Effects of a Leukotriene Antagonist on the Early Hemodynamic Manifestations of Group B Streptococcal Sepsis in Piglets¹

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ABSTRACT. In order to evaluate the influence of leukotrienes on group B streptococcal (GBS) sepsis we studied the effect of a leukotriene antagonist, FPL 57231, on the early hemodynamic changes occurring secondary to an infusion of live GBS. Paralyzed, mechanically ventilated piglets received a continuous intravenous infusion of bacteria (5×10^8 org/kg/min) while systemic arterial pressure and pulmonary artery pressure were measured continuously. Cardiac output was measured by thermodilution; and plasma samples for determination of thromboxane B_2 and 6-keto-PGF $_{1\alpha}$ were taken at preset intervals. In addition to GBS, treatment animals received a continuous infusion of FPL 57231 starting 15 min after the bacterial infusion was begun. Study animals as a whole responded to bacteria within 15 min with a marked elevation in pulmonary artery pressure from 13.6 ± 4 to 44.6 ± 6 mm Hg ($p < 0.001$), and a decline in PaO $_2$ (79 ± 9 to 44 ± 5 mm Hg) ($p < 0.001$) and a cardiac output (0.27 ± 0.07 to 0.15 ± 0.06 liter/min/kg) ($p < 0.0001$). In animals treated with FPL 57231 these changes were reversed or significantly attenuated by 60 min. In the control group pH deteriorated significantly to 7.17 ± 0.1 compared to baseline values ($p < 0.01$) by 60 min, while treatment group animals maintained a pH of 7.3 ± 0.23 . Thromboxane B_2 and 6-keto PGF $_{1\alpha}$ were similar in both groups and did not change during the study period. In addition, survival was significantly longer in treatment (191 ± 44 min) compared to control animals (100 ± 32 min) ($p < 0.01$). These data suggest that FPL 57231 may attenuate the early cardiovascular changes accompanying GBS infection and positively influence survival without modifying thromboxane or 6-keto PGF $_{1\alpha}$ levels. These results imply that leukotrienes influence the early hemodynamic manifestations of GBS sepsis. (*Pediatr Res* 20: 1004-1008, 1986)

Abbreviations

CO, cardiac output
GBS, group B streptococci
Ppa, pulmonary artery pressure
Ppaw, pulmonary wedge pressure
Pra, right atrial pressure

Psa, systemic arterial pressure
PVR, pulmonary vascular resistance
SVR, systemic vascular resistance
Tx B_2 , thromboxane B_2
5-HETE, 5-hydroxy-6,8,11,14 eicosatetraenoic acid
6-keto PGF $_{1\alpha}$, 6-keto prostaglandin F $_{1\alpha}$

Investigators have documented beneficial effects of nonsteroidal antiinflammatory agents on hemodynamics (1, 2) and survival (2, 3) in animal models using group B streptococcal toxin (1) and live organisms (2, 3). It has been shown that products of the cyclooxygenase pathway are responsible for many of the cardiovascular alterations seen in the first 2 h after either toxin (1) of live bacteria (2) are infused. Treatment with indomethacin during this period has resulted in improvement not only in the deleterious hemodynamic effects of GBS (1, 2) (*i.e.* increased Ppa and PVR, and decreased CO), but has also resulted in improved survival (2, 3). In addition, measurements of Tx B_2 , the stable metabolite of thromboxane A $_2$, and 6-keto PGF $_{1\alpha}$, the stable metabolite of prostacyclin, have shown that improvement in cardiovascular function is associated with a return of these products of arachidonic acid metabolism to baseline levels (1, 2).

After these initial hemodynamic and biochemical changes there is an increase in pulmonary capillary permeability, and alterations in Ppa and PVR which have been attributed to the lipoxygenase products of arachidonic acid metabolism (1, 4). However, when considering the many actions and interactions of prostaglandins, thromboxane, and leukotrienes (5), some in mimicry and some in opposition to each other, it is not unreasonable to expect important interactions between these substances, especially when they are derived from a common substrate and share elements of the same biochemical pathway. It is possible that many of the early hemodynamic changes attributed to thromboxane A $_2$ may in part be related to increased levels of leukotriene C $_4$ and D $_4$.

