Thromboxane Receptor Blockade (SQ 29,548) in Group B Streptococcal Toxin Challenge in Young Lambs¹

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ABSTRACT. Early-onset neonatal group B β -hemolytic streptococcus (GBS) infection exhibits pathophysiologic characteristics of a toxic shock syndrome, in which a cascade of inflammatory mediators are involved. Thromboxane A₂ (TXA₂) is thought to play an important role as a mediator of the pulmonary response to GBS toxin, because high lung lymph concentrations of a TXA₂ metabolite have been observed after GBS toxin injections in sheep. The aim of this study was to evaluate the effects of a selective antagonist of the TXA₂-prostaglandin endoperoxide receptor (SQ 29,548). Six unanesthetized young lambs, each serving as its own control, were given SQ 29,548 or vehicle control followed by GBS toxin challenge. Hemodynamic and lung function (lung mechanics, lung volume, ventilation) responses were followed for 5 h. When compared with the control studies, treatment with SQ 29,548 significantly altered the response to GBS toxin. SQ 29,548 reduced the increase in pulmonary and systemic vascular resistance, improved cardiac output and stroke volume, improved dynamic lung compliance but not airway resistance, and improved oxygenation. The attenuating effect of SQ 29,548 was most pronounced during the first phase of toxin response (15–90 min after toxin infusion), but significant treatment effects were also seen during the second phase (120–300 min after toxin infusion). This study demonstrates that TXA₂ is an important mediator of the response to GBS toxin and is responsible for hemodynamic and lung function changes. Thromboxane receptor blockade may offer a potential therapeutic approach to infants with severe early-onset GBS sepsis. (*Pediatr Res* 35: 571–579, 1994)

PG, prostaglandin
P_{LA}, left atrial pressure
P_{PA}, pulmonary arterial pressure
PVR, pulmonary vascular resistance
R_L, lung resistance
TX, thromboxane

Abbreviations

C_{dyn}, dynamic compliance
CO, cardiac output
CVP, central venous pressure
FRC, functional residual capacity
GBS, group B β -hemolytic streptococcus
MSP, mean systemic pressure
NC, nitrogen clearance
P_{cw}, capillary wedge pressure

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Early-onset infection by GBS in the newborn infant is still a potentially life-threatening condition with high mortality (1). This disease is characterized by severe respiratory distress, pulmonary hypertension, lung injury with increase in pulmonary capillary permeability, lung edema, progressive respiratory failure, circulatory collapse, and death (2, 3). Early-onset GBS infection exhibits pathophysiologic characteristics of a toxic shock syndrome, in which a cascade of different inflammatory mediators such as prostaglandins, platelet-activating factor, protease, oxygen radicals, and cytokines are engaged (4). An exotoxin from culture medium of GBS type III obtained from infants with early-onset GBS infection has been isolated and characterized as the agent primarily involved in this condition (5, 6). GBS toxin is a polysaccharide with an approximate molecular mass of 200 000 D. Previous studies have suggested that mannose phosphodiester groups constitute functionally active sites on the toxin and that, when injected into sheep, GBS toxin accumulated predominately in the lung (7). Intravenous infusion of picomole amounts of GBS toxin has been shown to induce inflammation also in tumor neovasculature in mice, which like humans are susceptible to GBS as neonates (8).

The isolation of the GBS toxin has made it possible to develop an animal model to study the pathophysiologic events in this disease. This has previously been done by our group in both sheep (9, 10) and young lambs (2, 10–12). A biphasic response to the toxin has been found. During the first phase, there is increased lung resistance, decreased dynamic lung compliance, increased ventilation, hypoxemia, pulmonary hypertension, and fever. The second phase is characterized by less prominent changes in these variables together with increased lung lymph protein clearance compatible with increased vascular permeability. An increase in lung lymph concentration of TXB₂, which is a stable metabolite of TXA₂, coincides with the first phase of the toxin reaction (13, 14). Preventing TXA₂ formation by indomethacin abolished pulmonary hypertension during the first phase, implying that TXA₂ might be one of the mediators responsible for the severe hemodynamic and lung function changes seen in the GBS toxic shock syndrome (13).

The advantage of using a bolus injection of GBS toxin as done in our lamb model is that it makes it possible to better study the different pathophysiologic components participating in the GBS

sepsis reaction. In fulminant human sepsis or in animal models using infusion of GBS bacteria, all these pathophysiologic events are going on at the same time, because it can be suspected that there is a continuous production of toxin by the GBS bacteria.

The aims of this study were to perform a comprehensive determination of the role of TXA₂ as a mediator of the GBS toxin response and to test the potential pharmacologic effects of a selective antagonist of the TXA₂-prostaglandin endoperoxide receptor, [1S-[1 α ,2 β (5Z),3 β ,4 α]-7-[3-[(2-[(phenylamino)carbonyl]hydrazino)methyl]-7-oxabicyclo(2.2.1)hept-2-yl]-5-heptenoic acid (SQ 29,548) (15), during GBS toxin challenge in the unanesthetized young lamb model. The study was designed to investigate the interrelationship between ventilatory and circulatory pathophysiology leading to respiratory insufficiency in GBS sepsis in a way not previously done.

