Thromboxane Receptor Blockade Prevents Pulmonary Hypertension Induced by Heparin-Protamine Reactions in Awake Sheep

Gilles Montalescot, MD, PhD, Edward Lowenstein, MD, Martin L. Ogletree, PhD, Elizabeth M. Greene, BA, Dwight R. Robinson, MD, Karen Hartl, MS, and Warren M. Zapol, MD

We used competitive thromboxane A2–prostaglandin endoperoxide receptor blockade (SQ 30,741) as a probe to evaluate the role of thromboxane in ovine pulmonary vasoconstriction associated with protamine reversal of heparin anticoagulation. Control heparin-protamine reactions induced rapid release of thromboxane into arterial plasma (more than 1 ng/ml plasma), a 2.5-fold increase of pulmonary artery pressure, a 20% decrease of Pao2, and a 30% reduction in arterial white blood cell concentration. After giving SQ 30,741 despite similar thromboxane release into arterial plasma after heparin-protamine challenge, acute pulmonary hypertension was significantly reduced when 94% of pulmonary vascular smooth muscle thromboxane receptors were occupied with SQ 30,741 (p<0.01 at 1 minute after protamine versus control heparin-protamine reaction) and was completely abolished by a 10 mg/kg i.v. bolus (p<0.0001 at 1 minute after protamine versus control). Peripheral leukopenia was not affected by SQ 30,741 prophylaxis, but hypoxemia was prevented. We conclude that thromboxane causes pulmonary vasoconstriction in ovine heparin-protamine-induced pulmonary hypertension. Pulmonary vasoconstriction and hypoxemia can be completely prevented by thromboxane receptor blockade. (Circulation 1990;82:1765–1777)

Use of protamine has greatly increased because neutralization of heparin anticoagulation is necessary for certain clinical procedures. However, protamine administration after cardiopulmonary bypass, cardiac catheterization, hemodialysis, plasmapheresis, and vascular surgery has been associated with numerous adverse reactions.1-5 Anaphylaxis associated with immunoglobulin E antibody has been documented in protamine-insulin-dependent diabetic patients.6 Protamine reversal of heparin anticoagulation may also cause catastrophic pulmonary artery hypertension and bronchoconstriction leading to acute right ventricular failure and cardiovascular collapse.1,2,4,7 This reaction is associated with sharply elevated plasma thromboxane levels without histamine release.3,7 Animal models have been developed to elucidate the mechanisms of pulmonary vasoconstriction induced by heparin-protamine reactions.8-10 A hemodynamic reaction similar to the human nonimmunological reaction associated with protamine reversal of heparin consistently occurs in the sheep and pig. In these animals, acute pulmonary vasoconstriction is always accompanied by the simultaneous release of thromboxane into arterial plasma.9,9,11 Other mediators released into plasma during the heparin-protamine reaction may cause acute pulmonary vasoconstriction. These include C3a, C4a, C5a (humans); C3a, C4a, and oxygen free radicals (sheep); and prostaglandin (PG) F2a (pigs).9 In animal models, indomethacin pretreatment blocks both the synthesis of vasoconstrictor prostanoids and the acute pulmonary vasoconstriction induced by protamine reversal of heparin anticoagulation.8,9 Pretreatment of heparinized sheep with a thromboxane synthetase inhibitor partially inhibited the elevation of pulmonary

See p 1877

Failure of protamine in this model is due to the inability of the human antithrombin III to neutralize the heparin remaining in the plasma.12-14 This is analogous to the human heparin-protamine reaction, where heparin anticoagulation is not reversed completely by protamine.12-14

From the Department of Anesthesia (G.M., E.L., E.M.G., W.M.Z.) and the Arthritis Unit of the Department of Medicine (D.R.R.), Massachusetts General Hospital and Harvard Medical School, Boston; Department of Pharmacology (M.L.O.), Squibb Institute for Medical Research, Princeton, New Jersey.

Supported by US Public Health Service grants HL-32591, HL-42997, and AR-79427. G.M. was the recipient of a grant from the Fondation pour la Recherche Médicale, the Société de Réanimation de Langue Française, and the Université de Paris XII, France.

Address for correspondence: Warren M. Zapol, MD, Department of Anesthesia, Massachusetts General Hospital, Boston, MA 02114.

Received November 14, 1989; revision accepted June 12, 1990.
artery pressure after protamine administration. This inhibitor prevented the release of thromboxane into plasma, but the plasma level of PGF$_{2\alpha}$ increased 10-fold above the baseline value after protamine administration. In contrast, there was no PGF$_{2\alpha}$ release during heparin-protamine control reactions (without pretreatment). Inhibition of thromboxane synthetase probably increased diversion of the eicosanoid precursor PGH$_2$ to production of vasoconstrictor PGF$_{2\alpha}$. Although Morel et al. hypothesized that thromboxane was the mediator causing pulmonary vasoconstriction during the heparin-protamine reaction, they could not prove this.

