Group B Streptococcal Sepsis in Piglets: Effect of Combined Pentoxifylline and Indomethacin Pretreatment

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ABSTRACT. Group B streptococcus (GBS), a common neonatal gram-positive pathogen, causes similar pathophysiology in human newborns and neonatal animal models of sepsis. Animal models of GBS sepsis demonstrate a two-phase response: 1) an acute phase (<1 h) of increased pulmonary artery pressure (P_{pa}) and reduced arterial oxygen pressure (PaO_2) that is associated with increased serum thromboxane B_2 (TxB_2) and 2) a late phase (2–4 h) of persistently increased P_{pa} and reduced PaO_2 , reduced systemic arterial pressure, and progressive fall in cardiac output that is associated with increased serum TxB_2 , 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$), and tumor necrosis factor- α (TNF $_{\alpha}$). We hypothesized that pretreatment of piglets with both pentoxifylline (PTF), an inhibitor of TNF $_{\alpha}$ production and activity, and indomethacin (INDO) would 1) inhibit GBS-induced TxB_2 , 6-keto-PGF $_{1\alpha}$, and TNF $_{\alpha}$ and 2) prevent both the acute- and late-phase physiologic responses of GBS sepsis. Combined PTF and INDO pretreatment of anesthetized, mechanically ventilated piglets infused with GBS (1.25×10^9 colony forming units/kg/h) for 4 h prevented GBS-induced increases in P_{pa} at 1 h (GBS+PTF+INDO: 1.8 ± 0.07 kPa versus GBS alone: 4.7 ± 0.1 kPa) and markedly attenuated increases in P_{pa} at 4 h (GBS+PTF+INDO: 2.1 ± 0.1 kPa versus GBS alone: 4.4 ± 0.1 kPa). PTF+INDO treatment prevented GBS-induced reductions in both mixed venous oxygen pressure and PaO_2 at 1, 2, and 4 h (GBS+PTF+INDO: 11.5 ± 0.4 kPa versus GBS alone: 7.1 ± 0.4 kPa), and attenuated GBS-induced declines in cardiac output. PTF+INDO treatment significantly attenuated GBS-induced serum TNF $_{\alpha}$ polypeptide levels (ELISA, pg/mL) at 4 h (GBS+PTF+INDO: 143 ± 45 versus GBS alone: 502 ± 147) and blocked GBS-induced increases in serum TxB_2 and 6-keto-PGF $_{1\alpha}$ (all levels < 10 pg/0.1 mL by RIA). INDO pretreatment alone prevented GBS-induced increases in serum TxB_2 and 6-keto-PGF $_{1\alpha}$ levels but did not significantly inhibit GBS-induced TNF $_{\alpha}$ production. INDO pretreatment alone did not attenuate GBS-induced increases in P_{pa} at 4 h, nor prevent late-phase reductions in both PaO_2 and mixed venous oxygen pressure. PTF+INDO treatment of GBS sepsis in piglets is superior to treatment with INDO or PTF alone. Inhibition of both blood eicosanoid and TNF $_{\alpha}$ production may provide adjunctive therapy for human newborns with sepsis and pulmonary hypertension. (*Pediatr Res* 31: 222–227, 1992)

Abbreviations

cfu, colony forming unit
CO, cardiac output
GBS, group B streptococcus
INDO, indomethacin
PGI $_2$, prostaglandin I $_2$
6-keto-PGF $_{1\alpha}$, 6-keto-prostaglandin F $_{1\alpha}$
 P_{pa} , pulmonary artery pressure
 P_{sa} , systemic arterial pressure
PTF, pentoxifylline
PVR, pulmonary vascular resistance
TNF $_{\alpha}$, tumor necrosis factor- α
 TxB_2 , thromboxane B $_2$
 TxA_2 , thromboxane A $_2$
 PaO_2 , arterial oxygen pressure
 P_w , capillary wedge pressure
SVR, systemic vascular resistance

GBS sepsis in human neonates is associated with hypoxemia, pulmonary hypertension, shock, and high mortality (1–3). Neonatal animal models of i.v. GBS infusion show similar features, but with the delineation of both an acute and late phase response (4, 5). Acute features (<1 h) include pulmonary hypertension, hypoxemia, and reduced CO. The acute phase of GBS infusion in animals is associated with increased serum TxB_2 levels, a stable metabolite of the potent vasoconstrictor TxA_2 (5, 6). Inhibition of TxA_2 synthesis by indomethacin (INDO), a cyclooxygenase inhibitor, or dazmegrel, a thromboxane synthase inhibitor, prevents the acute pulmonary hypertension and hypoxemia of GBS bacteremia in piglets (5, 6). Dimethylthiourea also attenuates GBS-induced acute pulmonary hypertension and hypoxemia in piglets, suggesting that hydroxyl radicals contribute to the acute features of GBS sepsis (7).