MATERIALS AND METHODS

Animal preparation. Six full-term lambs were instrumented 1 to 2 wk after birth. Polyvinylchloride catheters (1.0 mm inner diameter, 1.8 mm outer diameter) were placed in a femoral artery and a tarsal vein. A catheter was also placed in the right atrium via the right brachial vein for measurement of CVP. Through a left thoracotomy, catheters were inserted directly into the main pulmonary artery and left atrium, and an electromagnetic flow cuff (C and C Instruments, Culver City, CA) for measurement of CO was placed around the main pulmonary artery. The lambs were then allowed to recover for an average of 3 to 4 d, after which two pleural balloons (Silastic envelopes) for measurement of pleural pressure were placed in the right pleural space through a small thoracotomy. A tracheostomy was performed and a Swan-Ganz sheath was placed in an external jugular vein. After the operations, all lambs received gentamicin (6 mg/kg/d) and carbenicillin (150 mg/kg/d) during the whole study period. Lambs were allowed to recover for 3 to 4 d before the first study was performed. The second study was performed 4 to 5 d later.

The average age of the lambs was 22 d (range 18–28 d) during the control study and 23 d (range 17–37 d) during the SQ 29,548 study. Their mean weight was 8.9 kg (range 5.9–12.0 kg) during the control study and 8.7 kg (range 6.6–10.9 kg) during the SQ 29,548 study.

Experimental protocol. Six paired GBS toxin experiments were performed in alternating order, with each lamb serving as its own control. The unanesthetized lambs were standing and spontaneously breathing air in a loose sling. SQ 29,548 or vehicle control was prepared by dissolution in 95% ethanol (10 g/L) and addition of 2 mM Na₂CO₃ until the final concentration of SQ 29,548 1 g/L was obtained. After at least 1 h of baseline measurement, SQ 29,548 or vehicle control was started as an i.v. bolus of 2 mg/kg followed by a continuous infusion of 2 mg/kg/h. This infusion rate of SQ 29,548 had previously been shown to block TXA₂ receptors during *Escherichia coli* endotoxin challenge in sheep (16). After another hour of baseline measurements, 38 pmol of GBS toxin per kilogram mixed in 30 mL of normal saline was infused i.v. during 12 min. Each animal received an identical dose of GBS toxin for the SQ 29,548 and control experiment. The response to GBS toxin was monitored for 5 h after the beginning of GBS toxin infusion.

Hemodynamic measurements were performed every 15 min from the beginning of GBS toxin infusion until 1.5 h and at 30-min intervals thereafter. Lung mechanics and lung volume measurements were performed at 15, 30, 38, 45, and 60 min during the first hour and at 30-min intervals between 1 and 5 h after the start of GBS toxin infusion. Ventilatory parameters were measured every 30 min until 2 h and thereafter every hour. Arterial PO₂, PCO₂, pH, and body temperature were measured at the same intervals. Measurements of white blood cell count, granulocytes, lymphocytes, and packed red cell volume (hematocrit) were obtained at 1, 1.5, 2, 3, 4, and 5 h. Measurements of

TXB₂ and 6-keto-PGF_{1 α} in plasma were made hourly during the study. The animals were killed after completion of the studies.

The research protocol was approved by the Vanderbilt University Animal Care Committee.

Hemodynamic measurements. Mean pressures were continuously recorded from the pulmonary artery (P_{PA}), left atrium (P_{LA}), right atrium (CVP), and aorta (MSP) by pressure transducers (model 1290A, Hewlett-Packard, Andover, MA). P_{CW} was measured periodically through a 5 Fr Swan-Ganz catheter placed in wedge position in the pulmonary artery. CO was continuously monitored via the electromagnetic flow cuff around the main pulmonary artery (Narcomatic flowmeter model RT-510, Narco Biosystems, Inc., Houston, TX). CO was normalized to body weight, and the normalized CO was used to calculate vascular resistance. Total PVR was calculated as (P_{PA} – P_{LA})/CO. PVR confined to the precapillary bed was calculated as (P_{PA} – P_{CW})/CO and that confined to the postcapillary bed as (P_{CW} – P_{LA})/CO. Systemic vascular resistance was calculated as (MSP – CVP)/CO.