The purpose of the present studies was to determine whether preventing the physiological response to thromboxane would abolish pulmonary vasoconstriction associated with protamine reversal of heparin. We used SQ 30,741, a newly developed thromboxane A$_2$-PGH$_2$ (TxA$_2$-PGH$_2$) receptor antagonist. Our strategy was first to confirm that heparin-protamine reactions could be repeated at brief intervals in the same sheep without altering the hemodynamic or mediator response and second to provoke successive heparin-protamine reactions to evaluate the effect of pretreatment with SQ 30,741 given in increasing doses. We measured arterial plasma concentrations of eicosanoids and the TxA$_2$-PGH$_2$ receptor antagonist. To assess quantitatively the affinity of SQ 30,741 for sheep pulmonary vascular smooth muscle TxA$_2$-PGH$_2$ receptors, we used the prostaglandin endoperoxide analogue U 46,619, which mimics the actions of thromboxane on vascular smooth muscle and produces pulmonary vasoconstriction in sheep. In this manner, we determined the efficacy, the effective plasma concentrations, and the duration of action of this TxA$_2$-PGH$_2$ receptor antagonist in the setting of protamine reversal of heparin in the sheep.

Methods

Sheep Preparation

Suffolk sheep weighing 20–25 kg were anesthetized by mask with 1–2% halothane (Halocarbon Laboratories, Hackensack, N.J.) in oxygen and were surgically instrumented by use of sterile techniques. The right femoral artery was cannulated with a catheter advanced to the midthoracic aorta for intermittent blood sampling and systemic blood pressure monitoring. The studies were performed on 10 awake sheep 24 hours after anesthesia and catheterization. A thermodilution pulmonary artery catheter (Edwards Laboratories, Santa Ana, Calif.) was passed under local anesthesia by an 8F introducer sheath through the external jugular vein into the pulmonary artery to measure mean pulmonary artery pressure, pulmonary occlusion pressure, and central venous pressure.

Hemodynamic Measurements

Systemic blood, central venous, pulmonary artery, and pulmonary occlusion pressures were continuously monitored as previously described. Thermodilution cardiac output was determined by injecting 5 ml iced saline into the right atrium, and the mean value of triplicate determinations was computed. Stroke volume, pulmonary vascular resistance, and systemic vascular resistance were calculated by standard formulas.

Eicosanoid Measurements

Plasma levels of TxB$_2$, PGF$_{2\alpha}$, and 6-keto-PGF$_{1\alpha}$ were determined by radioimmunoassay using antiserum obtained from Dr. L. Levine. Four milliliters of arterial blood were collected in glass test tubes containing 0.05 ml 15% EDTA and 100 µg indomethacin. Samples were immediately transferred to ice and centrifuged at 2,000 g for 10 minutes at 4°C. Plasma was aspirated and stored in polypropylene tubes at −70°C until assayed.

SQ 30,741 Plasma Levels

Five-milliliter samples of arterial blood were collected in iced glass test tubes containing 143 IU heparin and were centrifuged at 2,000 g for 10 minutes at 4°C. Plasma was transferred to polypropylene tubes and stored at −70°C. Plasma concentrations of SQ 30,741 activity were measured with a radioreceptor assay for TxA$_2$-PGH$_2$ receptor ligands using human platelet membranes for receptors and the TxA$_2$-PGH$_2$ receptor antagonist 3H-labeled SQ 29,548 as radioligand. In brief, human platelet membranes were prepared as described by Hedberg et al. and aliquots were stored at −70°C. With C$_{18}$ reversed-phase chromatography, SQ 30,741 was extracted from samples of experimental sheep plasma and sheep plasma spiked with known concentrations of SQ 30,741 that ranged from 0.1 to 2,000 nM. The methanolic eluant was concentrated by Speed-Vac centrifugation and reconstituted in 50 mM Tris-buffered saline plus 0.05% ascorbic acid. Experimental sample extracts were reconstituted with one fifth of the original plasma volume, and an eight-point, 4,000-fold dilution series was prepared in duplicate. All samples were tested for inhibition of specific binding of 3H-labeled SQ 29,548 (5 nM) to human platelet membranes. Sample concentrations were measured relative to the fitted concentration-inhibition standard curve for known SQ 30,741 concentrations. In validating the receptor binding assay for SQ 30,741, we found that there was an average measurement error of 1.2% when SQ 30,741 concentrations inhibited 3H-labeled SQ 29,548 binding between 31% and 70% at concentrations ranging from 50 to 200 nM. Dilutions of experimental sample extracts that inhibited binding between 30% and 70% were used to calculate the original sample concentration of SQ 30,741 based on the concentration of SQ 30,741 detected in the binding assay and correction for the dilution factor. In other unpublished studies, we found that SQ 30,741 activities measured by radioreceptor assay ex vivo were in close agreement with those determined by inhibition of
U 46,619-induced platelet shape change. In clinical studies, SQ 30,741 concentrations measured ex vivo by GC-MS correlated well \( (r=0.90) \) with those determined by radioreceptor assay.

**Other Variables**

Total white blood cell counts were determined by a gas chromatography-mass spectroscopy Coulter counter (Coulter Electronics, Hialeah, Fla.). Hematocrit level was determined at the beginning and after each challenge. Arterial blood gas tensions and pHs were measured with a polarograph (Corning Medical and Scientific, Medfield, Mass.). The activated clotting time of whole blood was measured by a Hemochrom 400D System (International Technidyne, Edison, N.J.)\(^{17}\). The bleeding time was measured by Duke’s method standardized with a template. The back of each sheep’s ear was shaved and sterilized with alcohol. We made an incision with a bleeding time device (Simplate, General Diagnostics, Morris Plains, N.J.), avoiding the surface veins, and we started a timer. Surface blood was blotted with filter paper every 30 seconds until bleeding ceased, and the time required for cessation was recorded.

