In Vivo Characterization of Synthetic Thromboxane A₂ in Canine Myocardium

Henry H. Holzgreve, Lewis V. Buchanan, and Stuart Bunting

Recently, total chemical synthesis of thromboxane was achieved. The in vitro activity of synthetic thromboxane A₂ is indistinguishable from biologically generated material. The present study describes the in vivo characterization of synthetic thromboxane A₂ on the regional blood flow distribution of the canine heart. Local injections of synthetic thromboxane A₂ into the coronary vasculature caused marked reductions in coronary blood flow, measured by both radiolabeled microsphere injection and an electromagnetic flow device. The threshold concentration required to bring about this effect varied greatly between dogs and ranged from 0.125 μg/kg to 2.0 μg/kg. Similarly, the dose of thromboxane A₂ required to aggregate dog platelets in vitro varied from 30 ng/ml to 1,000 ng/ml. Bolus injections of 2 μg/ml thromboxane A₂ into the circumflex or left anterior coronary artery resulted in a simultaneous reduction in platelet count in coronary sinus blood of 83 ± 5.2% (mean ± SEM, n = 4, p = .0005). Both flow reduction and platelet effects were transient and localized. The time taken from onset to recovery of the response to control levels was 77 ± 6.0 seconds (mean ± SEM) for flow and 70–80 seconds for platelet count. Injections of thromboxane A₂ caused a small but significant increase in heart rate with no change in systemic blood pressure. In conclusion, the in vivo actions of synthetic thromboxane A₂ are consistent with the vasoconstrictor and platelet aggregatory effects seen in vitro, but dogs vary considerably in their sensitivity. (Circulation Research 1987;60: 290–296)

In 1969, Piper and Vane1 described the existence of an unstable material released during antigenic challenge of isolated sensitized guinea pig lung that contracted rabbit aortic strips in vitro and designated it as RCS, or rabbit aorta contracting substance. Vargaftig and Dao2 demonstrated that RCS was also produced by the injection of arachidonic acid into sensitized guinea pig lung and that this effect was blocked by the addition of nonsteroidal antiinflammatory drugs. Later, Vargaftig and Zirinis3 showed that human and rabbit platelets released RCS during in vitro aggregation. RCS from guinea pig lung and human platelets was further characterized by Svensson et al4 and found to consist of at least two factors — PGG₂ and/or PGH₂, and an unstable factor with a half-life of 30 seconds at 37°C. The major activity of RCS was attributed to this unstable component. In 1975, Hamberg et al5 accomplished the structural elucidation of a labile component of RCS derived from human platelets through the conversion of PGG₂, with a half-life of approximately 30 seconds. Because this compound was isolated from platelets and had a novel structure, the name thromboxane A₁ was proposed. Since that time, the role of this unstable metabolite of arachidonic acid in ischemic heart disease has been extensively investigated.6–11 These studies, however, were limited to the use of biologically generated TXA₁, stable TXA₂ analogs such as U-46,619 and carbocyclic TXA₂,12 or the assay of thromboxane B₂ (TXB₂) — the stable breakdown product of TXA₁.

While yielding much information regarding the potential role of TXA₂, each of these methodologies leave certain questions unanswered. For example, the use of biologically generated material does not allow for precise quantitative analysis of the amount of TXA₂ actually applied and/or the presence of other biologically active contaminants. Stable thromboxane analogs are intrinsically different from the parent material, again making interpretation of results difficult. The most detailed work has been accomplished by radioimmunoassay of TXB₂; however, TXB₂ levels do not necessarily reflect the involvement of TXA₂ associated with a given pathological event.

Recently, Bhagwat et al13 have synthesized the molecule proposed by Hamberg et al5 and have shown its activity in vitro to be identical to biologically generated material. The availability of synthetic TXA₂ has permitted us to assess its effects on regional blood flow in the canine heart. The results with exogenously administered compound show that TXA₂ is capable of eliciting vasoconstriction and platelet aggregation in the canine coronary vasculature.