Lung function measurements. The technique for measurements and calculations of lung function in this study has previously been evaluated and described in detail in lambs (17) and newborn infants (18). Ventilatory flow in the cuffed ET tube inserted through the tracheostoma was measured by a heated pneumotachograph (Hewlett-Packard, No. 0) connected to a pressure transducer (model MP 45-1-871, ± 2 cm H₂O, Validyne Engineering Corp., Northridge, CA) and a carrier demodulator (model CO 1914, Validyne Engineering Corp.). Transpulmonary pressure was measured between the airway (the proximal part of the ET tube) and the pleural balloon (Silastic envelope) in the right pleural space by a pressure transducer (model MP 45-1-871, ± 50 cm H₂O) and a carrier demodulator. Nitrogen concentration in breathing gas was measured continuously in the proximal part of the ET tube by a nitrogen analyzer (model 505 Nitrolyzer, Med Science Electronic, Inc., St. Louis, MO). A system of tubing (dead space 7 mL) was connected to the lamb's ET tube with a continuous bypass flow with a positive end-expiratory pressure of 4 cm H₂O. Multiple-breath nitrogen washout tests were performed during 100% oxygen breathing to measure FRC and ventilatory parameters. The ventilatory flow, pressure, and nitrogen signals were all sampled into a portable computer unit (NEC, Portable Powermate, NEC Technology, Boxborough, MA) and a 12-bit analog-digital converter (model DT 280, Data Translation, Marlborough, MA) with a sample rate of 200 Hz for further calculations. Lung C_{dyn} and R_i were calculated from the sampled ventilatory flow and transpulmonary pressure signals. The computerized system for calculation of lung mechanics has previously been described in detail (19, 20).

FRC was calculated by dividing the expired lung nitrogen volume by the end tidal nitrogen concentration before the initiation of oxygen breathing. FRC was normalized to body weight. From the nitrogen lung volume elimination curve, a dilution factor that made it possible to estimate ventilatory dead space and alveolar ventilation could be derived. A lung model was used to describe the nitrogen elimination pattern (distribution of ventilation) by fitting one, or the sum of two or three, exponential equations to the lung nitrogen elimination curve (21). The number of equations giving the best least-square fit to the original washout curve was chosen as the best description of the nitrogen elimination pattern (distribution of ventilation). Not all nitrogen washout curves could be satisfactorily described by this exponential lung model, such as when the exponential estimate deviated too much from the original lung nitrogen elimination curve.

The gas mixing efficiency of the ventilatory system was calculated as an NC index. NC index was defined as the ventilatory volume necessary to dilute the nitrogen lung volume from 90 to 10% of its total value, divided by the FRC.

Laboratory analysis. Arterial blood gas determinations were made using a model 158 pH/blood gas analyzer (Ciba Corning

Diagnostic Corp., Medfield, MA). White blood cells were counted with a model ZBI Coulter counter (Coulter Electronics, Hialeah, FL) specifically adjusted for counting sheep cells. A blood smear was stained with Wright's stain to determine the number of granulocytes and lymphocytes per 100 white cells. Analysis of TXB₂ and 6-keto-PGF_{1 α} in plasma was performed in duplicate using an enzyme immunoassay method (22, 23) with MAb, anti-IgG antibodies, enzymatic tracers, and specific eicosanoid antisera obtained from Cayman Chemicals (Ann Arbor, MI).

TX agonist. Before each study was completed, the efficacy of SQ 29,548 as a TX receptor antagonist was tested by an i.v. injection of the TX agonist 9,11-dideoxy-11 α ,9 α -epoxymethano-PGF_{2 α} (U 46,619, Upjohn Company, Kalamazoo, MI) in a dose of 5 μ g/kg. An average increase in P_{PA} of 2.5 mm Hg occurred when the agonist was given during SQ 29,548 treatment, whereas an average increase in P_{PA} of 54 mm Hg was observed in the control study.

Isolated effect of SQ 29,548. The isolated effect of SQ 29,548 on all physiologic variables was tested in three separate lambs that were 18 to 25 d old. These lambs were instrumented in the same way as those included in the study. We found no isolated effect by SQ 29,548 on any of the measured variables during a 6-h study period, preceded by 2-h baseline measurements, except for a marginal decline in total PVR.

Statistics. We assessed the effect of SQ 29,548 within a period by using a repeated measures analysis of variance when all data were available. The repeated measures analysis of variance combines all data for a period into a single statistical test of the effect of treatment in a period. When some data were missing, we averaged the difference between SQ 29,548 and control for each time available for an animal and then tested whether this average difference was significantly different from zero. This is algebraically equivalent to the test used in the repeated measures analysis of variance when data are complete. Repeated measures analysis of variance was used to determine overall treatment effect during the whole 5-h study period and during each phase of the GBS toxin reaction separately.

To show how results changed from baseline within a treatment arm or between groups at a single time point, we used paired *t* tests and show these results in the figures and tables. The results of these tests are intended to help clarify when differences between groups are most pronounced. The results of the individual tests at specific time points are not used in determining whether there is an overall difference between the two treatment arms.

We claim an overall treatment effect only if repeated measures analysis of variance (or the equivalent single test of treatment effect when data were incomplete) showed a statistically significant difference at *p* < 0.05 for the whole 5-h study period. A significant effect in an individual phase of the GBS toxin response is based on the repeated measures analysis of variance (or equivalent test) for that phase.

RESULTS

Biphasic response. In general, the vascular changes after GBS toxin infusion showed a biphasic response where the phases showed different characteristics. The first phase of the reaction (0.25 to 1.5 h) reached its peak 30 to 60 min after the beginning of toxin infusion. The second phase of the reaction (2 to 5 h) reached its maximum after 2 to 3 hours.