**In Vivo Experimental Protocols**

This study was approved by the subcommittee on animal research at Massachusetts General Hospital. To avoid studying infected or sick animals, animals were excluded on the basis of the following criteria before each heparin-protamine challenge: 1) a pulmonary artery pressure equal to or greater than 22 mm Hg, 2) a pulmonary artery temperature greater than 40.2°C, and 3) a white blood cell count less than 4,000/mm\(^3\) or greater than 12,000/mm\(^3\). A timed protocol was used to induce the heparin-protamine reactions\(^{8,11}\); after baseline measurements were obtained (−6 minutes), bovine lung heparin (Lyphomed, 200 IU/kg) was administered intravenously as a bolus (−5 minutes). Another set of control measurements was obtained (−1 minute), and then protamine sulfate (2 mg/kg, Eli Lilly, Indianapolis, Ind.) was given as an intravenous bolus 1 minute later (0 minutes). The subsequent sampling and measurement intervals were at 1, 2, 3, 5, 10, and 20 minutes as described elsewhere\(^{11}\). In addition, we recently demonstrated that heparin-protamine challenges could be repeated because the course and magnitude of these reactions did not differ when three challenges were repeated at 90-minute intervals. Thromboxane release into plasma and pulmonary vasoconstriction were reproducible over three sequential heparin-protamine challenges in the same animal\(^{18}\).

Stock solutions of U 46,619 (Upjohn, Kalamazoo, Mich.) were prepared in ethanol at a concentration of 1 mg/ml. We administered an intravenous bolus of U 46,619 at increasing amounts until we obtained a 20-mm Hg increase of mean pulmonary artery pressure. We waited until hemodynamic measurements returned to baseline values before each subsequent drug injection. We used the following U 46,619 doses: 0.0015, 0.015, 0.04, 0.075, 0.15, 0.3, 0.5, 0.75, 1, 1.5, 2.5, 3, 5, 7.5, 10, and 15 µg/kg. Stock solutions of SQ 30,741 (Squibb, Princeton, N.J.) were prepared in ethanol at a concentration of 100 mg/ml. Further dilutions in isotonic saline were adjusted with sodium bicarbonate 8.3% to obtain a pH of 7.5 before administration. The infusion was administered intravenously with a compact infusion pump (Harvard Apparatus, Millis, Mass.).

In three sheep, we evaluated the effect of 2 mg/kg SQ 30,741 on the heparin-protamine reaction (Figure 1). An initial control heparin-protamine reaction was induced. After the hemodynamic values returned to baseline, increasing doses of U 46,619 were administered to establish a control dose–response curve. Ninety minutes after the initial control heparin-protamine reaction, TxA\(_2\)-PGH\(_2\) receptor blockade and a second heparin-protamine challenge were begun as follows (Figure 1): after the −6-minute baseline measurement, we administered the SQ 30,741 i.v. bolus (2 mg/kg) and then a heparin bolus (200 IU/kg) and an infusion of SQ 30,741 (2 mg/kg/hr) (−5 minutes). An intravenous bolus of protamine was administered 5 minutes later (0 minutes), and hemodynamic measurements and blood samples were obtained. By 35 minutes, hemodynamic values had returned to baseline in all experiments, and the animal was rechallenged with increasing doses of U 46,619 during continuation of the infusion of SQ 30,741 (Figure 1). Last, at 90 minutes, a third heparin-protamine challenge was begun during the infusion of SQ 30,741. The SQ 30,741 infusion was discontinued after the last hemodynamic measurement and blood sample were obtained, 20 minutes later (110 minutes).

In seven other sheep, we evaluated the effect of a larger dose of SQ 30,741 on the heparin-protamine reaction. A similar protocol was used; a bolus of 10 mg/kg was followed by an infusion of SQ 30,741 (10 mg/kg/hr) (Figure 1). Because most of the pulmonary vasoconstriction reactions were inhibited by this dose, a fourth heparin-protamine challenge was begun 40 minutes after stopping the infusion of SQ 30,741 to prove that the sheep remained responsive to heparin-protamine challenge. In this study, we measured the bleeding time before and 30 minutes after starting the infusion of 10 mg/kg/hr of SQ 30,741 to assess the antithrombotic activity of SQ 30,741 at this dose. Two sheep did not complete the entire study because they did not fulfill all of the inclusion criteria between the second and third heparin-protamine challenges. One animal developed a fever and a second sheep did not return to hemodynamic baseline values. Thus, the results of a bolus of 10 mg/kg are reported in seven sheep, but measurements during the 10 mg/kg/hr infusion were obtained in five sheep.

Blood was sampled for determination of plasma SQ 30,741 levels at −4 (immediately after bolus administration), 1 (the hemodynamic peak of the
second heparin-protamine reaction), 10, 35 (the beginning of the second U 46,619 challenge), and 95 minutes (the hemodynamic peak of the third heparin-protamine reaction).

**Determination of Fractional TxA<sub>2</sub>-PGH<sub>2</sub> Receptor Antagonism**

We calculated fractional receptor antagonism to correlate the extent of TxA<sub>2</sub>-PGH<sub>2</sub> receptor occupancy by SQ 30,741 with the level of inhibition of the heparin-protamine pulmonary vasoconstriction reaction. This required maintaining a constant plasma concentration of receptor blocker. Therefore, receptor occupancy was calculated during infusion of SQ 30,741 at 2 and 10 mg/kg/hr. The U 46,619 dose-response curves were plotted for both the control and both infusion doses.