The purpose of this study was to investigate the role of leukotrienes in the early cardiovascular manifestations of GBS sepsis in a highly lethal animal model using piglets by evaluating the effect of the leukotriene receptor antagonist, FPL 57231, on hemodynamics. Plasma levels of Tx B_2 and 6-keto PGF $_{1\alpha}$ were also examined to detect possible nonspecific effects of this antagonist.

MATERIALS AND METHODS

Animal model. Ten Yorkshire piglets were anesthetized with pentobarbital (30 mg/kg, intraperitoneally). A femoral artery

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and vein were cannulated and used for Psa measurement and blood sampling. The left external jugular vein was cannulated and the catheter advanced into the right atrium for measurement of pressure (Pra) and injection of ice cold saline which was used for measurement of CO. A 5F Swan-Ganz thermodilution catheter was introduced into the right external jugular vein and advanced under fluoroscopy into the left pulmonary artery. CO was measured by thermodilution using a cardiac output computer (95510-A, Edwards Laboratory, Santa Ana, CA). The Swan-Ganz catheter was also used to measure Ppa and Ppaw. Heparinized normal saline (10 U/ml) was infused continuously through the pulmonary artery catheter. Vascular pressures were measured with pressure transducers (model P23 ID; Gould-Statham Instruments, Hato Rey, PR) and recorded on a multi-channel recorder (model 5 polygraph, S2-925T25, Grass Instrument, Quincy, MA).

A tracheostomy was performed and a 3.5-mm endotracheal tube placed. The animals were ventilated with a time-cycled, pressure-limited, infant ventilator (Sechrist, Model IV-100 B Infant Ventilator, Sechrist Industries, Anaheim, CA). Peak inflation pressure was set at 12 cm H₂O; PEEP at 2 cm H₂O, and the respiratory rate at 40 breaths/min. Animals were ventilated with room air. Ventilatory parameters were not altered during the study period. Arterial blood gases were measured prior to, 15, 30, and 60 min after bacterial infusion was started (pH/Blood Gas Analyzer 113, Instrumentation Laboratories, Lexington, MA). Rectal temperature was continuously monitored with a thermistor probe (Yellow Springs Instrument CO., Yellow Springs OH), and maintained at 38.0° C by means of a servocontrolled radiant warmer.

Animals were paralyzed with pancuronium bromide using an initial dose of 0.2 mg/kg intravenously followed by an infusion of 0.4 mg/kg/h. After a 60-min stabilization period, baseline cardiovascular measurements (Psa, Ppa, CO, Ppaw, and Pra) and arterial blood gases were obtained prior to bacterial infusion. This value will be hereafter referred to as baseline.

Bacterial preparation. Group B α -hemolytic streptococci (type Ic) were isolated from the blood of an infant who developed early onset sepsis at the University of Miami/Jackson Memorial Hospital Neonatal Intensive Care Unit. Bacteria for each animal experiment were incubated 18 h in Todd-Hewitt broth at 37° C. The broth culture was centrifuged at 1700 rpm for 30 min and the bacterial pellet was resuspended in sterile Ringer's lactate solution with 5% dextrose to a concentration determined by serial viable counts to be 1.46×10^9 colony forming units per ml.

Induction of sepsis. Bacterial infusion was begun immediately after baseline values were obtained. Bacteria were infused through a femoral vein at a rate calculated to deliver approximately 5×10^8 organisms/kg/min. This infusion was continued until the animal died or 4 h had elapsed.

The piglets were then randomly assigned to a treatment group composed of five animals (mean $\pm$ SD; weight, 2849 ± 622 g; age, 11 ± 2 days) who received the leukotriene antagonist, FPL 57231 (1 mg/kg/min for 30 min, followed by a dose of 0.5 mg/kg/min $\times$ 30 min) (Fisons plc., Loughborough, U.K.) via a femoral vein beginning after the 15-min values were obtained. The drug was entirely dissolved in 10 ml of sterile water. Five piglets (weight, 3331 ± 780 g; age, 11 ± 4 days) were assigned to the control group and received 10 ml of normal saline over the same time period.

