synthesis.

Accordingly, this study was undertaken to evaluate the effects of dazoxiben (UK-37-248), an imidazole-containing compound that selectively inhibits TX synthetase, on cyclic flow reductions (CFRs) and platelet aggregation in dogs with experimentally stenosed coronary arteries. The preparation used in this study is similar to the one described previously by Folts and his colleagues and to that used by Aiken et al. It is thought that platelet and red blood cell aggregates form in severely stenotic coronary arteries and then break loose. This phenomenon produces CFRs, which are characterized by progressive declines in flow interrupted by sudden and usually spontaneous restorations of flow. We hypothesized that selective inhibition of TXA₂ synthesis with dazoxiben can abolish these CFRs by inhibiting platelet TXA₂ production.

**Methods**

**Surgical preparation.** Mongrel dogs (17 to 27 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg iv) and ventilated artificially with room air. Aortic and venous catheters were inserted in each via the common carotid artery and external jugular vein, respectively. A thoracotomy was performed in the fifth left intercostal space and the heart was suspended in a pericardial cradle. A Königsberg pressure transducer was inserted into the left ventricular apex and another catheter was inserted into the left atrium for injecting tracer microspheres. A segment of the left anterior descending (LAD) or left circumflex (LCX) coronary artery was dissected away from surrounding tissue gently and a pulsed Doppler flow probe (manufactured by Dr. C. J. Hartley, Houston) was placed on the coronary artery proximal to where the constriction would subsequently be positioned. Also, when possible, a small polyethylene catheter was inserted into the distal end of a diagonal branch of the LAD below the site of constriction, enabling the pressure gradient across the stenosis to be measured. The experimental preparation is illustrated in figure 1.

**Experimental protocol.** After instrumentation, dogs were allowed to stabilize for ½ hr. Control hemodynamic measurements included those of heart rate, arterial blood pressure, left ventricular dp/dt, and phasic and mean coronary flow, all of which were recorded continuously on a Hewlett-Packard (Model 7755B) 8-channel recorder. The Doppler flow probes were calibrated on coronary arteries in a separate group of four dogs. Regional myocardial blood flow was measured with 15 µm carbonized microspheres labeled with 125I, 46Sc, 113Sn, 85Sr, 97Nb, and 89Co. Microspheres were sonicated and vortexed for several minutes and 1 to 3 million spheres were injected into the left atrium over an 8 to 10 sec period. Starting 10 sec before and continuing for 90 sec after microsphere injection, reference arterial blood was withdrawn from the carotid artery at a constant rate of 7.8 ml/min with a Harvard infusion/withdrawal pump. The order of isotope injection was changed randomly.

After obtaining control measurements, the hyperemic response after a 10 sec total occlusion was measured. Then a hard plastic cylindrical constrictor similar to that described by Folts et al. was placed on the coronary artery 5 to 15 mm distal to the Doppler flow probe. The cyclic flow pattern, which is indicative of platelet aggregation and spontaneous release, was produced after identifying a constrictor of suitable internal diameter that completely (or nearly so) eliminated the hyperemic response after a 10 sec occlusion. Once attained, the cyclic coronary flow pattern was observed for 1 hr during which coronary blood flow was monitored continuously by Doppler flow probe. Regional myocardial blood flow measurements were obtained when coronary blood flow was approximately at its nadir and after a spontaneous restoration of flow. During the hour of observation, the frequency of these CFRs was recorded, as were the zenith and nadir of coronary blood flow.

After 1 hr dazoxiben* (2.5 mg/kg in 20 ml saline) was administered to 28 dogs intravenously over 1 min. Hemodynamic variables and CFRs were monitored continuously for another hour and changes in the pattern of coronary blood flow were compared with those occurring during the first hour. Another group (n = 13 dogs) received an equal volume of saline and was observed for an additional 1 hr period to determine whether spontaneous changes in the cyclic coronary flow pattern occurred in the absence of the drug. To further test the hypothesis that selective reductions in TXA₂ production at the site of the stenosis are primarily responsible for abolition of CFRs, the stable endoperoxide analog U46619 was given, intra-atrially or topically (1 to 5 µg), in an effort to restore CFRs after their abolition by dazoxiben in seven dogs. To determine maximal decreases in coronary blood flow, regional myocardial blood flow, and distal coronary pressure, the constricted coronary artery was occluded totally at the end of each experiment, and all measurements (including those of regional myocardial blood flow) were repeated within 3 min after occlusion. Next, to delineate the ischemic zone of the left ventricle, 10 ml of 10%

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*Generously provided by Dr. Pedro Urquilla at Pfizer Pharmaceuticals, Groton, CT.