Materials and Methods

Animal Preparation

Experiments were performed in mongrel dogs of either sex (15–20 kg) that were fasted for 24 hours prior to surgery. Anesthesia was induced with sodium brevital (10 mg/kg i.v.) and maintained with α-chloralose (100 mg/kg i.v.) and morphine sulfate (3 mg/kg i.m.). Additional doses of α-chloralose and/or mor-
phine were given as needed to maintain level III anesthesia throughout the experiment. Dogs were intubated with a Murphy No. 8 endotracheal tube and placed on a Harvard Apparatus respirator set at nominal rate of 13 breaths/min and a tidal volume of approximately 20 ml/kg. After instrumentation, a positive end-expiratory pressure (PEEP) of 10 cm H2O was introduced. Arterial blood gases were serially monitored and the respirator adjusted to maintain the arterial pH, PCO2, and PO2 within the normal physiological range. Supplemental O2 was introduced as necessary when respirator adjustments failed to correct PO2 deficiencies. Lead II of the electrocardiogram was monitored continuously. A femoral vein was isolated and a polyethylene catheter inserted for the administration of fluids and pharmacologic agents. This intravenous line was kept open with normal (0.9%) saline solution infused at a rate sufficient to maintain left atrial filling pressure between 1–3 mm Hg and aortic blood pressure at a constant level as determined during the control state. Lidocaine was employed as needed to control ventricular ectopy.

The left and right femoral arteries were isolated and catheters introduced and advanced to the aortic arch. An infusion/withdrawal pump (Harvard Apparatus, South Natick, Mass., Model 600) was used to simultaneously withdraw duplicate reference blood samples from these catheters during each microsphere injection.

With the dog in right lateral recumbency, a left fifth thoracotomy was performed, the lungs retracted, and the pericardium opened. The circumflex coronary artery was isolated approximately 1.0 cm from its origin and dissected free of epicardium for a distance of 1.5 cm. Arterial branches that arose in this region were ligated with sutures of 4-0 silk and severed. A 22-gauge angiocath (Deseret, Sandy, Utah) was inserted into the proximal lumen of the circumflex to monitor coronary perfusion pressure and for injection of pharmacologic agents. An electromagnetic flow probe (Carolina Medical Instruments, King, N.C.) was placed proximal to the angiocath in each animal. Zero calibration for the electromagnetic flow probe was performed by brief total occlusion of the instrumented vessel. An 18-gauge angiocath (Deseret, Sandy, Utah) was placed into the lumen of the ascending aorta for measurement of systemic arterial pressure. A flared polyethylene tube was placed in the left atrial appendage for pressure measurement and for injection of radiolabeled microspheres. The coronary sinus was catherized according to the method of Carlson and Utley, used in dogs to assess the effects of intracoronary injections of synthetic TXA2 on platelet count.

**Determination of Regional Myocardial Blood Flow**

Serial changes in regional myocardial blood flow were determined by the radioactive microsphere technique as previously described by Heyman et al. Microspheres 10 μm in diameter (New England Nuclear Corporation, Boston, Mass.) were labeled with 113mSn, 103Ru, 95Nb, and 46Sc. Each aliquot was calibrated to contain approximately 1–2 × 10⁶ spheres suspended in 0.9% saline solution containing 0.01% polysorbate 80 (Twean 80) and diluted to a final volume of 5 ml. Prior to injection, the dog received 250 U/kg sodium heparin. The spheres were vigorously agitated by passing them back and forth between two 6-ml syringes connected by a three-way stopcock. Microscopic examination of a drop of the sphere suspension revealed no significant clumping. As an additional check, to demonstrate adequate mixing, duplicate reference blood samples withdrawn simultaneously from 2 separate arterial catheters were shown to be similar in counts per milliliter per minute. Flow determinations not fulfilling these criteria were discarded.

The microsphere suspension was injected as a bolus over 5–10 seconds into the left atrium, after which the injection catheter was flushed with 10 ml 0.9% saline solution. During the injection, aortic pressure, coronary blood flow, heart rate, and distal coronary pressure were continuously recorded. Any physiologic deviation from the immediate preinjection steady state during the sphere injection was noted and that flow considered invalid. Duplicate timed withdrawals from the 2 femoral artery/aortic arch catheters were begun 15–20 seconds prior to the sphere injection and continued for 90 seconds postinjection. Blood samples from each individual withdrawal were divided among 3 or 4 tared tubes, weighed and corrected for the specific gravity of canine blood (1.04 g/ml) and the precise reference withdrawal rate calculated in ml/min. At the end of the experiment, the animal was killed by electrically inducing ventricular fibrillation, and the heart removed. The left ventricle was separated from the remainder of the heart, trimmed of epicardial fat and vessels, and prepared for analysis by a modification of the method of Marcus et al. The left ventricle is divided into 4 layers from base to apex. Each layer is then subdivided into 8 segments. Each segment is further subdivided into an epicardial, midwall, and endocardial section.