Aortic and Ppa were measured continuously throughout the baseline and study periods. CO, Ppaw, and Pra were measured prior to and during the bacterial infusion and at 15, 30, 45, and 60 min after infusion of GBS was begun. Pulmonary vascular resistance was calculated using the formula:

$$\frac{\bar{P}_{pa} - \bar{P}_{paw}}{\text{CO/kg}}$$

and systemic vascular resistance calculated using the formula:

$$\frac{\bar{P}_{sa} - \bar{P}_{ra}}{\text{CO/kg}}$$

Blood samples (1 ml) from control and treatment animals were collected for radioimmunoassay of TxB₂ and 6-keto PGF_{1 α} in tubes containing aspirin and EDTA (0.41 and 1.95 mg/ml for 1 ml blood, respectively). Plasma from these samples was extracted with ethanol, centrifuged to remove precipitated protein, and dried under nitrogen before being reconstituted in 0.5–1.0 ml of phosphate-buffered saline containing 0.1% gelatin. The method used for their determination has been described previously (2).

Data analyses. Data are expressed as mean $\pm$ SEM. Statistical analyses included repeated measure analysis of variance (6), and the paired and unpaired *t* tests. Weight and age were compared by a two sample *t* test, while change from baseline to the point 15 min after the infusion of GBS was started was compared with a paired *t* test. In addition, the paired *t* test was used to compare the baseline with 60 min values. Both prebacteria and 15-min postinitiation of GBS baseline values were compared between groups, using an unpaired *t* test, and found not to be statistically different. These baseline values were combined to present an overall picture of the response to GBS in the piglet. Repeated measure analysis of variance was used to compare the pattern of response to treatment over time from a point 15 min after GBS started to the subsequent points for the two groups for all variables (Ppa, PVR, SVR, Psa, Pra, Ppaw, heart rate, CO, pH, PaCO₂, and PaO₂).

RESULTS

Hemodynamic measurements at baseline and 15 min after the start of GBS infusion were comparable in control and FPL 57231-treated animals. Ppa increased significantly in both groups (Fig. 1) and was markedly elevated compared to baseline at 15 min ($p < 0.01$). Arterial oxygen tension (Fig. 1) was significantly lower than baseline at 15 min ($p < 0.001$).

After 15 min of bacterial infusion, Ppa in control animals remained fairly stable (Fig. 1). Animals treated with FPL 57231, displayed a gradual reduction in Ppa to values which are significantly lower than control animals from 30 to 60 min ($p < 0.01$), but which were still higher than pre-GBS infusion baseline ($p < 0.02$).

Arterial oxygen tension in the control animals remained low throughout the study period. In contrast, PaO₂ in the FPL 57231 group increased toward baseline values over the study period and was significantly different in the two groups from 30 to 60 min ($p < 0.05$). However, it remained lower than baseline ($p < 0.02$).

After the initial marked reduction at 15 min ($p < 0.01$) CO continued to decline in control animals throughout the experiment (Fig. 2). CO in the FPL 57231-treated animals increased and was significantly greater than that of control animals during the period from 30 to 60 min. In addition, the 60-min value in this group was not statistically different from baseline.

PVR and SVR increased significantly ($p < 0.001$) with GBS infusion (Fig. 3). Values in the control group remained elevated during the study period while there were significant decreases in PVR ($p < 0.01$) and SVR ($p < 0.05$) in FPL 57231-treated animals. There were no statistical difference in SVR or PVR between baseline and 60-min values.

The pH in both groups decreased ($p < 0.001$) by 15 min, but thereafter diverged. At the end of the study period animals in the treatment group had a mean pH of 7.30 ± 0.23 while the control group had a pH of 7.17 ± 0.10 ($p < 0.01$). The same pattern was found when base excess was compared ($p < 0.01$). There were no statistically significant differences between groups in PaCO₂.

Ppaw was compared between groups. This pressure rose significantly for the groups as a whole from 3.8 ± 3.8 to 9.6 ± 1.7

Fig. 1. Responses of mean pulmonary artery pressure and PaO_2 to infusion of GBS. Effect of FPL 57231 on these measurements in the treatment group compared to the control group are shown. Duration of FPL 57231 infusion is noted by connected arrows. Significant differences between groups are indicated.