**FIGURE 1.** Schematic diagram of cardiac preparation for studying CFRs. Stippled area represents area made ischemic by coronary constrictor and intravascular platelet aggregation. Ao = aorta; PA = pulmonary artery; RV = right ventricle; LV = left ventricle.
by guest on November 12, 2017

LABORATORY INVESTIGATION—CORONARY ARTERY DISEASE

(weight/volume) patent blue violet (Alphazurine) was injected quickly into the left atrium, followed by a 5 ml flush and electrical fibrillation of the heart. Ischemic left ventricular tissue was sampled from that area well within the perimeter of unstained left ventricular myocardium.

Effects of dazoxiben on intravascular TXA₂ production in vivo. Blood samples for determination of the intravascular production of TXA₂ and PGI₂ were taken from the aortic and distal coronary catheters before coronary constriction, during two CFRs (as coronary blood flow was declining before dazoxiben was given), and 10 min after the administration of 2.5 mg/kg dazoxiben. Both catheters were filled with heparin sodium (from beef lung; 1000 units/ml) immediately after placement and after each blood sample was obtained. After discarding blood contained in the void space of each catheter, blood from both aortic and distal coronary arterial catheters was allowed to flow directly into chilled Vacutainer tubes (Becton-Dickinson; 12 ml samples) containing indomethacin (10 μg) and heparin (1500 units). TXA₂ levels were measured from blood samples obtained when maximal platelet TXA₂ production was believed to be occurring, such as during CFRs. Blood was collected from the aortic and distal coronary catheters starting 1 to 2 min before the anticipated restoration of coronary blood flow. To avoid spuriously high TXA₂ levels in the aortic samples due to intracatheter clotting, the entire aortic blood sample was collected immediately, while blood flow from the distal coronary catheter was allowed to continue. In some cases, when coronary blood flow approached zero, flow from the distal catheter practically ceased. In these cases, to avoid intracatheter clotting, platelet activation, and spurious TXA₂ production, coronary blood flow through the stenosed coronary was restored by lightly tapping the constrictor. During blood collections, the Vacutainer tubes were kept in an ice-water slurry. The third set of blood samples was obtained between 10 to 15 min after dazoxiben administration. In 10 of the dogs the flow rate from the distal coronary arterial catheter was measured by dividing the volume of blood obtained by the collection time (pg/ml plasma × ml/min = pg/ min), which enabled calculations of production to be made. Blood samples were centrifuged (4° C) at 1500 rpm for 10 min, and the plasma was separated and frozen until the batch analyses for TXB₂ and 6-keto-PGF₁α levels were performed.

Morphologic studies. The LAD of each of five dogs was suture-ligated on both sides of the plastic constrictor at a point in time when a reduction in cyclic blood flow had occurred and blood flow through the vessel was at its nadir. Suture ligation also was performed without plastic constrictors in two control coronary arteries. The segments of arteries were removed and fixed in 10% formalin in phosphate buffer. The segments were dehydrated and embedded in methacrylate in a longitudinal orientation. Longitudinal sections were cut and stained with toluidine blue. The sections were examined and photographed by light microscopy.

Effects of systemically administered dazoxiben on platelet TXA₂ production in vitro. To confirm that dazoxiben possesses the ability to inhibit TXA₂ production, the production of TXA₂ was measured in vitro in platelet-rich plasma prepared from blood drawn from a separate group of five dogs. Three of these dogs were anesthetized with sodium pentobarbital and ventilated artificially. A segment of the LAD was isolated after exposing the heart via a left thoracotomy to reproduce the experimental preparation we used as closely as possible. A 20 min stabilization period was allowed and 18 ml of blood was collected from the antecubital vein of each dog before and 5 and 30 min after the administration of dazoxiben (2.5 mg/kg iv). Two other dogs were lightly restrained in a standing sling. Each blood sample was replaced with an equal volume of saline. The blood samples were quickly, but gently, transferred to plastic tubes containing 2 ml of 3.5% sodium citrate, pH 7.4, and this was followed by two gentle inversions to ensure adequate mixing. Centrifugation at 150 g for 10 min resulted in a plasma suspension of platelets. After removal of the platelet-rich plasma, the remaining blood was centrifuged at 1500 g for 10 min to obtain platelet-poor plasma.