The resulting 96 samples were weighed and counted, together with the reference bloods and pure samples of each nuclide, in a Packard Auto Gamma Scintillation Spectrometer. The multichannel analyzer was set for the following regions of interest:

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Energy (keV)</th>
<th>Activity (dpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>141Ce</td>
<td>85–180</td>
<td></td>
</tr>
<tr>
<td>113Sn</td>
<td>340–440</td>
<td></td>
</tr>
<tr>
<td>103Ru</td>
<td>450–570</td>
<td></td>
</tr>
<tr>
<td>95Nb</td>
<td>650–818</td>
<td></td>
</tr>
<tr>
<td>46Sc</td>
<td>820–1300</td>
<td></td>
</tr>
</tbody>
</table>

Myocardial blood flow was calculated with the equation:

\[
Qm = \frac{(Cm \times 100 Qr)}{Cr}
\]

where \(Qm\) = myocardial blood flow (ml/min), \(Cm\) = myocardial tissue counts (counts/min/g), \(Qr\) = reference blood withdrawal rate (ml/min), and \(Cr\) = counts in the reference arterial sample (dpm). Flow per gram
of myocardium was obtained by dividing blood flow by the sample weight.

All blood flow calculations were performed on an IBM 3081J computer, using a matrix inversion program to calculate the correct isotopic activity for each radionuclide.

**TXA₂ Preparation**

TXA₂ free acid was prepared fresh prior to the start of each experiment by saponification of thromboxane 1-15-macrolactone (kindly supplied by Professor Clark Still, Columbia University, N.Y.), using KOSiMe₃.¹⁵,¹⁷ Thromboxane A₂ macrolactone (200–1,000 µg) and KOSiMe₃ (4:1, wt:wt) were dissolved in anhydrous ether to a final concentration of 3 µg/µl TXA₂. The saponification mixture was maintained at room temperature overnight, or until saponification was complete as determined by assaying for TXA₂ activity on platelets. The reacted TXA₂ was then stored on ice until use. Prior to injection, each dose of TXA₂ was assayed for potency by testing its ability to induce aggregation at the appropriate concentration (40–60 ng/ml) in human platelet-rich plasma (PRP).

**Platelet Assays**

Human whole blood was collected over 3.8% (wt: vol) trisodium citrate at a ratio of 9:1 (blood/citrate). PRP was harvested by centrifuging the blood at 200g for 20 minutes at 25°C. The PRP was removed and stored in plastic tubes closed to air.

Canine PRP was prepared by collecting whole blood into trisodium citrate, 2.5% (wt: vol) at a ratio of 9:1. The PRP was prepared by centrifuging the citrated blood at 800g for 1 minute at 25°C. The PRP obtained from each centrifugation.

All platelet aggregations were performed in 0.5 ml aliquots of PRP in a Payton 300 BD-5 dual channel aggregometer (Payton Associates, Buffalo, N.J.) at 37°C with a stirring rate of 900 rpm. Human PRP was used without further treatment. Canine PRP assays were carried out in the same manner, with and without the addition of Ca²⁺ to a final concentration of 1 mM.

**Experimental Protocol**

**NUTRIENT BLOOD FLOW STUDIES.** 1. Four of the 9 dogs receiving TXA₂ also served as a vehicle control group. After being instrumented as previously described, the animals were allowed to stabilize, and the first dose of microspheres injected into the left atrium. After baseline hemodynamics were recorded, 15 µl of the saponification vehicle (KOSiMe₃-ether) was injected into the proximal circumflex coronary artery (Cx) via a 22-gauge angiocath followed by a 2-ml saline flush. The second dose of microspheres was injected 45 seconds after administration of the vehicle. Hemodynamics were followed throughout the course of the vehicle injection and administration of the microspheres.

Following a stabilization period, 2 µg/kg of TXA₂ was injected directly into the Cx as previously described. The amount of saponification vehicle varied according to the individual concentration of each TXA₂ preparation, but in all cases was less than 10 µl. Physiological parameters were continuously monitored throughout the injection interval. The third set of microspheres were injected 45 seconds after the administration of TXA₂.

After the hemodynamic parameters had returned to preinjection values, the proximal Cx was totally occluded, and a fourth set of microspheres injected to demarcate the Cx distribution in each animal.

2. Five dogs underwent the same experimental protocol, excluding the administration of the vehicle control. This variation was undertaken to identify any cumulative effects of repeated vehicle administration.

