Cardiovascular and Pulmonary Effects of Thromboxane B₂ in the Dog

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SUMMARY The hemodynamic properties of thromboxane B₂ (TxB₂), a product of prostaglandin endoperoxide metabolism, have not been thoroughly described. TxB₂ is a bronchoconstrictor, but its effects on the systemic circulation and circulating platelets are unknown. Its precursor, thromboxane A₂ (TxA₂), is a potent vasoconstrictor as well as a platelet-aggregating agent. Using intact anesthetized dogs, we investigated the effects of TxB₂ on pulmonary artery pressure (PAP), airway pressure (AP), systemic arterial pressure (SAP), and myocardial contractility (MC). Vascular responses were evaluated in relation to changes in platelet population and aggregability. Intravenous TxB₂ (25 and 50 μg/kg) increased AP (mean 62% and 69%) and PAP (50% and 86%), respectively, whereas SAP and MC responses were inconsistent. Left ventricular injections (25 μg/kg) also increased AP (36%) and PAP (36%). Intraventricular administration of TxB₂ produced a consistent elevation of SAP (10%) with a concomitant fall in MC (11%). These vascular responses were not consistent with alterations in platelet number or aggregability. A tachyphylactic response to TxB₂ developed in AP and PAP at both dose levels and with both routes of administration. Intravenous and intraventricular TxB₂ (25 μg/kg) produced a parallel decreasing response in PAP, suggesting the possible saturation of TxB₂ binding sites or the depletion of a catabolic enzyme in the lung.

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THROMBOXANE B₂ (TxB₂), a metabolite of thromboxane A₂ (TxA₂) (Hamberg et al., 1975), is a major product of prostaglandin endoperoxide metabolism (Hamberg and Samuelsson, 1974a, 1974b). The conversion of TxB₂ from endoperoxide has been demonstrated in guinea pig lung (Hamberg and Samuelsson, 1974a), cerebral cortex (Wolfe et al., 1976), and spleen (Samuelsson, 1976), in human platelets (Hamberg et al., 1975) and umbilical artery (Tuvemo et al., 1976), and in rat cerebral cortex (Wolfe et al., 1976). TxA₂ is a potential regulator of vascular and pulmonary homeostasis (Kolata, 1975; Svensson and Hamberg, 1976) and has been implicated in the regulation of coronary blood flow under pathological conditions (Svensson and Hamberg, 1976; Ellis et al., 1976). Unlike TxA₂, which has a short half-life (t₁ = 32 seconds in aqueous solution at 37°C; Hamberg et al., 1975), TxB₂ is chemically stable (Hamberg et al., 1975), and consequently, if biologically active, may be responsible for some of the vascular effects of its parent compound.

The purpose of this study was to establish the effects of TxB₂ on the systemic vasculature in dogs and to correlate these findings with possible changes in circulating platelet concentration and aggregability.

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Methods

Mongrel dogs (n = 16) of either sex and weighing 10.5–21.5 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and intubated with auffed endotracheal tube. Ventilation was maintained with a positive pressure respirator. Airway pressure (AP) was monitored via a needle inserted in the respirator tube and attached to a pressure transducer (Statham P23Db). Drugs were administered either intravenously or intra-arterially. For the former, a catheter was placed in the right femoral vein. Intra-arterial TxB₂ was administered through a catheter (Elecath, 100 cm) threaded in a retrograde manner from the left femoral artery into the left ventricle. A left thoracotomy was performed at the level of the 4th intercostal space, and a Walton-Brodie strain gauge arch was sutured onto the right ventricular wall. Pulmonary artery pressure (PAP) was monitored via a catheter placed in a branch of the left pulmonary artery and positioned in the direction of the right ventricle. Pulmonary artery pressure (PAP) was monitored via a catheter placed in a branch of the left pulmonary artery and positioned in the direction of the right ventricle.

Stock solutions of TxB₂, prostaglandin E₂ (PGE₂), and prostaglandin F₆ (PGF₆) were prepared in ethanol (1 mg/ml) and stored at –20°C. Solutions were prepared fresh on the day of use. Samples of TxB₂ were dried under nitrogen and resuspended in isotonic saline at a concentration of 500 μg/ml. Solutions of PGE₂ and PGF₆ were dried under nitrogen and diluted to a concentration of 100 μg/ml.

TxB₂ was administered three times at each dose level (50 and 25 μg/kg, iv, and 25 μg/kg, ia). The 25- and 50-μg/kg doses were not given to the same dog.
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THROMBOXANE B₂ (25-µg/kg)

FIGURE 1 A: Effects of TxB₂ (25 µg/kg) administered intravenously. Arrow indicates point of TxB₂ injection. B: Effects of TxB₂ (25 µg/kg) administered intra-arterially. Panels A and B represent responses from different dogs.