Prostaglandin measurements. TXB₂, the stable breakdown product of TXA₂, 6-keto-PGF₁α, the stable metabolite of PGI₂, and PGE₂ were measured by the method of Dray et al., as modified by Campbell et al. After extraction and chromatographic purification, 0.1 ml of the sample was combined with 0.1 ml of H₂O-TXB₂, 3H-6-keto-PGF₁α, or ³H-PGE₂, and 0.1 ml of specific antiserum in a 12 x 75 mm culture tube. After incubation overnight at 4° C, the antibody-bound and antibody-free prostaglandins were separated with dextran-coated charcoal. The bound radioactivity was counted with a Beckman liquid scintillation spectrometer.

Effects of dazoxiben on canine coronary arterial prostacyclin and PGE₂ release in vitro. To determine the effects of dazoxiben on blood vessel prostacyclin release, canine coronary arterial rings were incubated in 1 ml of medium 199 (Gibco) containing 0.2% bovine serum albumin at 37° C in a Dubnoff metabolic shaking water bath (Precision Scientific, Chicago) in an atmosphere of 95% O₂ and 5% CO₂. Dazoxiben (1 x 10⁻⁶M) or its vehicle was added to the incubation medium and the samples were incubated for 10 min. After 10 min, indomethacin (5 x 10⁻³M) was added to stop prostaglandin synthesis and the media was removed and stored at -20° C until assay. The tissue was blotted dry and weighed. The quantities of 6-keto-PGF₁α and PGE₂ were determined in the diluted incubation medium by radioimmunoassay as previously described. The results are expressed in nanograms per milligram of tissue.

Hemodynamic effects of dazoxiben. To determine if dazoxiben exerts hemodynamic effects independent of its influence on platelet production of TXA₂, heart rate, arterial blood pressure, left ventricular maximum dp/dt, coronary blood flow, and regional myocardial blood flow were monitored before and after administration of dazoxiben in five open-chest dogs anesthetized with 30 mg/kg sodium pentobarbital. Dazoxiben was given in cumulative doses of 1.0, 2.5, and 5.0 mg/kg iv at 5 min intervals.

Materials. Sodium pentobarbital (50 mg/ml) was purchased from Abbott Laboratories (Chicago); l-epinephrine was purchased from Elkins-Sinn, Inc.; and Alphazurine (patent blue violet), arachidonic acid, and indomethacin were obtained from Sigma Chemical Company (St. Louis). The cyclic endoperoxide analog (U46619) was kindly provided by the Upjohn Co. (Kalamazoo, MI).

Statistical analyses. All values are expressed as mean ± SE. Student’s group t test was used to determine if significant differences existed between dazoxiben-treated and saline controls and a paired Student’s t test was used to determine if a significant change in the value of a parameter occurred within animals after saline or dazoxiben administration. Comparisons involving more than two groups were made by analysis of variance and Duncan’s multiple-range or Newman-Keuls test. In all cases a p value < .05 was considered to indicate a significant difference.

Results

Dazoxiben effects on CFRs. Among 41 dogs studied, CFRs similar to those described previously were observed in 28. Several different constrictors were tested in most dogs before one was found to produce CFRs. Placement of the constrictor reduced mean and
phasic coronary blood flows 42 ± 6% and 53 ± 5%, respectively, in saline-treated (control) dogs and 35 ± 4% and 48 ± 5% in dazoxiben-treated dogs (p = NS for both). Compared with control (preconstriction), the hyperemic response after a 10 sec total coronary occlusion was blunted or abolished (236 ± 11% versus 82 ± 7% of control [basal] coronary blood flow; p < .001). Figure 2 is a tracing from a representative dog in which a coronary stenosis caused a decrease in distal coronary pressure and resting coronary blood flow. Subsequently, CFRs developed and continued unabated for several hours in saline-treated dogs. Except for reducing distal coronary pressure, these CFRs did not produce major hemodynamic changes.