**DOSE-RESPONSE EFFECTS.** Dose-response curves were attempted in 4 preparations. Doses of TXA₂ ranging from 0.25 to 8.0 µg/kg were administered as previously described. Microspheres were injected 45 seconds after each bolus of TXA₂. In all cases, the preparation was allowed to return to control values between doses of TXA₂.

**PLATELET ASSAYS.** The effect of intracoronary synthetic TXA₂ on coronary sinus platelet count was compared in 4 additional animals. In vitro platelet aggregations were performed on each dog studied. Coronary sinus blood was collected as previously detailed at 20 and 10 seconds prior to injection, and thereafter at 0, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 180, 240, and 300 seconds. At time zero, 2 µg/kg synthetic TXA₂ was injected as an intracoronary bolus as previously described. Platelet counts were performed on a Baker 810 Whole Blood Platelet Counter (Allentown, Penn.). Blood samples were collected from the coronary sinus at 10-second intervals into pretreated EDTA tubes (Becton Dickinson, Rutherford, N.J.) with a Minipuls 2 roller pump (Gilson Instruments, Middleton, Wis.). Blood withdrawal was maintained at 5.0 ml/min throughout each determination. Radiolabelled microspheres were not used in this group to eliminate the possibility of particulate contamination in the sampled effluent.

**Data Analysis**

Flow criteria were used to divide the heart into 2 distinct regions. A central circumflex region (CCX) was defined as that area of the heart in which nutrient blood flow of the endocardium fell to less than 0.15 ml/min/g during the total Cx occlusion. Likewise, a peripheral control region (PCR) was identified as that area of the heart in which no diminution of flow occurred during the same microsphere injection, i.e. an area not perfused by the circumflex artery.

In all dogs, a paired t test was used to test for differences before and after administration of TXA₂. A p value of less than 0.05 was considered significant. All data presented are mean ± SEM.

**Results**

**Effects on Myocardial Blood Flow**

**CONTROL GROUP.** The effect of the saponification vehicle on myocardial blood flow was examined in 4 dogs. There was no significant change in blood flow or
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ENDO

FIGURE 1. Effects of synthetic TXA₂ on myocardial blood flow — CCX region (A): CCX region demonstrates marked reduction in both epicardial (EPI) and endocardial (ENDO) blood flow 45 seconds after intracoronary injection of TXA₂ into proximal circumflex coronary artery (mean ± SEM).

Effects of synthetic TXA₂ on myocardial blood flow — PCR region (B): While flow increased in PCR region 45 seconds after TXA₂ administration, it was not statistically significant. EPI = epicardial, ENDO = endocardial, mean ± SEM.

in any of the monitored physiological parameters when 15 µl of the saponification vehicle was injected directly into the Cx.

TXA₂ GROUP. The changes in myocardial blood flow 45 seconds after intracoronary administration of 2 µg/kg TXA₂ are summarized in Figure 1.

In the PCR region, both epicardial and endocardial flow increased (.82 ± .08 to 1.11 ± .18 and 1.09 ± .12 to 1.53 ± .25 ml/min, respectively), but the change was not significant.

In the CCX region, nutrient blood flow fell significantly in both the epicardium and endocardium (1.04 ± .20 to .55 ± .08 p = 0.05, and 1.19 ± .12 to .48 ± .12 ml/min/g, p = 0.0008, respectively).

These observations are confirmed by the electromagnetic flow data. Mean electromagnetic flow fell from 25.9 to 6.6 ml/min/g (p = 0.0003) during the peak TXA₂ effect. The duration of effect of TXA₂, defined as that period of time required for mean electromagnetic flow to return to preinjection values, in the 9 dogs receiving a 2 µg/kg bolus was 77 ± 6 seconds. A complete hemodynamic summary is presented in Table 1.

Heart rate increased significantly in all cases (85 ± 10 to 102 ± 10 bpm, p = 0.0075). As with other TXA₂ effects, this increase was transient, usually persisting for less than 3 minutes. A typical physiological trace is depicted in Figure 2.

Dose-Response Data

Attempts to obtain a dose-response relationship to intracoronary TXA₂ were usually unsuccessful. Doses of synthetic TXA₂ ranging from 0.25 to 8.0 µg/kg failed to produce dose-related effects. The threshold dose response was defined as the amount of material necessary to produce a 20% reduction in the electromagnetic flow of the affected artery, persisting for longer than 15 seconds. This value ranged from 0.25 µg/kg to greater than 1.0 µg/kg. Dose-response data were compiled for 6 separate dogs, while each animal received equivalent amounts of TXA₂, confirmed by platelet aggregation assays, each dog tested produced a unique response. Of the 6 dogs studied, only 2 demonstrated a definitive dose-dependent response. Even then, the EC₅₀s were considerably different (0.5 µg/kg and 2.5 µg/kg).