In each sheep we evaluated the dose of U 46,619 that induced a 10-mm Hg increase of mean pulmonary artery pressure during the control period and the infusion period of SQ 30,741. U 46,619 dose ratios (DR) for a standardized 10-mm Hg increase of mean pulmonary artery pressure were calculated using the ratios of infusion to control periods. The fractional vascular smooth muscle TxA<sub>2</sub>-PGH<sub>2</sub> receptor blockade by SQ 30,741 (F) was calculated using the formula F is equal to (DR minus 1) divided by DR, which was defined to quantify competitive antagonism for a single receptor from shifts in dose–response curves. Variance of TxA<sub>2</sub>-PGH<sub>2</sub> receptor antagonists.<sup>20,21</sup> The results were averaged at each infusion level (2 mg/kg/hr, n=3 and 10 mg/kg/hr, n=5). Dose ratio and SQ 30,741 concentration data were used to generate a Schild plot, from which pA<sub>2</sub>, slope, and the drug receptor dissociation constant (K<sub>a</sub>) were determined.<sup>22</sup>

**Statistics**

All data are expressed as mean±SEM. Changes of variables over time both within and between study groups were evaluated using multivariate analysis of variance for repeated measures as implemented in the SAS statistical programs (Version 6.0, SAS Institute Inc., Cary, N.C.). Planned contrasts of the results in the control group with the results in the drug-treated group were performed using an F test and two-tailed t tests. Baseline values of each variable in both groups were compared using a one-way analysis of variance and a t test. Planned comparisons were tested individually, and an alpha level of 0.05 was considered significant. The TxB<sub>2</sub> levels were logarithmically transformed before analysis to maintain the assumption of homogeneity of variance.

**Results**

**Arterial Plasma SQ 30,741 Levels**

Mean peak plasma SQ 30,741 levels 1 minute after the bolus (−4 minutes) were 8.3±3.1 µM after the 2 mg/kg dose and 72.0±4.4 µM after the 10 mg/kg dose. Arterial plasma SQ 30,741 levels then rapidly
decreased, and plasma levels were \(3.2 \pm 0.9\) and \(29.5 \pm 4.5\ \mu M\) (with the 2 and 10 mg/kg doses, respectively) at 1 minute when the peak of the heparin-protamine reaction occurred. Stable levels of SQ 30,741 were maintained during the infusion after 35 minutes when the second set of U 46,619 challenges was administered and a third heparin-protamine reaction was induced (Figure 2).

**U 46,619 Dose-Response Curves**

Intravenous injections of increasing doses of the \(\text{TxA}_2-\text{PGH}_2\) receptor agonist U 46,619 produced transient rises of mean pulmonary artery pressure above the baseline value. This effect was measured before (control U 46,619 challenge) and during the constant infusion of the \(\text{TxA}_2-\text{PGH}_2\) receptor antagonist at a rate of 2 mg/kg/hr or 10 mg/kg/hr (U 46,619 rechallenge) (Figure 3). Inhibition of pulmonary vasoconstriction by SQ 30,741 was characterized by a parallel rightward shift of the U 46,619 dose-response curves without any reduction of the maximum hypertensive response. The magnitude of these shifts corresponded to a fourfold and a 17-fold reduction in the potency of U 46,619 during the 2- and 10-mg/kg/hr infusions of SQ 30,741, respectively. This is equivalent to a 79% mean antagonism of \(\text{TxA}_2-\text{PGH}_2\) receptors during the 2- and 10-mg/kg/hr infusion (\(F = 0.79\)) and 94% antagonism of these receptors during the 10-mg/kg/hr infusion of SQ 30,741 (\(F = 0.94\)). At these infusion rates, steady-stage SQ 30,741 levels averaged 1.5 and 8 \(\mu M\), respectively (Figure 2). These concentration and dose ratio data were analyzed by Schild plot,\(^2\) which gave a \(pA_2\) of 6.3 and a slope of \(-1.00\). This analysis yielded an apparent \(K_s\) value of 0.5 \(\mu M\) for antagonism by SQ 30,741 of pulmonary vasoconstrictor responses to U 46,619 in sheep.

**Hemodynamic Effects of Heparin-Protamine Challenge**

There were no changes from baseline values (−6 minutes) of any hemodynamic variable after SQ 30,741 administration (−1 minute). The marked increase of pulmonary artery pressure (1 minute) elicited by protamine reversal of heparin anticoagulation in the absence of \(\text{TxA}_2-\text{PGH}_2\) receptor blockade was significantly reduced by a 2-mg/kg bolus of SQ 30,741 (\(p = 0.009\)) and completely inhibited by a 10-mg/kg bolus (\(p < 0.0001\)) (Table 1 and Figure 4). SQ 30,741 infusion at a rate of 2 mg/kg/hr did not significantly affect the 1-minute peak pulmonary artery pressure caused by protamine injection. SQ 30,741 infusion at 10 mg/kg/hr significantly reduced the pulmonary hypertension (\(p < 0.05\) at 1 minute and \(p < 0.0001\) at 2 minutes after protamine injection). Increased pulmonary occlusion pressure caused by distal pulmonary vasoconstriction and the increase of central venous pressure reflecting right ventricular failure during control heparin-protamine challenges were inhibited after \(\text{TxA}_2-\text{PGH}_2\) receptor blockade (Table 1). Cardiac output and stroke volume did not significantly change with the heparin-protamine reaction either in controls or after a 2- or 10-mg/kg bolus of SQ 30,741. After protamine administration pulmonary vascular resistance increased significantly from baseline values in the control periods (threefold increase) as well as during the 2- and 10-mg/kg/hr infusion periods (twofold increase) (Figure 5). Protamine induced no significant change from baseline values of pulmonary vascular resistance after a bolus of either 2- or 10-mg/kg SQ 30,741 (Figure 5). The 10-mg/kg/hr infusion was stopped immediately after the end of the third heparin-protamine challenge (110 minutes), and a fourth heparin-protamine challenge (control) was induced 40 minutes later. In all sheep, this fourth heparin-protamine challenge elicited acute and profound pulmonary vasoconstriction similar to the first control heparin-protamine challenge (data not shown).