P_{PA}, P_{CW}, and P_{LA} (Fig. 1). P_{PA} and P_{CW} in the control study showed a typical biphasic response with a significant increase in pressure compared with baseline. The first phase showed the highest pressure increase but with shorter duration compared with the second phase. There was a significant overall treatment effect by SQ 29,548 during the whole study period (*p* < 0.01 repeated measures analysis of variance), mostly because of the significant treatment effect in the first phase (*p* < 0.001, not adjusting for possible baseline difference; *p* < 0.01, adjusted for

Fig. 1. A, P_{PA}; B, P_{CW}; C, P_{LA} in six young lambs during a control study and during SQ 29,548 treatment. Values are mean $\pm$ SEM. *, Significant difference (paired *t* test, *p* < 0.05) between individual time points and baseline value at zero time. +, Significant difference (paired *t* test, *p* < 0.05) between groups at an individual time point. Repeated measures analysis of variance showed a significant treatment effect on P_{PA} (*p* < 0.01 overall; *p* < 0.0001 first phase) and P_{CW} (*p* < 0.05 first phase).

baseline). The treatment effect during the second phase was statistically significant with use of the unadjusted baseline (*p* < 0.05). If adjustment was made for the possible difference at baseline, this effect was no longer statistically significantly different. Pulmonary P_{CW} was significantly lower with SQ 29,548 only during the first phase (*p* < 0.05, repeated measures analysis of variance). P_{LA} did not change significantly over time, nor was the response different in the presence of SQ 29,548.

Heart rate, CO, and stroke volume (Fig. 2). There was no overall treatment effect on heart rate during the study on the basis of the repeated measures analysis of variance. However, in the control study the animals showed a significant increase in heart rate late in the first phase and during the second phase of the reaction, whereas after SQ 29,548 treatment the animals

Fig. 2. A, Heart rate; B, CO; C, stroke volume in six young lambs during a control study and during SQ 29,548 treatment. Values are mean $\pm$ SEM. *, Significant difference (t test, $p < 0.05$) between individual time points and baseline value at zero time. +, Significant difference (paired t test, $p < 0.05$) between groups at an individual time point. Repeated measures analysis of variance showed a significant treatment effect on stroke volume ($p < 0.05$ overall; $p < 0.01$ first phase; $p < 0.05$ second phase).

responded sooner with modest tachycardia during both the first and second phase of the toxin reaction. CO decreased significantly compared with baseline 45 min after the beginning of the toxin infusion and remained significantly decreased until after 3 h in the control study. After SQ 29,548 treatment, the animals not only managed to preserve their CO but also showed a significant increase during the first phase of the reaction. The overall treatment effect on CO by SQ 26,548 during the whole

study was marginally significant ($p = 0.06$, repeated measures analysis of variance). The decrease in CO in the control study was mainly caused by a significant reduction in stroke volume, which stayed low during the whole reaction. During SQ 29,548 treatment, the stroke volume was largely preserved. The difference in stroke volume was significant over the entire period ($p < 0.05$), in particular during the first phase ($p < 0.01$; second phase, $p < 0.05$).

MSP, CVP, and vascular resistance (Fig. 3). MSP was higher in the control study compared with the SQ 29,548 study during the first phase of the reaction ($p < 0.05$) but similar in the second

Fig. 3. A, Mean systemic arterial pressure; B, CVP; C, systemic vascular resistance for five lambs during a control study and during SQ 29,548 treatment. Values are mean $\pm$ SEM. *, Significant difference (paired t test, $p < 0.05$) between individual time points and baseline value at zero time. +, Significant difference (paired t test, $p < 0.05$) between groups at an individual time point. Repeated measures analysis of variance showed significant treatment effects on mean systemic arterial pressure and systemic vascular resistance during the first phase after GBS toxin ($p < 0.05$).

phase. No major changes were seen in CVP. Only at 30 min after the start of the toxin infusion was the CVP significantly higher in the control study compared with the SQ 29,548 study.

Systemic vascular resistance increased significantly in the control study but not after SQ 29,548 treatment. The two studies were significantly different in the first phase ($p < 0.05$).

PVR (Fig. 4). The major treatment effects of SQ 29,548 were seen on the PVR. In the control study, total PVR significantly increased compared with baseline during both the first and

second phase of the reaction. The increase in resistance was higher with shorter duration during the first phase compared with the second phase. Although PVR also increased with SQ 29,548, the increase was later and much smaller and there was an overall treatment effect of SQ 29,548 ($p < 0.05$) during the whole study period, mainly because of a highly significant treatment effect during the first phase of the reaction ($p < 0.01$; second phase, $p > 0.1$). The PVR increase during the first phase of the toxin reaction was abolished by the treatment.

A significant increase compared with baseline in PVR was found on both sides of the capillary bed in the lung in the control study. The major PVR change was in the arterial part of the pulmonary circulation, but there were also increases on the venous side. Treatment with SQ 29,548 substantially altered PVR on both sides of the capillary bed, but the treatment effect was statistically significant only in the precapillary bed ($p < 0.05$ overall, $p < 0.01$ in phase 1). The major effect was during the first phase of the reaction that was completely blunted.