Fig. 2. Response of CO to the infusion of GBS. FPL 57231 infusion is noted by connected arrows.

mm Hg ($p < 0.001$). The Ppaw in control animals remained essentially unchanged while the Ppaw in the treatment group fell to values that were not significantly different from baseline. Right atrial pressure did not change significantly during the study period, nor were there statistical differences between groups.

Mean Psa and heart rate increased in both groups with GBS infusions, but were not statistically different over time from each other or from prebacterial infusion baseline at 60 min.

Mean TxB_2 values (Fig. 4) were significantly increased at 15 min after bacterial infusion began ($p < 0.01$) compared to baseline values in both groups. These values remained high during the study period, and groups were not significantly different.

Plasma concentrations of 6-keto $\text{PGF}_{1\alpha}$ were not different when baseline was compared to 15 min. These values remained fairly stable throughout the study period. In addition the ratio of

Fig. 3. Responses of pulmonary and systemic vascular resistances to infusion of GBS. FPL 57231 infusion in the treatment group is indicated by connected arrows.

Fig. 4. Levels of TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ in treatment and control groups are shown.

6-keto $\text{PGF}_{1\alpha}$ to TxB_2 was not statistically different between groups.

Survival (mean $\pm$ SD) was 100 ± 32 min in the control animals and 191 ± 44 min in the treatment group ($p < 0.01$). Two of

five controls and all five treatment animals were alive after 2 h of bacterial infusion.

DISCUSSION

The hemodynamic changes observed with an infusion of GBS or its extracellular toxin (*i.e.* an initial period of marked pulmonary hypertension followed by a later phase characterized by increased pulmonary vascular permeability and increased $\bar{P}_{pa}$) are similar to those produced by an infusion of endotoxin (2, 4, 7). The initial phase of pulmonary hypertension is prevented by indomethacin given before and after the infusion of bacteria. The later phase is not affected by indomethacin, yet is prevented by steroids (8, 9). In addition, recent evidence suggests that the increase in pulmonary hypertension and PVR and the decrease in CO noted in this late phase of GBS sepsis are reversed by a leukotriene antagonist (2, 10).

One of the important effects of steroids when used in experimentally induced sepsis is the prevention of granulocyte depletion. This treatment has been shown to prevent the late occurring pulmonary vascular changes secondary to GBS toxin or endotoxemia (8, 9, 11). A decrease in granulocyte numbers may occur within 15 to 30 min of initiation of experimental toxemia (1, 8, 9, 11). This granulocytopenia, which appears to reach its nadir by 60 min (12), is associated with pathologic findings in the pulmonary vasculature characterized by capillaries which are filled with granulocytes undergoing degranulation and by disruption of the integrity of the endothelial basement and internal elastic membranes (12). This breakdown of granulocytes and their resulting products appear capable of injuring vascular endothelium (13, 14), a finding that may explain the late phase increased vascular permeability (12).

Furthermore, the observed increased level of 5-HETE a major product of leukocyte 5-lipoxygenation, may also reflect increased lipoxygenase activity and leukotriene production (15–18) in adult sheep treated with endotoxin, suggesting an early role for leukotrienes in the pathophysiology of sepsis (12, 18). This increase in 5-HETE has been noted to occur within the 1st h after initiation of endotoxin infusion (18) and to reach its peak concentration following the initial period of severe pulmonary hypertension (19). The appearance of 5-HETE (18) in lung lymph during endotoxemia is suggestive evidence that lipoxygenase metabolites of arachidonic acid may be involved in the pulmonary vascular manifestations of sepsis. In addition, production of 5-HETE and other chemoattractants (*e.g.* leukotriene B_4) by leukocytes trapped in the lung might perpetuate pulmonary leukostasis and thereby contribute to the pathogenesis of lung injury. The concept that lipoxygenase products may participate in ongoing leukostasis is also suggested by the neutropenia associated with intravenous administration of leukotriene D_4 in primates (20).