The 50-µg/kg doses were administered at random intervals, and the 25-µg/kg doses were given at 20-minute intervals.

AP, PAP, systemic arterial pressure (SAP), and myocardial contractility (MC) were allowed to return to control levels before subsequent doses were administered. PGE₂ was given after the last dose of TxB₂. PGF₂₅ was given when control values were reached after PGF₂₅.

Blood (18 ml) for platelet studies was drawn from the right femoral vein into a plastic syringe (20 ml) containing 2 ml of trisodium citrate (0.13 M). The blood specimen was centrifuged (140 g) for 5 minutes at room temperature. The platelet-rich plasma (PRP) was removed from the sedimented cells. Platelet concentrations were estimated with a Coulter counter (model ZB1). For each aggregation test, a sample (1 ml) of PRP was placed in a siliconized glass cuvette containing a magnetic stirring bar. The cuvette was placed in the 37°C spectrophotometer and stirred at 900 rpm. After 1 minute, a selected dose of ADP in 20 µl saline was injected into the PRP. Changes in absorbance at 600 nm were monitored with a modified Beckman CU monochromator with a Gilford photometer. Percent aggregation was calculated from the change in absorbance at peak aggregation.

In vitro effects of TxB₂ on platelet aggregation were tested with blood samples (5 ml) drawn from four unanesthetized dogs. For each sample, 1 ml of PRP was tested sequentially with 20 µl of saline containing one of the following: (1) 37.5 µg TxB₂, (2) 375.0 µg TxB₂, or (3) 0.5 µg ADP. We used 37.5 µg to approximate the concentration of TxB₂ seen in an average 15-kg dog given a 25-µg/kg injection of TxB₂. This assumes a blood volume of 1100 ml, a hematocrit of 50%, and dilution in the 20µl of saline. The 375.0-µg dose is an order of magnitude higher and confirmed the response to 37.5 µg.

Results

The 25-µg/kg intravenous dose of TxB₂ elevated AP by a mean of 62% and PAP by 50% (Fig. 1A). Progressively diminished responses were noted with repeated administrations. SAP and MC showed inconsistent responses. In contrast, the intra-arterial administration of 25 µg/kg (Fig. 1B) produced a smaller elevation of AP (mean 39%) and PAP (31%). Subsequent to the second dose, all PAP responses diminished with repeated administration. However, a consistent elevation of SAP (10%) with a concomitant fall in MC (11%) was observed. The SAP response was present with each subsequent dose.

The larger TxB₂ dose (50 µg/kg) administered intravenously produced a greater increase in AP (60%) and PAP (86%). Subsequent doses elicited progressively diminished responses in both parameters, as had been the case with the 25-µg/kg dose. Inconsistent responses were also observed in SAP and MC.

Table 1 compares results between TxB₂ at both doses and routes of administration with PGE₂ and PGF₂₅. A 5-µg/kg dose of PGF₂₅ elevated AP, PAP, and SAP. PGE₂ (5 µg/kg) lowered AP and SAP and raised PAP.

"Time to onset" (Table 1) designates the initiation of a hemodynamic response in any of the monitored parameters. Intra-arterial TxB₂ had a relatively short time to onset because the injection was made through a catheter located in the left ventricle. The intravenous injection was made via

<table>
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<tr>
<th>Dose</th>
<th>n</th>
<th>µg/kg</th>
<th>Time to onset (sec)</th>
<th>AP (%)</th>
<th>PAP (%)</th>
<th>SAP (%)</th>
</tr>
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<tbody>
<tr>
<td>TxB₂</td>
<td>5</td>
<td>50</td>
<td>4.6 ± 0.9</td>
<td>+69.0 ± 19.2</td>
<td>+86.0 ± 8.5</td>
<td></td>
</tr>
<tr>
<td>TxB₂</td>
<td>5</td>
<td>25</td>
<td>7.2 ± 0.7</td>
<td>+62.0 ± 17.0</td>
<td>+50.0 ± 6.0</td>
<td></td>
</tr>
<tr>
<td>TxB₂</td>
<td>6</td>
<td>25</td>
<td>4.0 ± 0.9</td>
<td>+38.8 ± 8.9</td>
<td>+31.3 ± 4.1</td>
<td>+10.1 ± 1.1</td>
</tr>
<tr>
<td>PGF₂₅</td>
<td>5</td>
<td>5</td>
<td>−15.9 ± 5.4</td>
<td>+22.3 ± 4.7</td>
<td>−33.4 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>PGF₂₅</td>
<td>8</td>
<td>5</td>
<td>7.1 ± 0.5</td>
<td>+26.4 ± 5.4</td>
<td>+73.1 ± 12.9</td>
<td>+29.4 ± 11.0</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE.
a catheter placed in the inferior vena cava. The SAP response following intra-arterial injection was earlier than the PAP response following intravenous injection because of the shorter distance TxB$_2$ traveled to the site of vascular reactivity. These doses of TxB$_2$ (25 and 50 µg/kg) caused the elevation of AP and PAP to be sustained for at least 10 minutes. Peak elevations occurred after approximately 45 seconds and then gradually returned to baseline.