Lung mechanics (Fig. 5). After GBS toxin infusion in the control study, there was a significant decrease in C_{dyn} compared with baseline during the first phase of the reaction. This decrease in C_{dyn} coincided with the increase in P_{PA} and PVR. After SQ 29,548 treatment, no significant changes in C_{dyn} were observed. There was a significant overall treatment effect ($p < 0.05$) by the blocker mainly because of the difference in the first phase ($p < 0.05$; second phase, $p > 0.1$). Calculations of specific compliance (C_{dyn}/FRC) also showed that SQ 29,548 completely blocked the reaction by the toxin during the first phase (data not shown). No significant respiratory rate dependence on the measurements of C_{dyn} could be found.

The changes in R_i showed a biphasic response with significant increase compared with baseline in R_i during both the first and second phase in the control study. After SQ 29,548 treatment, the changes in R_i persisted but to a lesser extent. However, there was no statistical evidence that SQ 29,548 affected R_i response after GBS toxin. Calculations of specific conductance ($1 \cdot R_i^{-1} \cdot FRC^{-1}$) did not reveal a statistically significant treatment effect by SQ 29,548 on the airway reactivity to GBS toxin infusion (data not shown).

FRC (Fig. 5). No significant difference was found in FRC between the treatment and control studies. A tendency toward an increase in FRC during the study compared with baseline was found after SQ 29,548 treatment, whereas after an initial small increase no FRC changes were found in the control study.

Ventilation (Table 1). An increase from baseline in both total and alveolar ventilation (calculated from the nitrogen washout curve) was found in both studies but was more prominent early during the toxin challenge. No treatment effect by SQ 29,548 was found on ventilation.

Gas mixing efficiency (NC index) was unaffected by GBS toxin infusion both with and without SQ 29,548 treatment. For estimation of the lung nitrogen elimination pattern (distribution of ventilation), a total of 92 curves in the control study and 90 curves in the SQ 29,548 study were subject to description by our exponential lung model (21). In the control study, 35% of the washout curves could best be described by a single exponential component, and a two-component model fit best in 40% of the curves. In 25% of the curves, exponential estimation of the washout pattern was unsatisfactory according to our lung model. The corresponding numbers in the SQ 29,548 study were 30% (one component), 43% (two components), and 27% (unsatisfactory description). No specific change in exponential patterns could be observed during the course of the studies, and there were no significant differences between the control and SQ 29,548 treatment.

Gas exchange and body temperature (Table 2). There was a significant decrease in oxygenation compared with baseline in the control study. There was a much smaller decrease with SQ 29,548 treatment ($p < 0.05$ overall). This difference was most pronounced during the first phase of the reaction ($p < 0.01$) and

Fig. 4. *A*, Total PVR; *B*, PVR in precapillary vascular bed; *C*, PVR in postcapillary vascular bed in six young lambs during a control study and during SQ 29,548 treatment. Values are mean $\pm$ SEM. *, Significant difference (paired t test, $p < 0.05$) between individual time points and baseline value at zero time. +, Significant difference (paired t test, $p < 0.05$) between groups at an individual time point. Repeated measures analysis of variance showed a significant treatment effect on total PVR and precapillary PVR (both $p < 0.05$ overall, $p < 0.01$ first phase).

Fig. 5. *A*, Lung C_{dyn} ; *B*, R_L to ventilatory flow; *C*, FRC in six young lambs during a control study and during SQ 29,548 treatment. Values are mean $\pm$ SEM. *, Significant difference (paired t test, $p < 0.05$) between individual time points and baseline value at zero time. +, Significant difference (paired t test, $p < 0.05$) between groups at an individual time point. Repeated measures analysis of variance showed a significant treatment effect on C_{dyn} ($p < 0.05$ overall, $p < 0.05$ first phase).

coincided with the pulmonary vascular and lung mechanical changes (second phase, $p > 0.1$). No significant changes or differences between the studies were observed in arterial PCO_2 and base excess. The body temperature increased significantly during both control and SQ 29,548 studies in comparison with baseline.

White blood cells and packed red cell volume (Table 3). There was a significant decrease compared with baseline in white blood cell count after GBS toxin infusion in both studies. Both lymphocytes and granulocytes were affected. There was a statistically significant difference in total white blood cell count between the two studies in the second phase ($p < 0.05$). In the control study, some hemoconcentration occurred during both the first and second phase of the reaction, but a statistically significant difference compared with baseline in packed red cell volume was seen only during the second phase. There was no hemoconcentration during the first phase of the SQ 29,548 studies.

Prostaglandins (Table 4). TXB_2 plasma concentration was significantly increased compared with baseline during the whole study period in both studies. There were no statistically significant differences in serum concentrations whether the animal was treated or not treated with SQ 29,548. The serum concentration of 6-keto- $PGF_{1\alpha}$ showed a slight increase at 1 and 2 h after the toxin infusion. There was no significant difference between the studies.