There was a close relationship between the frequency shift (in kHz) obtained from the pulsed Doppler flowmeter and actual coronary blood flow, as measured by collecting timed samples from a catheter advanced retrogradely to a point just distal to the flow probe. The correlation between actual volume flow (in ml/min) and the signal produced by the pulsed Doppler flowmeter was highly significant (p < .001), yielding a straight line (y = 31.3x ± 5.8; r = .982) and a y intercept (zero absolute flow) of 5.8 ml/min. Due to differences in the sizes of the dogs and in the coronary arteries chosen for constriction (LAD vs LCX), changes in coronary flow were normalized to control or preconstriction conditions.

Progressive coronary occlusion compromises perfusion in the subendocardium more than in the subepicardium. Therefore, radiolabeled microspheres were used to measure changes in transmural blood flow during CFRs. Since there were no differences between control and dazoxiben-treated dogs with respect to blood flow to any region or transmural layer before dazoxiben treatment, the values shown in table 1 represent the pooled values of both groups. Both subendocardial and subepicardial blood flow fell significantly during nadirs of coronary blood flow during CFRs (table 1). Blood flow in the subepicardium increased to control values during restoration of flow. Total coronary occlusion at the end of the experiment produced significantly greater declines in both subepicardial and subendocardial blood flow. Blood flow in nonisch-
hemic left ventricular myocardium did not change significantly.

The changes in the frequency (number of CFRs/hr) and severity (average of three lowest nadirs of coronary blood flow) of CFRs before and after saline or dazoxiben administration are shown in table 2. There was no difference in the original severity of constriction or in the frequency or magnitude of CFRs between saline- and dazoxiben-treated dogs during the first hour of observation. The frequency of CFRs and the nadir of coronary blood flow remained unchanged after saline administration (18.7 ± 5.7% to 13.4 ± 4.1%). Dazoxiben reduced the frequency of CFRs significantly (table 2). In fact, CFRs were abolished completely in 18 of 28 dogs. The nadir of coronary blood flow also increased significantly after dazoxiben administration (8.6 ± 2.2% to 48.8 ± 5.4% of control coronary blood flow).

**Restoration of CFRs with U46619.** Figure 3 illustrates an original tracing in which CFRs, abolished completely by dazoxiben, were restored by the intra-atrial administration of U46619, a stable endoperoxide analog that exerts TX-like effects on platelet aggregation and coronary vascular resistance. 21 U46619 was given only to dogs in which CFRs were abolished completely by dazoxiben. Five of seven dogs given the stable endoperoxide analog in this manner had restoration of their CFRs.

**Hemodynamic changes during CFRs.** Table 3 shows the hemodynamic changes that occurred during CFRs with dazoxiben and saline treatment. There were no significant differences for any hemodynamic variable between the dazoxiben-treated and saline-treated groups. However, with time there were significant decreases in aortic systolic and diastolic pressures and in left ventricular dp/dt in the dazoxiben-treated animals.

**Hemodynamic effects of dazoxiben.** Dazoxiben did not produce hemodynamic changes in control animals without coronary constrictions. Figure 4 illustrates that dazoxiben, at doses of 1.0, 2.5, and 5.0 mg/kg, did not affect heart rate, arterial (systolic or diastolic) pressure, coronary blood flow, or left ventricular dp/dt, nor did it affect the transmural distribution of blood flow in five open-chest, anesthetized dogs.

**Effects of dazoxiben on plasma aortic and distal coronary arterial TXB₂ and 6-keto-PGF₁α levels during intravascular platelet aggregation.** Figure 5 shows the plasma TXB₂ levels proximal (aortic) and distal to the coronary stenosis, before and during CFRs, and after the administration of dazoxiben. Levels of TXB₂ in plasma sampled from the distal coronary catheter increased significantly from a control value of 71 ± 18 to 352 ±

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**TABLE 1**

Regional myocardial blood flow (ml/min/g) in ischemic and nonischemic left ventricular (LV) myocardium before and during CFRs and after total coronary occlusion

<table>
<thead>
<tr>
<th>LV region</th>
<th>Level</th>
<th>Control (preconstriction)</th>
<th>CFR Flow restorations</th>
<th>Total LAD occl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic</td>
<td>Epi</td>
<td>1.00±0.08\textsuperscript{a}</td>
<td>0.37±0.06\textsuperscript{b}</td>
<td>1.03±0.16\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Endo</td>
<td>0.90±0.09\textsuperscript{a}</td>
<td>0.26±0.07\textsuperscript{b}</td>
<td>0.72±0.12\textsuperscript{a}</td>
</tr>
<tr>
<td>Nonischemic</td>
<td>Epi</td>
<td>0.89±0.08</td>
<td>0.34±0.07</td>
<td>0.86±0.08</td>
</tr>
<tr>
<td></td>
<td>Endo</td>
<td>0.99±0.10</td>
<td>0.89±0.07</td>
<td>0.90±0.09</td>
</tr>
</tbody>
</table>