**Plasma Eicosanoid Levels**

Each heparin-protamine challenge was associated with a significant increase of arterial plasma \(\text{TXB}_2\) levels above baseline values. These acute increases were similar in the first heparin-protamine reactions (control), the second heparin-protamine reactions (after a bolus of SQ 30,741), and the third heparin-protamine reactions (during infusion of SQ 30,741), irrespective of the dose (2 and 10 mg/kg) (Figure 6).

There was no change from baseline of plasma \(6\)-\(\text{PGF}_{2\alpha}\) and \(6\text{-keto-PGF}_{2\alpha}\) levels in either control challenges or after drug treatment (data not shown).

**Blood Gas Tensions**

Two minutes after protamine administration, the arterial oxygen tension significantly decreased in control heparin-protamine reactions and returned to baseline values by 10 minutes. In contrast, there was no significant change of \(\text{PaO}_2\) during heparin-protamine challenges with SQ 30,741 pretreatment at all doses. No significant changes of \(\text{PaCO}_2\) or \(\text{pH}\) were measured in control or drug-treated challenges.

**Hematologic Data**

An acute and profound leukopenia always occurred after heparin-protamine challenge. At 1 minute, there were no differences between drug-treated and control studies, and the mean white blood cell concentration was 70% of the baseline value (Figure 7). The leukopenia was transient, always returning to baseline values by 10 minutes.

The hematocrit level was unchanged during the study in all groups.

**Activated Clotting Time and Bleeding Time**

At −6 minutes, in the animals receiving the 2-mg/kg bolus followed by 2-mg/kg/hr infusion of SQ 30,741, the control, bolus, and infusion activated clotting times were 119±9, 120±3, and 104±3 seconds, respectively. Four minutes after heparin administration, the activated clotting time increased to more than 300 seconds in all challenges. Five minutes after protamine administration, the acti-
**Figure 2.** Plot of arterial plasma SQ 30,741 levels. Bolus administration and beginning of the infusion (−5 minutes) were followed by samples at −4, 1, 10, 35, and 95 minutes. Arrows indicate second and third heparin-protamine challenges.

**Figure 3.** U 46,619 dose-response curves. ΔPpa, increase of mean pulmonary artery pressure from baseline value.
vated clotting times returned to the baseline values of 122±3, 116±3, and 109±4 seconds in control, bolus, and infusion periods, respectively.

In the control, 10-mg/kg bolus, and 10 mg/kg/hr infusion periods, the activated clotting times at -6 minutes were 117±5, 116±7, and 104±5 seconds, respectively. The clotting times increased to more than 300 seconds in all sheep after heparin administration. At 5 minutes, protamine reversal of heparin anticoagulation normalized the clotting time in these three groups to 115±5, 110±6, and 114±7 seconds, respectively.

In the five sheep in which the bleeding time was measured before administration of SQ 30,741, bleeding time averaged 4.8±0.5 minutes. After administration of a 10-mg/kg bolus of SQ 30,741 and a 10-mg/kg/hr infusion, the bleeding time at 30 minutes in the same animals was 4.3±0.3 minutes (no difference from control period).

**Discussion**

The major result of this study is that the pulmonary hypertension (Figure 4) and hypoxemia (Table 2) associated with the heparin-protamine reaction in sheep may be completely prevented by the specific TxA2-PGH2 receptor blocker SQ 30,741. In contrast, the transient leukopenia (Figure 7) associated with this drug interaction is not altered by competitive antagonism of TxA2-PGH2 receptors. Thus, thromboxane appears to be the chief mediator causing pulmonary vasoconstriction induced by the heparin-protamine reaction in sheep. Furthermore, thromboxane release into plasma was similar when heparin-protamine challenges were repeated at 90-minute intervals (Figure 6) as reported in another study from our laboratory demonstrating that the course and magnitude of plasma thromboxane increase and pulmonary hypertension were not altered when heparin-protamine challenges were repeated at short-term intervals.18 Pulmonary vascular smooth muscle receptor blockade requires a high degree of TxA2-PGH2 receptor antagonism because 80% saturation of receptors was ineffective and because 94% occupancy of TxA2-PGH2 receptors by SQ 30,741 was necessary to reduce significantly the mean peak pulmonary artery and pulmonary occlusion pressures and pulmonary vascular resistances. Complete prevention of pulmonary vasoconstriction was associated with higher SQ 30,741 plasma levels after intravenous administration of a 10-mg/kg bolus.