DISCUSSION

This study showed that hemodynamic changes dominated after GBS toxin infusion, and these changes were mainly affected by the SQ 29,548 treatment. As has been described previously, the response to GBS toxin was biphasic (10, 11). In the pulmonary circulation, we found a significant increase in the P_{PA} and PVR in the control study. Treatment of the animal with SQ 29,548 showed a significant treatment effect, abolishing the first phase of the pulmonary vascular reaction. Our results, linking TXA_2 to pulmonary vasoconstriction during the first phase, support work performed by others (24–31). The second phase of the pulmonary vascular reaction was not significantly attenuated by SQ 29,548 treatment, suggesting that TXA_2 is mainly involved in the first part of the response. The marked increase in serum concentration of TXB_2 during the first phase compared with the second phase of the reaction is further support for this idea.

The isolated effect of a prolonged SQ 26,548 infusion was tested in three separate animals. Except for a marginal decline in total PVR, which did not affect the results of the study, we found no isolated effect on any physiologic variable that could be attributed only to SQ 29,548. Also, during baseline measurements before toxin infusion, no effect of SQ 29,548 could be observed. This means that the pathophysiologic differences noted in this study between control and SQ 29,548 treatment are most likely attributed to the effect of SQ 29,548 on changes induced by the GBS toxin rather than a direct effect of SQ 29,548 alone.

In previous histologic examinations of lung tissue after GBS toxin infusion, signs of severe blood congestion have been found (2, 10). An increase in the lung microvascular pressure due to venous constriction is the probable cause. We have previously shown that the flow of lung lymph with low protein concentration significantly increases in lambs during the first phase of the GBS toxin reaction (11, 12). An increase in pulmonary microvascular pressure is clearly implicated by the increased protein-poor lymph flow rate during the first phase of the reaction. This increase in pulmonary microvascular pressure could be caused by an increase in pulmonary venous constriction. This has been extensively studied in the sheep model after *E. coli* endotoxin challenge (32). In that study, the fraction of the PVR attributed to the pulmonary veins was significantly increased during the toxin reaction. In our study, we also partitioned the response in PVR into precapillary and postcapillary compartments, which was not done previously in the GBS model. In the control study, the fraction of the total PVR, which could be attributed to an increase in venous constriction, was between 25 and 30% during both the first and second phase of the reaction, whereas the baseline value was about 15% of total PVR. This indicates that pulmonary venous tone is increased during the GBS toxin reaction. The increase in the fraction of the total PVR attributed to

Table 1. *Ventilatory measurements (mean + SEM)**

Time	Study	Baseline	½ h	1 h	1½ h	2 h	3 h	4 h	5 h
Breathing frequency (breath/min)	CTL	47 ± 4.5	56 ± 5.6	58 ± 8.6	58 ± 7.3	53 ± 7.3	61 ± 10.6	72 ± 12.2	63 ± 6.0†
	SQ	46 ± 4.1	40 ± 9.1	51 ± 2.6	69 ± 9.2	54 ± 6.5	70 ± 9.1†	61 ± 4.1†	60 ± 9.3
Tidal volume (mL/kg)	CTL	5.7 ± 0.4	6.1 ± 0.8	6.5 ± 0.9	6.7 ± 0.8	6.6 ± 0.7	6.5 ± 0.7	5.1 ± 0.4	5.3 ± 0.4
	SQ	5.9 ± 0.5	6.7 ± 0.8	5.3 ± 0.4	5.6 ± 0.4	6.2 ± 0.6	5.3 ± 0.4	5.4 ± 0.6	5.7 ± 0.5
Total ventilation (mL/min/kg)	CTL	266 ± 23	347 ± 59	410 ± 35†	375 ± 38†	337 ± 38	376 ± 44†	374 ± 66	330 ± 34†
	SQ	260 ± 18	306 ± 27	361 ± 55	337 ± 50	345 ± 45†	358 ± 38	317 ± 33	301 ± 25
Alveolar ventilation (mL/min/kg)	CTL	114 ± 12	185 ± 57	177 ± 29	163 ± 17†	139 ± 7	153 ± 17	135 ± 23	117 ± 8
	SQ	104 ± 13	150 ± 28	163 ± 19†	137 ± 16	128 ± 15	133 ± 17†	147 ± 21	123 ± 14
NC index	CTL	6.4 ± 0.4	5.1 ± 0.7	5.8 ± 0.4	5.9 ± 0.4	6.2 ± 0.5	5.7 ± 0.1	7.6 ± 0.6	7.2 ± 0.5
	SQ	7.3 ± 1.1	5.7 ± 0.7	5.6 ± 0.5	6.3 ± 0.7	6.9 ± 1.1	6.5 ± 0.6	6.8 ± 0.8	6.5 ± 0.9

* CTL, control; SQ, SQ 29,548.