All time points except total LAD occlusion were before saline or dazoxiben administration. Regional myocardial blood flow was measured during total coronary occlusion at the end of the study. Within the same region and transmural layer of the left ventricle, values with the same letter superscript are not significantly different from each other (ANOVA, Duncan’s multiple-range test; p < .05, n = 25 dogs).

Endo = endocardium; epi = epicardium; occl = occlusion.

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**TABLE 2**

Frequency and severity of CFRs before and after saline or dazoxiben administration

<table>
<thead>
<tr>
<th></th>
<th>Frequency (CFRs/hr)</th>
<th>Severity (nadir of CBF % control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before drug</td>
<td>After drug</td>
</tr>
<tr>
<td>Saline (n = 13)</td>
<td>8.8±0.8</td>
<td>9.0±1.0</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Dazoxiben (n = 28)</td>
<td>10.1±0.8</td>
<td>3.2±1.0</td>
</tr>
<tr>
<td></td>
<td>p &lt; .001</td>
<td>p &lt; .001</td>
</tr>
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</table>

The average of three lowest nadirs were used to obtain a mean value for this parameter. When total abolition of CFRs occurred after dazoxiben administration, the three lowest values for CBF were used to compute a posttreatment value in this group of dogs.
126 pg/ml during CFRs. Aortic TXB\(_2\) levels also increased significantly during CFRs (73 ± 12 to 145 ± 32 pg/ml). Abolition of the CFRs by dazoxiben was accompanied by a normalization of both aortic and distal coronary arterial TXB\(_2\) to control levels. The levels of 6-keto-PGF\(_{1\alpha}\) in distal coronary arterial blood also increased 259\%, from control (preconstriction) levels of 133 ± 22 to 344 ± 41 pg/ml. In addition to decreasing TXB\(_2\) levels, dazoxiben left elevated PGI\(_2\) values (343 ± 82 pg/ml) unaltered (figure 5, B). The ratio of 6-keto-PGF\(_{1\alpha}\) to TXB\(_2\) concentration, which is independent of flow rates from the distal coronary catheter at the three time points, is shown in figure 5, D. The 6-keto-PGF\(_{1\alpha}\)/TXB\(_2\) ratio declined from a control value of 3.04 ± 0.67 to 2.9 ± 0.9 with CFRs; this ratio increased significantly to 7.1 ± 1.6 after dazoxiben administration. The increased ratio of 6-keto-PGF\(_{1\alpha}\)/TXB\(_2\) after dazoxiben was due to the reduction in the TXB\(_2\) concentration.

**Morphologic observations.** Periconstrictor segments of LAD obtained at the nadir of blood flow contained microthrombi primarily composed of masses of aggre-

**TABLE 3**

<table>
<thead>
<tr>
<th>Hemodynamic changes in dogs with coronary arterial stenoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRT</td>
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<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>AOS (mm Hg)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>AOD (mm Hg)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>LV dP/dt(_{max}) (mm Hg/sec)</td>
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</tbody>
</table>

Dazoxiben (2.5 mg/kg, in 20 ml saline \([n = 28\) dogs\]) or saline \([n = 13\) dogs\]) was given immediately after measurements were obtained at 60 minutes. AOS = aortic systolic pressure; AOD = aortic diastolic pressure; LV dP/dt\(_{max}\) = maximal rate of rise of left ventricular pressure; TRT = treatment. \(^a\)Significantly different from control \((p < .05, ANOVA and Duncan's multiple range test).\)

\(^b\)Significantly different from the variable immediately above it.
FIGURE 4. Results of administration of dazoxiben to five open-chest, pentobarbital anesthetized dogs without coronary constrictors in cumulative doses of 1.0, 2.5, and 5.0 mg/kg. There was no significant change in any hemodynamic parameter.

gated platelets (figure 6). The arterial walls exhibited multiple foci of endothelial denudation and damage to the internal elastic lamella. Platelets and leukocytes (neutrophils) were frequently adherent to the vessel wall in these areas. The control vessels contained loosely arranged erythrocytes and a few other blood elements, but no aggregates of platelets were identified.