**Mediators in the Heparin-Protamine Reaction**

A number of studies have previously demonstrated complement activation by heparin-protamine challenge.23,24 Because complement-derived anaphylatoxins such as C3a and C5a can cause pulmonary vascular smooth muscle contraction, they may have directly mediated the pulmonary vasoconstriction induced by heparin-protamine reactions. However, our data demonstrate the exclusive vasoconstrictor role of TxA2-PGH2 receptor agonists in the heparin-

**Table 1: Hemodynamic Measurements**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ppa (mm Hg)</th>
<th>Pvo (mm Hg)</th>
<th>Pv (mm Hg)</th>
<th>Ppe (mm Hg)</th>
<th>HR (beats/min)</th>
<th>CO (l/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-6 min 1 min</td>
<td>-6 min 1 min</td>
</tr>
<tr>
<td>Bolus SQ 30,741 (2 mg/kg)</td>
<td>17.5±1.7</td>
<td>34.3±1.2*</td>
<td>25.5±1.8</td>
<td>10±0.1</td>
<td>102±0.12</td>
<td>5.0±0.2</td>
</tr>
<tr>
<td>Bolus SQ 30,741 (10 mg/kg)</td>
<td>19.0±1.3</td>
<td>23.3±1.4</td>
<td>10.5±0.6</td>
<td>10±0.3</td>
<td>100±0.11</td>
<td>5.3±0.6</td>
</tr>
<tr>
<td>Infusion SQ 30,741 (2 mg/kg/hr)</td>
<td>15.0±0.7</td>
<td>36.7±2.2</td>
<td>10.3±0.3</td>
<td>10±0.3</td>
<td>106±0.10</td>
<td>5.3±0.6</td>
</tr>
<tr>
<td>Control (n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-6 min 1 min</td>
<td>-6 min 1 min</td>
</tr>
<tr>
<td>Bolus SQ 30,741 (10 mg/kg/hr)</td>
<td>16.0±0.8</td>
<td>24.2±1.9</td>
<td>10.4±0.6</td>
<td>10±0.3</td>
<td>107±0.12</td>
<td>5.3±0.6</td>
</tr>
</tbody>
</table>

Ppa, pulmonary arterial pressure; Pvo, pulmonary venous pressure; Pv, central venous pressure; Ppe, systemic blood pressure; CO, cardiac output; HR, heart rate.

*Value at minute significantly differs from baseline value (-6 minutes) at the peak (1 minute) is significantly different than change from -6 to 1 minute in controls.
protamine reaction in sheep. In clinical studies, the release of complement anaphylatoxins has not always identified patients with pulmonary vasoconstriction: an increase of complement fragments C3a and C4a into arterial plasma occurred immediately after protamine injection in all patients with or without pulmonary artery hypertension.\textsuperscript{3,25,26} The C5a fragment, a potent leukoaggregating and leukoactivating anaphylatoxin, is probably responsible for the consistent leukopenia measured in heparin-protamine reactions in humans as well as animals.\textsuperscript{27} This was partially confirmed by Fehr and Rohr,\textsuperscript{28} who demonstrated that administration of cobra venom factor to rabbits, associated with depletion of complement, prevented leukopenia during subsequent heparin-protamine challenges. Thus, although complement activation and pulmonary leukosequestration are consistent events during the heparin-protamine reaction, they do not appear to play a direct role in producing pulmonary vasoconstriction.

PGF\textsubscript{2\alpha} is a far-less potent pulmonary vasoconstrictor\textsuperscript{29} than is thromboxane A\textsubscript{2}. There is little evidence of a role for this prostaglandin in the human heparin-protamine reaction. Others have reported a concomitant rise of PGF\textsubscript{2\alpha} with thromboxane after protamine neutralization of heparin anticoagulation in the pig.\textsuperscript{9} In our sheep study, plasma PGF\textsubscript{2\alpha} levels did not increase either during control heparin-protamine challenges or during heparin-protamine challenges after TxA\textsubscript{2}-PGH\textsubscript{2} receptor blockade with SQ 30,741. Morel et al\textsuperscript{8} measured an increase of PGF\textsubscript{2\alpha} only during heparin-protamine challenges of sheep pretreated with a thromboxane synthetase inhibitor. It is likely that this inhibitor redirected the precursor PGH\textsubscript{2} to the synthesis of prostaglandins other than thromboxanes including PGF\textsubscript{2\alpha}. This acute increase of PGF\textsubscript{2\alpha} may have been responsible for the increase of pulmonary artery pressure measured in these sheep. Our results indicate that PGF\textsubscript{2\alpha} plays no major role in the pulmonary vasoconstriction induced by the heparin-protamine reaction.

Evaluation of the TxA\textsubscript{2}-PGH\textsubscript{2} Receptors

To address the role of eicosanoids in pulmonary artery hypertension induced by heparin-protamine reactions, previous studies used nonspecific probes such as cyclooxygenase inhibitors\textsuperscript{8,9} and thromboxane synthetase inhibitors.\textsuperscript{8} The inhibitory effect of SQ 30,741 on pulmonary artery pressure was specifically due to the blockade of TxA\textsubscript{2}-PGH\textsubscript{2} receptors because this agent does not influence prostaglandin synthetic enzymes such as thromboxane synthetase or prostacyclin synthetase.\textsuperscript{21} Our data validate the hypothesis that TxA\textsubscript{2}-PGH\textsubscript{2} receptor activation causes pulmonary vasoconstriction during the heparin-protamine reaction. The prostaglandin endoperoxide analogue