† $p < 0.05$ compared with baseline.Table 2. *Gas exchange and body temperature (mean + SEM)**

Time	Study	Baseline	½ h	1 h	1½ h	2 h	3 h	4 h	5 h
Arterial Po ₂ (kPa)	CTL	11.1 ± 0.2	7.9 ± 0.5†	8.6 ± 0.9†	9.8 ± 0.4†	10.3 ± 0.4†	10.5 ± 0.4	9.6 ± 0.5†	9.8 ± 0.5†
	SQ‡	11.5 ± 0.6	10.0 ± 0.2§	11.0 ± 0.6§	10.6 ± 0.5†	11.2 ± 0.7	10.7 ± 0.3	10.6 ± 0.6	10.2 ± 0.4†§
Arterial PCO ₂ (kPa)	CTL	5.4 ± 0.2	5.4 ± 0.2	7.0 ± 1.6	5.6 ± 0.2	5.1 ± 0.2	5.0 ± 0.2	5.4 ± 0.3	5.0 ± 0.1
	SQ	5.3 ± 0.3	5.3 ± 0.4	5.5 ± 0.3	5.3 ± 0.1	5.1 ± 0.3	5.2 ± 0.2	5.1 ± 0.3	5.3 ± 0.3
Base excess (mEq/L)	CTL	0.9 ± 1.5	-1.4 ± 1.6	-7.0 ± 3.6	-4.6 ± 3.0	-4.3 ± 2.5	-3.9 ± 2.8	-2.0 ± 3.0	-1.5 ± 2.0
	SQ	2.2 ± 2.7	0.4 ± 2.1	2.5 ± 1.9	1.7 ± 1.6	-0.4 ± 1.5	0.7 ± 1.8	1.2 ± 2.5	3.1 ± 2.3
Body temperature (°C)	CTL	39.6 ± 0.2	40.0 ± 0.1†	40.3 ± 0.2†	40.1 ± 0.2†	40.1 ± 0.3†	40.6 ± 0.3†	40.2 ± 0.3†	39.8 ± 0.4
	SQ	39.3 ± 0.2	39.6 ± 0.2†	40.6 ± 0.1†	40.0 ± 0.1†	39.8 ± 0.3†	39.9 ± 0.3†§	39.4 ± 0.2§	39.3 ± 0.2

* CTL, control; SQ, SQ 29,548.

† $p < 0.05$ compared with baseline.‡ Treatment effect ($p < 0.05$ overall; first phase, $p < 0.01$).§ $p < 0.05$ between CTL and SQ at that time.Table 3. *Changes in blood cells (mean + SEM)**

Time	Study	Baseline	1 h	1½ h	2 h	3 h	4 h	5 h
White blood cell count (cells/mm ³)	CTL	10893 ± 1095	3244 ± 719†	3859 ± 618†	3539 ± 671†	3738 ± 950†	4272 ± 891†	5277 ± 1257†
	SQ‡	10685 ± 1555	6659 ± 2525†	6532 ± 1332	6771 ± 1045	5621 ± 671†§	5829 ± 788†	7505 ± 1114§
Granulocytes (cells/mm ³)	CTL	5091 ± 737	432 ± 297†	1013 ± 303†	821 ± 218†	2279 ± 828†	2779 ± 662†	3236 ± 741
	SQ	4519 ± 1191	2250 ± 1276†	2853 ± 906	3892 ± 953§	4105 ± 863§	3977 ± 845	5402 ± 1322
Lymphocytes (cells/mm ³)	CTL	5802 ± 1152	2804 ± 471†	2846 ± 562	2718 ± 521†	1459 ± 226†	1483 ± 234†	2283 ± 840
	SQ	6166 ± 620	4409 ± 1253	3660 ± 675†	2867 ± 369†	1493 ± 282†	1832 ± 258†	2093 ± 417†
Packed red cell volume (%)	CTL	27 ± 1.5	30 ± 2.4	28 ± 2.4	29 ± 1.9†	30 ± 2.1†	28 ± 2.2	27 ± 2.2
	SQ	28 ± 2.7	28 ± 2.5	27 ± 2.3	29 ± 2.3	29 ± 2.5†	29 ± 2.9	26 ± 2.4

* CTL, control; SQ, SQ 29,548.

† $p < 0.05$ compared with baseline.‡ Treatment effect ($p < 0.05$ second phase).§ $p < 0.05$ between CTL and SQ at that time.Table 4. *Plasma prostaglandins concentrations (mean + SEM)**

Time	Study	Baseline	1 h	2 h	3 h	4 h	5 h
TXB ₂ (ng/L)	CTL	117 ± 25	2687 ± 1001	1169 ± 337†	796 ± 227†	474 ± 115†	323 ± 61†
	SQ	107 ± 27	1111 ± 249†	554 ± 89†	341 ± 55†	270 ± 57†	302 ± 80†
6-keto-PGF _{1α} (ng/L)	CTL	163 ± 27	781 ± 396	732 ± 357	222 ± 72	150 ± 32	172 ± 55
	SQ	129 ± 25	332 ± 36†	310 ± 31†	169 ± 26	102 ± 16	132 ± 36

* CTL, control; SQ, SQ 29,548. 1 nmol TXB₂ or 6-keto-PGF_{1α} = 370.5 ng/L.† $p < 0.05$ compared with baseline.

the venous side of the lung circulation was abolished during the first phase of the reaction during SQ 29,548 treatment. SQ 29,548 treatment may therefore reduce blood congestion of the lung and reduce hemoconcentration, which also occurs in the GBS toxic shock syndrome.