Attempts to normalize each dose-response experiment with a standardized dose of U-46,619 (3 µg/kg) were also unsuccessful. The same variability that was noted for synthetic TXA₂ was seen with each U-46,619 challenge (data not shown). U-46,619 is subject to the same dose-response variability that we observed for synthetic TXA₂.¹⁸-¹⁹

Platelet Effects

Bolus intracoronary injection of 2 µg/kg synthetic TXA₂ produced a profound thrombocytopenia in the coronary sinus blood. Figure 3 illustrates that this effect was of short duration with the peak reduction occurring at 30 seconds postinjection. Parallel systemic platelet counts were unaffected.

Table 1. Hemodynamic Summary

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>2 µg/kg TXA₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aₐ-Systolic (mm Hg)</td>
<td>102 ± 3.4</td>
<td>103 ± 1.6</td>
</tr>
<tr>
<td>Aₐ-Diastolic (mm Hg)</td>
<td>68.3 ± 3.9</td>
<td>73 ± 2.0</td>
</tr>
<tr>
<td>Aₐ X Pressure (mm Hg)</td>
<td>84 ± 3.0</td>
<td>83 ± 1.7</td>
</tr>
<tr>
<td>Cx-Systolic (mm Hg)</td>
<td>101 ± 3.6</td>
<td>91 ± 10.0</td>
</tr>
<tr>
<td>Cx-Diastolic (mm Hg)</td>
<td>70 ± 3.0</td>
<td>64 ± 8.0</td>
</tr>
<tr>
<td>Cx X Pressure (mm Hg)</td>
<td>84 ± 3.0</td>
<td>73 ± 8.2</td>
</tr>
<tr>
<td>Left Atrial pressure (mm Hg)</td>
<td>3.3 ± 0.5</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>82 ± 10.7</td>
<td>102 ± 10.6</td>
</tr>
<tr>
<td>EMF phasic (ml/min)</td>
<td>40 ± 5.8</td>
<td>31 ± 6.0</td>
</tr>
<tr>
<td>EMF X (ml/min)</td>
<td>16 ± 2.5</td>
<td>6.0 ± 2.1</td>
</tr>
<tr>
<td>Peak EMF reduction (%)</td>
<td>26 ± 3.1</td>
<td>16.8 ± 3.6</td>
</tr>
<tr>
<td>Duration* (sec)</td>
<td>. . .</td>
<td>72.3 ± 8.0</td>
</tr>
</tbody>
</table>

*Time for EMF flow to return to control value.

Control values were recorded immediately prior to TXA₂ administration. "2 µg/kg TXA₂" values were recorded 45 seconds after thromboxane injection, with the exception of "Peak EMF Reduction" and "Duration" entries, and represent hemodynamic status at time of microsphere injection. Aₐ = aortic arch, Cx = circumflex coronary artery, EMF = Cx electromagnetic flow, data ± SEM.
In the group used to study platelet changes, all dogs responded uniformly with a fall in coronary sinus platelet count while these same animals simultaneously exhibited variability in electromagnetic flow. The 4 dogs studied ranged in response from virtually no detectable flow alteration to total flow occlusion persisting for longer than 4 minutes following intracoronary administration of 2 μg/kg synthetic TXA2. This variability was consistent with that observed in the group of animals that received microsphere injections.

In vitro platelet aggregation data again demonstrated interdog variability. All animals tested did aggregate to synthetic TXA2; however, the threshold dose for irreversible platelet aggregation was unique for each animal studied. Figure 4 depicts in vitro aggregations for 4 separate dogs. Threshold synthetic TXA2 doses ranged from 30 ng/ml to 1 μg/ml. Three of the 4 dogs required the addition of 1 mM Ca2+ to achieve a positive response. The animal exhibiting 30 ng/ml sensitivity aggregated fully without added Ca2+.