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Plot of time course of mean pulmonary artery pressure (Ppa) during heparin-protamine (H-P) reactions. Top panel: Effect of SQ 30,741 after a bolus of 2 mg/kg or an infusion of 2 mg/kg/hr on the Ppa changes induced by protamine reversal of heparin anticoagulation. Bottom panel: Effect of SQ 30,741 after a bolus of 10 mg/kg or an infusion of 10 mg/kg/hr. *p<0.05, value after bolus administration of SQ 30,741 differs from that in control period; †p<0.05, value during constant infusion of SQ 30,741 differs from that in control period.}
\end{figure}
U 46,619 mimics the effect of thromboxane on smooth muscle and appears to share the same receptor.\textsuperscript{2,30} We evaluated the ability of SQ 30,741 to inhibit competitively pulmonary vascular TxA\textsubscript{2}-PGH\textsubscript{2} receptors by its effect on the U 46,619 dose-response curve. Saturation of 94\% of the TxA\textsubscript{2}-PGH\textsubscript{2} receptors with SQ 30,741 significantly reduced the pulmonary artery hypertension occurring after protamine reversal of heparin anticoagulation. This level of antagonism was obtained by infusing SQ 30,741 at a rate of 10 mg/kg/hr and producing a mean SQ 30,741 plasma level of 8 \( \mu \text{M} \). When the mean SQ 30,741 level in sheep plasma was threefold higher, it produced total inhibition of pulmonary vasoconstriction induced by the heparin-protamine reaction (6 minutes after a 10-mg/kg bolus of SQ 30,741 was administered; 1 minute; Figure 2). The short plasma half-life of SQ 30,741 and the rapid decrease of SQ 30,741 plasma levels suggest that complete inhibition of the pulmonary vasoconstriction can be obtained with a smaller dose injected only a few seconds before protamine administration.

The conclusion that SQ 30,741 is a weak antagonist of the pulmonary artery hypertension response to both U 46,619 and the heparin-protamine reaction in sheep is supported by comparison to in vivo studies of SQ 30,741 effects on rat and dog mesenteric vascular responses to U 46,619. In rats, 3 and 30 mg/kg/hr i.v. infusions of SQ 30,741 produced dose ratios of 202±53 and 1,260±580, respectively.\textsuperscript{31} In dogs, a 1-mg/kg/hr infusion produced a dose ratio of 18.5±2.\textsuperscript{32} This response is equivalent to the dose ratio produced by a 10-fold higher infusion rate in our sheep study. SQ 29,548, which is a TxA\textsubscript{2}-PGH\textsubscript{2} receptor antagonist structurally related to SQ 30,741, was also surprisingly weak as an antagonist of U 46,619-induced pulmonary artery hypertension in sheep.\textsuperscript{13}

Schild analysis of the U 46,619 dose ratio and SQ 30,741 concentration data yields a pA\textsubscript{2} of 6.3 with a slope of -1.00 and a \( K_b \) value of 0.50 \( \mu \text{M} \). The slope of unity is consistent with SQ 30,741 being a competitive TxA\textsubscript{2}-PGH\textsubscript{2} receptor antagonist. The apparent drug receptor dissociation constant (\( K_b \) value) is particularly useful for comparisons of drug potency among preparations. The apparent \( K_b \) value in the sheep is considerably higher than \( K_b \) values measured in vitro for inhibition by SQ 30,741 of platelet responses to U 46,619 in dogs (0.11 \( \mu \text{M} \)),\textsuperscript{15} monkeys (0.06 \( \mu \text{M} \)),\textsuperscript{21} and humans (0.09 \( \mu \text{M} \)).\textsuperscript{21} Whether tissue or species differences account for the range of SQ 30,741 potency in these preparations cannot be determined from our study. However, Schumacher et al\textsuperscript{21} demonstrated that comparable exposure to SQ 30,741 caused the same magnitude of antagonism of platelet and mesenteric artery smooth muscle TxA\textsubscript{2}-PGH\textsubscript{2} receptors in monkeys. Furthermore, Ogletree and Allen\textsuperscript{33} have demonstrated clear interspecies
differences in smooth muscle TxA2-PGH2 receptors. Thus, affinity differences are likely more related to species than to tissue differences of TxA2-PGH2 receptors, implying that the $K_i$ in sheep is greater than that in humans. Possibly, a smaller bolus or infusion dose would provide greater levels of pulmonary artery smooth muscle blockade in humans. However, plasma TxB2 levels in the clinical heparin-protamine reaction in humans are on average higher than TxB2 levels in the experimental heparin-protamine reaction in sheep.

The TxA2-PGH2 receptor blockade produced by SQ 30,741 is reversible, and pulmonary vasoconstriction induced by reversing heparin anticoagulation with protamine was completely restored 40 minutes after cessation of SQ 30,741 administration. The short duration of action of SQ 30,741 is sufficient to inhibit the heparin-protamine reaction without prolonged antithrombotic effects. We observed neither hemodynamic effects nor other side effects during or after administration of this TxA2-PGH2 receptor inhibitor to awake sheep.

Friedhoff et al. reported a small, but measurable, increase of the template bleeding time in healthy humans with 94% occupancy of the platelet TxA2-PGH2 receptors by another thromboxane antagonist (SQ 28,668). We found no detectable increase of the bleeding time during an infusion of SQ 30,741 at the highest dose level, corresponding to 94% occupancy of pulmonary vascular smooth muscle TxA2-PGH2 receptors in sheep.

**Source of Thromboxane**

Thromboxane release is central to the pulmonary vasoconstriction induced by the heparin-protamine reaction in sheep, but the precise cellular source of thromboxane remains unknown. We recently demonstrated that sheep platelets were not involved in this reaction because both acute pulmonary artery hypertension and thromboxane release occurred at a similar magnitude in control and 99% platelet-depleted sheep challenged with heparin and protamine. Pulmonary artery hypertension has been reported after infusion of heparin-protamine complexes into isolated cat lungs perfused with an acellular dextran containing perfusate, which suggests that blood elements are not involved, and resident lung cells may be responsible for the release of thromboxane. Endothelial cells or the recently described pulmonary intravascular macrophages, which are abundant in certain species such as the sheep and pig, may release the thromboxane.