After toxin infusion in the control study, there was a significant decrease in CO, which was caused by a significant reduction in stroke volume. The mechanism behind the cardiac decompensation in the control study could be secondary to hypovolemia and venous pooling, increased afterload, or a direct toxic effect on the myocardium mediated by TXA₂ (33). Treatment with SQ

29,548 preserved stroke volume during the whole study together with CO. The increased CO during the first phase of the GBS toxin response during SQ 29,548 treatment may have been related to increased body temperature and absence of an increase in systemic vascular resistance.

During the control study, lung mechanics showed a marked decrease in C_{dyn} during the first phase of the toxin reaction coinciding with the increase in PVR. No significant changes in C_{dyn} were found after SQ 29,548 treatment. It is plausible that the main cause of the reduced C_{dyn} during the first phase of the control study was interstitial edema and blood congestion due to

pulmonary venous constriction and an increase in lung microvascular pressure. It is also plausible that SQ 29,548 prevented pulmonary blood congestion by reducing the pulmonary venous constriction, which improved the mechanical properties of the lung. The improved lung function due to SQ 29,548 treatment during the first phase of the reaction also resulted in significantly better oxygenation compared with that in the control study.

Other factors such as changes in lung volume and respiratory rate could explain a reduction of C_{dyn} in the control study. However, FRC did not decrease and specific compliance (C_{dyn}/FRC) also showed a reduction during the first phase in the control study (data not shown). Uneven distribution of ventilation in combination with an increase in respiratory rate might cause a decrease in C_{dyn} . This is probably not the case here, because we found no change in the distribution of ventilation according to our lung model and no significant correlation between respiratory rate and decrease in C_{dyn} in either study. Furthermore, the NC index, which is supposed to reflect the gas mixing efficiency and the conditions in the small airways (34, 35), was not affected during either the control or the SQ 29,548 study; this favors uncompromised patency of alveoli and peripheral airways.

In this study, we also found a biphasic increase in resistance to ventilatory flow similar to what we found in a previous study (11). Even if there was an attenuation of R_i after SQ 29,548 treatment, this effect was not statistically significant. This means that TXA_2 is not the only mediator of the smooth muscle constriction of the proximal airways. In this study, the proximal airway constriction with increased R_i seemed to have little or no influence on ventilation, because FRC and gas mixing efficiency (as judged by the NC index) were unaffected in both studies. Total ventilation and alveolar ventilation, however, were increased both in the control study and after SQ 29,548 treatment. This probably reflects the increased metabolic demand at increased body temperature induced by the GBS toxin infusion.

During the second phase of the reaction, we found a significantly smaller decrease in total white blood cell count during the SQ 29,548 study compared with the control study, which could indicate reduced sequestration of white blood cells in the lung (10). SQ treatment may therefore also indirectly attenuate granulocyte-mediated lung injury (12).

In conclusion, treatment with a selective antagonist of the TXA_2 -prostaglandin endoperoxide receptor (SQ 29,548) in the GBS toxin lamb model primarily blocked the pathophysiologic changes during the first phase of the toxin reaction, demonstrating that TXA_2 is an important mediator of that phase. SQ 29,548 treatment led to significant reduction in the changes in pulmonary and systemic vascular resistance, improved CO and stroke volume, improved dynamic lung compliance, and improved oxygenation. TX receptor antagonists, such as SQ 29,548, may offer a potential therapeutic approach to infants with severe early-onset GBS sepsis. We propose that this should be investigated in animal models and humans with ongoing GBS sepsis.

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Announcement

American Cancer Society

Cancer Control Career Development Award for Primary Care Physicians

The American Cancer Society is pleased to announce the 1995 Cancer Control Career Development Award for Primary Care Physicians (CCCCDA). This award is intended to develop academic leaders in primary care specialties emphasizing cancer control: family practice, general internal medicine, obstetrics and gynecology, and pediatrics. Through the CCCCCDA, the Society seeks to support individuals in supervised programs that will develop the candidate's clinical and teaching expertise and his/her capacity to perform independent clinical research in cancer control. It is anticipated that physicians trained under these awards will improve cancer control through involvement in primary care practice, education, and research activities related to cancer control.

Candidates for first year Cancer Control Career Development Awards for Primary Care Physicians may not have an academic rank above that of Assistant Professor and must not be tenured or be the section head (or equivalent) in his/her discipline. These awards are intended to support the early development of academic careers which place emphasis on cancer control; physicians with well-established careers and substantial research funding should not apply. In addition, candidates for these awards must be citizens or permanent residents of the United States.

This is a 2-year award. The stipend for the award is \$25,000 for the first year and \$30,000 for the second. The application deadline is August 15, 1994 for awards to begin July 1, 1995.

Brochures and applications will be available in March 1994. *To request further information or application materials, please contact the office of:* Virginia Krawiec, MPA, Detection and Treatment Department, American Cancer Society, Inc., 1599 Clifton Road, NE, Atlanta, GA 30329-4251, telephone: 404-329-5734; fax: 404-325-1467.