Effects of systemically administered dazoxiben on PG and TX production by platelets in vitro. Arachidonic acid-induced TXA₂ and PGE₂ production by platelet-rich plasma prepared from blood samples taken before and after administration of dazoxiben were measured in five dogs. Exogenously added arachidonic acid stimulated PGE₂ and TXB₂ production in platelet-rich plasma prepared from control (predazoxiben) blood samples (figure 7). TXB₂ (but not PGE₂) production was suppressed or eliminated in platelet-rich plasma isolated from blood samples taken 5 and 30 min after dazoxiben.

Effects of dazoxiben on prostacyclin and PGE₂ production by canine coronary arteries in vitro. Table 4 shows the effects of dazoxiben on endogenous PGE₂ and PGI₂ (measured as 6-keto-PGF₁α) production in vitro in canine coronary arterial rings. Dazoxiben did not alter the production of PGE₂ or 6-keto-PGF₁α significantly.

Discussion

The results of this study show that, in our experimental preparation, the TX-synthetase inhibitor dazoxiben abolishes or attenuates CFRs presumably produced by alternating formation and dislodgement of platelet–red blood cell thrombi. These CFRs were accompanied by physiologically important changes in endocardial and epicardial blood flow. Thus, the ability to prevent CFRs would be expected to translate into an important preservation of blood flow and contractile function.

Morphologic examination of coronary arteries at the site of constriction revealed platelet thrombi with blood erythrocytic and leukocytic involvement. These

FIGURE 5. Changes in aortic and distal coronary arterial TXB₂ (A) and 6-keto-PGF₁α (B) levels before and during CFRs and after dazoxiben administration. Production of TXB₂ and 6-keto-PGF₁α (C) was calculated as the product of plasma concentration × blood flow rate from the distal coronary catheter. The ratio of 6-keto-PGF₁α/TXB₂ (D) in blood collected from the distal coronary arterial catheter was obtained from values of concentrations (n = 15). The n value for all except panel C (where n = 10) is 15 dogs. *Significantly different (p < .05) vs other two time points (ANOVA and Duncan’s multiple-range test).

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findings confirm the morphologic and radiographic observations of Folts et al.\textsuperscript{11, 24} and support the view that CFRs in the preparation are due to platelet aggregation. The ability of dazoxiben to eliminate stenosis-induced CFRs and to inhibit TXB\textsubscript{2} synthesis in vivo and in vitro argue strongly in favor of platelet aggregation as an important cause of the CFRs observed in this preparation.

The beneficial effects of dazoxiben are not primarily related to hemodynamic alteration since the drug did not produce any systemic hemodynamic effects or increase coronary blood flow in open-chest, anesthetized dogs not subjected to coronary constriction. Folts et al.\textsuperscript{24} have also shown that coronary vasodilators do not reverse CFRs in similar experimentally stenosed coronary arterial preparations.

In this study CFRs were clearly associated with 5- and 2\textsuperscript{1/2}-fold increases in the plasma concentrations of TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1α}, respectively, in blood collected in a catheter draining the distal coronary arterial bed. Figure 5, C shows the changes in the “production” rate (concentration \times distal coronary flow rate) of TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1α} before and during CFRs and after dazoxiben administration. There was no significant change in the production of either TXB\textsubscript{2} or 6-keto-PGF\textsubscript{1α} during the CFRs. Therefore, the increase in concentration of TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1α} must be more closely related to a reduction in coronary blood flow than to a major increase in production de novo.