It seems reasonable to postulate from these data that the 5-lipoxygenase products (5-HETE) which are produced and are released as granulocyte numbers decrease early on in endotoxemia, may also produce alterations in hemodynamics. Leukotriene C_4 and D_4 have been shown to increase $\bar{P}_{pa}$, PVR, and SVR, and decrease CO (21–23). In the present study we attempted to examine the hemodynamic effects of leukotrienes in the early phase (first 60 min) of GBS sepsis using a competitive antagonist of leukotriene C_4 and D_4 , FPL 57231 (24, 25). FPL 57231 appears to be a highly specific competitive receptor antagonist of leukotriene D_4 *in vivo* (24, 25). The parent compound, FPL 55712, is one of a series of chromone-2-carboxylic acids, whereas FPL 57231 is a propionic acid analog of FPL 55712 (25). In addition, FPL 57231 appears to have a narrower range of antagonism than FPL 55712 (25).

FPL 57231 when given after the onset of sepsis caused a sudden improvement in CO and oxygenation, and a decline in PVR, SVR, and $\bar{P}_{pa}$ to approximately baseline levels. In addition, it caused a progressive decline in $\bar{P}_{pa}$. FPL 57231 also mitigated the fall in pH seen in control animals, and lengthened

survival. These data suggest that leukotrienes, specifically C_4 and D_4 , are released and are biologically active early on in the septic animal. Furthermore, these changes occurred without modification of TxB_2 , or 6-keto $PGF_{1\alpha}$ levels.

The etiology of the myocardial depression in this model of sepsis is not clear (2, 26). Leukotrienes, specifically D_4 , have been shown to depress CO and cause coronary artery vasoconstriction (27–29). The possibility that TxA_2 effects cardiac function in GBS infection has been suggested (2, 26); however, in the present work a leukotriene antagonist resulted in a return of CO to baseline levels despite an elevated level of TxB_2 , suggesting that leukotrienes may also play a role in the myocardial dysfunction accompanying sepsis.

The apparent similarity in effect of TxB_2 and leukotriene C_4 and D_4 on CO and to a lesser extent on pulmonary artery pressure is not easily explained. It is possible that their properties and/or sites of actions are similar. It has been suggested that in some species the action of leukotriene C_4 and D_4 may, in part, be mediated by TxB_2 (30). This, however, appears to be related to species. In the pig (22) and in humans indomethacin does not block leukotriene action, suggesting that leukotriene C_4 and D_4 have relatively specific sites of action (31). Interestingly, it has been reported that a cyclooxygenase blocker may prevent enzymatic conversion of HPETE to HETE, both indicators of lipoxygenase activity, and hence interfere with lipoxygenation and possibly production of leukotrienes (32).

The potential for right to left shunting through fetal channels must be considered in a young animal model, however, this phenomenon appears to be minimal during room air breathing in the piglet by 48 h of age (33). In addition, work evaluating stressful experimental conditions such as sepsis (2, 34) and hypoxemia (35) have not shown evidence of shunting. Furthermore, during the increase in $\bar{P}_{pa}$ in the present study the peak pulmonary arterial systolic pressure was less than the systemic arterial diastolic pressure. Based on this pressure differential, it is unlikely that there was any significant ductal shunting or any flow right to left via the foramen ovali.

Caution must be exercised in attempting to extrapolate the results of this experiment to the clinical setting and to other experimental models. This model is rapidly lethal, unlike most cases of neonatal GBS disease. In addition, the absence of extrapulmonary right to left shunting also distinguishes this animal model from the human neonate. In contrast to other models, the one used also differs in that it uses a continuous infusion of organisms rather than a bolus of either toxin or bacteria and, in fact, this may result in an infusion of both toxin and live bacteria. The presence of an ongoing bacterial stimulus may account for the persistent elevation in plasma TxB_2 concentration seen in control animals, in contrast to the peak and later decline in concentration of TxB_2 reported by others (36, 37).

In conclusion, the findings of the present study suggest that FPL 57231, a leukotriene antagonist, significantly improves CO and oxygenation, decreases $\bar{P}_{pa}$, and improves survival in a highly lethal model of group B β -hemolytic streptococcal sepsis, suggesting that leukotriene C_4 and D_4 play an early role in hemodynamics. These effects appear to be independent and not mediated through TxB_2 or 6-keto $PGF_{1\alpha}$ for the levels of the latter substances were unchanged during the study period.

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