**Adverse Reactions After Protamine Administration in Patients**

Numerous mediators are released after protamine reversal of heparin anticoagulation, but plasma his-
tamine levels do not increase in either humans or sheep demonstrating acute pulmonary artery hypertension.\textsuperscript{1,3,7} True IgE-associated anaphylaxis to protamine has been demonstrated in patients with protamine-insulin-dependent diabetes mellitus, but anaphylaxis is far less common than pulmonary artery hypertension.\textsuperscript{6} Anaphylactic reactions are associated with histamine release. Thus, the human and sheep acute pulmonary vasoconstrictor response is associated with a different mediator profile and appears to be a nonimmunological event. At present, no predictive factor has been identified to indicate patients at high risk for acute pulmonary vasoconstriction. The onset of acute pulmonary artery hypertension with circulatory collapse, bronchoconstriction, and hypoxemia requires rapid symptomatic treatment. The optimal therapy is still unclear, but epinephrine and isoproterenol have been administered to restore adequate systemic blood pressure in patients with the heparin-protamine reaction.\textsuperscript{1,3,4} No safe prophylactic therapy has been available because hemorrhagic and renal side effects of cyclooxygenase-inhibiting drugs do not allow their routine prescription, especially before cardiopulmonary bypass.\textsuperscript{37,38} Furthermore, cyclooxygenase inhibitors may shunt the arachidonic acid cascade from the cyclooxygenase to the lipoxygenase pathway, causing the release of leukotrienes. We recently demonstrated that reversal of low molecular weight heparin with a different heparin antagonist (polybrene) did not cause thromboxane release and pulmonary hypertension in sheep.\textsuperscript{18} This combination may provide safer

**Figure 7.** Plots of time course of white blood cell counts (WBC) during heparin-protamine (H-P) reactions. Top panel: No effect of SQ 30,741 occurred with either a bolus of 2 mg/kg or as an infusion of 2 mg/kg/hr on the leukopenia induced by protamine reversal of heparin anticoagulation. Bottom panel: No effect of SQ 30,741 occurred after a bolus of 10 mg/kg. *p<0.05, value during constant infusion of SQ 30,741 at 10 mg/kg/hr differs from that in control period.

**Table 2.** Blood Gas Tensions and pH Data

<table>
<thead>
<tr>
<th></th>
<th>PaO\textsubscript{2} (mm Hg)</th>
<th>PaCO\textsubscript{2} (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-1 min 2 min</td>
<td>-1 min 2 min</td>
<td>-1 min 2 min</td>
</tr>
<tr>
<td>Control (n=3)</td>
<td>107±3 83±7*</td>
<td>42±5 49±6</td>
<td>7.40±0.01 7.37±0.03</td>
</tr>
<tr>
<td>Bolus SQ 30,741 (2 mg/kg)</td>
<td>123±5 116±3†</td>
<td>41±6 36±4</td>
<td>7.40±0.02 7.43±0.01</td>
</tr>
<tr>
<td>Infusion SQ 30,741 (2 mg/kg/hr)</td>
<td>90±10 87±6†</td>
<td>36±5 37±3</td>
<td>7.42±0.01 7.43±0.02</td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>96±4 83±3*</td>
<td>40±1 42±1</td>
<td>7.40±0.01 7.39±0.01</td>
</tr>
<tr>
<td>Bolus SQ 30,741 (10 mg/kg)</td>
<td>97±3 98±4†</td>
<td>41±1 40±2</td>
<td>7.40±0.01 7.40±0.01</td>
</tr>
<tr>
<td>Infusion SQ 30,741 (10 mg/kg/hr)</td>
<td>94±2 95±4†</td>
<td>40±2 41±2</td>
<td>7.40±0.01 7.40±0.01</td>
</tr>
</tbody>
</table>

*Value at 1 minute significantly differs from baseline value (-6 minutes) within the same group; tchange from baseline (-6 minutes) to peak value (1 minute) significantly differs from the change from -6 to 1 minute in controls.
neutralization of anticoagulation in patients, especially after cardiopulmonary bypass. Thromboxane receptor blockade appears to offer yet another promising strategy for preventing the occasional but catastrophic pulmonary artery hypertension occurring in patients after heparin neutralization by protamine sulfate. The competitive Txa2-PGH2 receptor antagonist SQ 30,741 is very effective in sheep, and its short half-life is an advantage in preventing the acute heparin-protamine reaction. This drug may be infused into patients immediately before protamine administration without prolonged antithrombotic effects. Further studies are needed to evaluate both approaches in preventing heparin-protamine reactions in humans to accurately define their effectiveness and tolerance.

Acknowledgments

We thank David Hoaglin, PhD, and Kevin Stanek, BS, for statistical assistance, and Cynthia Fitzgibbon, BS, for technical assistance.

References


**KEY WORDS** • pulmonary vasoconstriction • hypoxemia • prostaglandins • thromboxane receptor antagonists • heparin • protamine • SQ 30,741
Thromboxane receptor blockade prevents pulmonary hypertension induced by heparin-protamine reactions in awake sheep.

G Montalescot, E Lowenstein, M L Ogletree, E M Greene, D R Robinson, K Hartl and W M Zapol

_Circulation_. 1990;82:1765-1777
doi: 10.1161/01.CIR.82.5.1765

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/82/5/1765

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/