Needleman et al.\textsuperscript{25} have presented evidence from in vitro studies that endoperoxides formed by imidazole (a less potent TXA\textsubscript{2}-synthetase inhibitor) treated platelets can be converted to PGI\textsubscript{2} by the endothelium of exogenously added bovine coronary arterial segments or microsomes. Aiken et al.\textsuperscript{4} have provided indirect evidence that conversion of platelet-derived cyclic endoperoxides to PGI\textsubscript{2}, does occur and plays a significant role in the antithrombotic effects of OKY 1581, a selective TXA\textsubscript{2}-synthetase inhibitor. However, the fact that distal coronary levels of PGI\textsubscript{2} increased with CFRs in our study and remained at the same level after dazoxiben administration, while TXA\textsubscript{2} levels also increased during CFRs and returned to normal after dazoxiben, argues strongly for an important role of TXA\textsubscript{2} in the production of platelet aggregation in this preparation. The ability of an exogenously administered analog of endoperoxide to restore CFRs after their abolition by dazoxiben also supports the concept that TXA\textsubscript{2}, per se, contributes in an important way to the intravascular platelet aggregation in severely stenosed and (at least partially) deendothelialized coronary arteries. Collectively, the findings of the present and previous studies seem to indicate that CFRs are associated with increases in TXA\textsubscript{2} concentration and that their abolition by a selective TXA\textsubscript{2}-synthetase inhibitor is associated with both a decrease in platelet TXA\textsubscript{2} synthesis and a beneficial intracoronary balance in the ratio of PGI\textsubscript{2} to TXA\textsubscript{2} concentration.

FIGURE 6. Morphologic characteristics of coronary arteries. A. Periconstrictor segment of LAD obtained at a point of marked CFR contains a platelet thrombus (original magnification \times 60). B, Higher magnification view shows the small granular platelets, some of which are attached to the damaged arterial wall, which is devoid of endothelium (in other areas there were adherent neutrophils) (original magnification \times 370). C. Control coronary artery contains loosely arranged erythrocytes and a few leukocytes, but no platelet aggregates (original magnification \times 370). Toluidine blue-stained sections of methacrylate-embedded coronary arteries.
TABLE 4
Effects of dazoxiben on PGE₂ and 6-keto PGF₁α production by canine coronary arterial segments in vitro

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dazoxiben</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>arterial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>segments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGE₂ (ng/mg)</td>
<td>0.20±0.02</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>6-keto-PGF₁α</td>
<td>3.37±0.25</td>
<td>4.52±0.51</td>
</tr>
</tbody>
</table>

Dazoxiben was added in vitro to yield a final concentration of 10⁻⁶M.

FIGURE 7. Arachidonate-induced TXA₂ and PGE₂ production by platelets isolated from blood obtained from five dogs before and 5 and 30 min after systemically administered dazoxiben (2.5 mg/kg). The three following concentrations of arachidonate were used: 3 × 10⁻⁴M (———), 7.5 × 10⁻⁴M (—○—), and 7.5 × 10⁻³M (——△——). Dazoxiben completely suppressed TXA₂ production, while PGE₂ production was not decreased.

Wang et al.²⁶ maintain that the adult dog lacks TXA₂ receptors in the coronary vasculature. Also, Smith et al.²⁷ have demonstrated in the superfused cat coronary artery that dazoxiben does not display many TXA₂-receptor antagonist properties. Thus, there is little evidence to support the notion that antagonism of TXA₂-induced coronary vasoconstriction plays an important role in this preparation. Also, the ability of systemically administered dazoxiben to inhibit arachidonate-stimulated TXA₂ (and not PGE₂) production in platelets, coupled with the lack of effect on canine coronary arterial PGI₂ synthesis in vitro, is strong evidence that dazoxiben selectively inhibited TXA₂ production in the concentrations used in the present study. Recently, Schumacher and Lucchesi²⁸ have reported that dazoxiben also reduces in vivo thrombotic activity.

The role of coronary thrombosis as either a primary or secondary event in the pathogenesis of acute myocardial infarction is becoming increasingly recognized,²⁹ and previous studies have suggested a potential role for thromboxane in perpetuating the syndrome of unstable angina pectoris in patients.³⁰ Folts et al.¹¹ have demonstrated previously that aspirin diminishes the frequency of CFRs in this same experimental preparation, and a recent multicenter clinical trial conducted by the Veterans Administration has demonstrated that the equivalent of one aspirin per day reduces mortality and the frequency of myocardial infarction in patients with unstable angina pectoris.³⁰ In view of the effectiveness of dazoxiben in abolishing existing cyclic coronary flow changes and associated increases in TXA₂ production in this experimental preparation, this drug (and other similar TX-synthetase inhibitors) should provide relatively selective tools with which to explore the role of increases in TXA₂ levels as well as alterations in the balance between TXA₂ and prostacyclin in initiating and/or perpetuating certain ischemic heart disease syndromes. The possible benefit of TX-
synthetase inhibitors in treating selected patients with unstable angina pectoris needs to be determined.

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