**Discussion**

The results presented confirm that synthetic TXA2 has the predicted in vivo biological activity. A 2 μg/kg intracoronary injection resulted in local reduction in nutrient blood flow resulting from a transient vasoconstriction in the affected artery. Not surprisingly, this effect was most pronounced in the endocardium, an observation consistent with the action of most coronary vasoconstrictors. Similar local increases in vascular resistance have been reported in vivo for the stable analogs of thromboxane. The short duration of action of a single bolus injection is consistent with the known biological half-life of TXA2. The rapid in vivo degradation of the synthetic TXA2 is seen in the transient effects on heart rate, electromagnetic flow reduction, and coronary sinus thrombocytopenia, all returning to preinjection levels within 3 minutes. We cannot exclude, however, that release of prostacyclin (previously described following injection of U-46,619) was reducing the magnitude of the response to TXA2.

While the data presented confirm that the in vivo biological activity of synthetic TXA2 is consistent with the properties observed in vitro, they raise questions that remain unresolved. Among these is the variability of potency of synthetic TXA2 between dogs.

The in vitro potencies of U-46,619, carbocyclic thromboxane A2 (c-TXA2), and synthetic TXA2 demonstrated similar nanomolar thresholds for inducing isolated vascular smooth muscle contraction. While there is little in vitro variability on vascular smooth muscle, each of these compounds demonstrates a unique activity in platelet aggregation assays. Synthetic TXA2 is a potent stimulus for the aggregation of human platelets, and our results show that it also induces aggregation of canine platelets. U-46,619 demonstrates uniform potency in human platelets but is usually unable to induce aggregation of canine platelets unless they are pretreated with 10 μM epinephrine. In contrast, c-TXA2 has been shown to be an inhibitor of arachidonic-acid- and endoperoxide-induced aggregation of human platelets. Mais et al have suggested that these variations provide further
In vivo canine platelet aggregations to synthetic TXA₂: Synthetic TXA₂-induced platelet aggregation in all dogs studied; however, threshold dose for irreversible aggregation varied from 30 to 1,000 ng/ml. All assays shown were performed in presence of added 1 mM Ca²⁺. Requirement for exogenously added Ca²⁺ was not absolute as dog No. 54 demonstrated identical response in absence of added calcium (data not shown).

The potency of intracoronary injections of synthetic TXA₂ in some dogs corresponds well with that reported by others for U-46,619. Interdog variability, however, was also observed with this analog; for example, Mehta et al reported in 1984 that coronary flow exhibited no overall significant change — increasing in 8 animals, decreasing in 6 others.

Serial assays of synthetic TXA₂ in human PRP indicate that after the saponification is complete, the synthetic TXA₂ generated remains stable for several hours at 0°C, making it unlikely that the observed variations in activity were due to instability of the stock solution of TXA₂. Even though the exact amount of TXA₂ was not analyzed chemically, it was assumed that when biological activity was maximum in the saponification mixture and did not decay during the course of the experiment that the lactone was 100% hydrolyzed.

Miwa et al have demonstrated a uniform population of TXA₂ receptors throughout the canine coronary vasculature. This observation tends to preclude variability in our model due to vessel size or exact injection site location. Taken together, these in vivo and in vitro studies suggest that the variability observed in our experiments was intrinsic to the dog and not the procedure.

Synthetic TXA₂ uniformly produced an in vivo coronary sinus thrombocytopenia following bolus intracoronary administration. The in vivo and in vitro sensitivities for synthetic TXA₂ spanned a wide range and did not correlate. The 4 cases presented had unique in vitro thresholds (platelet aggregation assay) ranging from 30 to 1,000 ng/ml, yet each animal had a similar in vivo response. Since the data do not allow for an in vivo dose comparison on platelets, the identical responses in coronary sinus thrombocytopenia could be due to maximal in vivo doses of synthetic TXA₂. Our data demonstrate the effects of exogenously added synthetic TXA₂ in a normal vascular bed. However, the response may be different in diseased vessels, or when TXA₂ is produced by the vessels themselves.

Our results demonstrate that TXA₂ is capable of causing vasoconstriction and platelet aggregation in vivo, but the question still remains whether sufficient material is produced to make a significant contribution to a particular pathological event. Canine platelets in vitro are able to respond to synthetic TXA₂, but their sensitivity varies considerably, as does the response of the coronary vasculature. Therefore, the interdog and intradog variability that has been previously noted for arachidonate metabolites and endoperoxides also pertains to synthetic TXA₂.

References
3. Varaghshyz BB, Zinnis P: Platelet aggregation induced by arachidonic acid is accompanied by release of potential inflammatory mediators distinct from PGE₂ and PGF₂α. Nature 1973; 244:114-116


KEY WORDS • thromboxane A₂ • coronary blood flow • vasoconstriction • myocardial ischemia • platelet aggregation • thrombosis
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