No consistent change in platelet counts or aggregability to ADP was observed in blood drawn prior to or during peak TxB$_2$ response. In vitro studies produced no evidence of platelet aggregation to TxB$_2$ at concentrations equivalent to blood levels achieved by experimental doses or at doses 10 times as great. ADP, however, evoked a normal aggregation response, indicating that the platelets were functional.

**Discussion**

These results show that TxB$_2$ is active in the systemic vasculature and that it elevates SAP. The data also confirm the report that TxB$_2$ increases AP (Wasserman and Griffin, 1977) and is a constrictor of the pulmonary vasculature (Kadowitz et al., 1977).

In preliminary studies the cardiovascular and pulmonary effects of TxB$_2$ were analyzed at dose levels ranging from 5–50 µg/kg. Doses of 25 and 50 µg/kg were selected because of their ability to produce consistent and reproducible results. TxB$_2$ at both doses (50 and 25 µg/kg) and with both routes of administration (intravenous and intra-arterial), sharply increased AP and PAP.

AP increased to a greater degree after intravenous than after intra-arterial administration of TxB$_2$. A probable explanation for this paradox resides in the vascular connections between the pulmonary and bronchial circulations. At normal PAP levels, the direction of flow through these anastomoses is generally from the bronchial to the pulmonary circulation. However, in circumstances in which PAP rises acutely, the pressure gradient may reverse, and flow occurs in the opposite direction. Thus substances that are administered by the intravenous route and produce pulmonary hypertension have direct access to the bronchial smooth muscle in relatively higher concentrations than would occur after administration via the intra-arterial route.

The effects on the systemic circulation were inconclusive when TxB$_2$ was given intravenously. To eliminate restriction of pulmonary venous return to the left heart resulting from intense pulmonary vasoconstriction, TxB$_2$ was administered intra-arterially directly into the left ventricle. This route of administration elevated SAP in all dogs tested. TxB$_2$ produces moderate elevation of SAP in comparison with its marked pressor effects on the pulmonary vasculature.

There was tachyphylaxis to TxB$_2$ in the pulmonary vasculature and bronchial smooth muscle. SAP and airway responses diminished with each subsequent administration of TxB$_2$. No tachyphylaxis to TxB$_2$ was observed in the SAP response. In fact, there was a slight rise in mean pressure over three administrations. The diminution in PAP response to intravenous TxB$_2$ (25 µg/kg) over three administrations was nearly parallel to the response seen with an equal dose of TxB$_2$ given intra-arterially. Parallel linear regression lines result from plotting mean responses in PAP against dose sequences from these two preparations (Fig. 2).

The parallel tachyphylactic response in PAP to TxB$_2$ indicates the possible saturation of TxB$_2$ binding sites or the depletion of a catabolic enzyme in the lung. The percent increase in PAP resulting from intravenous administration is greater than that resulting from intra-arterial injection. This may be due to dilution of TxB$_2$ or to the binding or metabolism of TxB$_2$ in the systemic circulation. Although TxB$_2$ is a significant vasoconstrictor, it is less potent than PGF$_{2}$α.

Platelet studies indicate no direct relationship between the pressor effects of TxB$_2$ and changes in platelet counts or aggregability. No consistent changes in platelet population occurred after TxB$_2$ administration. In vitro results were the same after initial TxB$_2$ administration and after all subsequent doses. In light of the consistent vascular responses, it seems unlikely that they can be correlated with alterations in platelet population or activity.

In conclusion, our study demonstrates that in
high doses: (1) TxB₂ increases systemic vascular resistance, (2) tachyphylaxis to TxB₂ develops in the airway and in pulmonary arterial smooth muscle, and (3) these actions are independent of increased platelet aggregation. Although many of the actions of TxA₂ and TxB₂ on smooth muscle are similar, it remains to be determined whether the less potent but more stable TxB₂ contributes to the constrictor action of TxA₂ on the pulmonary vasculature and bronchial smooth muscle.

Acknowledgments

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