The late features (2–4 h) of GBS infusion in animal models include persistent pulmonary hypertension, hypoxemia, progressive fall in CO, systemic hypotension, and lung injury (4–6). The late phase of GBS infusion is associated with increased serum levels of TxB_2 , 6-keto-PGF $_{1\alpha}$, a stable metabolite of the potent vasodilator PGI $_2$, and TNF $_{\alpha}$ (5, 6, 8, 9). Early INDO administration blocks the late phase increase in both serum TxB_2 and 6-keto-PGF $_{1\alpha}$ (5), but serum TNF $_{\alpha}$ levels were not measured in these studies. INDO treatment causes little improvement in late phase GBS-induced pulmonary hypertension (5), but partially attenuates late phase GBS-induced systemic hypotension, reduced CO, and hypoxemia (5). PTF, a methylxanthine that inhibits TNF $_{\alpha}$ production and activity (10–15), significantly reduces GBS-induced serum TNF $_{\alpha}$ polypeptide levels in neonatal piglets, but does not inhibit GBS-induced TxA_2 or PGI $_2$ produc-

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tion (8). However, PTF causes only a mild improvement in GBS-induced late-phase pulmonary hypertension and hypoxemia in piglets (8). These combined observations show that neither treatment with INDO nor PTF alone causes marked improvement in the late phase of GBS infusion in piglets. We speculate that arachidonic acid metabolites and TNF_α may act in an additive or synergistic manner to contribute to the late-phase features of GBS sepsis.

We hypothesized that combined PTF and INDO pretreatment would inhibit GBS-induced TxA_2 , PGI_2 , and TNF_α production and would prevent both the acute and late phase pathophysiologic responses of GBS sepsis in neonatal piglets.

MATERIALS AND METHODS

Animal preparation. Twenty piglets were anesthetized (30 mg/kg pentobarbital i.v.), paralyzed (0.3 mg/kg pancuronium bromide i.v.), anticoagulated with heparin (1000 IU i.v.), and mechanically ventilated via a tracheostomy tube with a large animal Harvard ventilator adjusted to deliver a tidal volume of 12 ± 2 mL/kg at a rate to maintain arterial carbon dioxide pressure at 4.6 to 5.3 kPa (35–40 mm Hg) during baseline conditions. The piglets from each experimental group were of similar age and weight: GBS alone, 3.1 ± 0.5 kg, 12 $\pm$ 2 d of age; GBS+INDO, 3.5 ± 0.8 kg, 13 $\pm$ 2 d of age; GBS+PTF+INDO, 3.6 ± 0.7 kg, 12 $\pm$ 2 d of age. All animals were ventilated with room air throughout each experiment. As previously described (16), catheters were placed in 1) the left external jugular vein for infusion of GBS, PTF, or INDO (double lumen 5 Fr Swan-Ganz, American Edwards Laboratories, Irvine, CA), 2) the aorta to measure P_{sa} and sample arterial blood for pH, blood gas tensions, and eicosanoid and TNF_α measurements, and 3) a branch of the left pulmonary artery (5 Fr Swan-Ganz thermodilution catheter) to measure P_{pa} and P_{w} and CO in triplicate by thermodilution using an Edwards 9520A CO computer (American Edwards Laboratories) and for sampling mixed venous blood. After instrumentation, anesthesia and muscle paralysis were maintained with pentobarbital (3 mg/kg i.v. every h) and pancuronium bromide (3 mg/kg i.v. every h), respectively. The piglets received sigh breaths to 30 cm H_2O every 20 min to minimize spontaneous development of atelectasis. Vascular pressures were measured using Hewlett-Packard 1280 transducers (Hewlett-Packard Co., Palo Alto, CA) referenced to mid-chest. Core temperature was maintained at $38.5 \pm 0.5^\circ\text{C}$ with an overhead radiant heat source.

GBS strain and preparation. The GBS strain is a type III clinical isolate made rifampin- and streptomycin-resistant (COH 31 r/s) (16). The culture conditions, mode of resuspension in sterile nonbacteriostatic saline, and tests of culture purity were performed as described (17). The GBS suspensions for infusion into piglets contained <0.03 endotoxin units/mL of endotoxin based on the limulus amoebocyte lysate assay (5 endotoxin units/mL = 1 ng/mL) (Associates of Cape Cod, Woods Hole, MA).

Eicosanoid assays. Two-mL arterial blood samples were obtained under each of the four experimental conditions. The blood samples were drawn into cold inhibitor solution containing indomethacin and sodium EDTA and centrifuged as previously described (9). The decanted plasma was frozen at -70°C until RIA for TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ were performed as described (9). TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ were assayed by measuring competitive inhibition of [^3H] TxB_2 to rabbit anti- TxB_2 binding or [^3H]6-keto- $\text{PGF}_{1\alpha}$ to anti-6-keto- $\text{PGF}_{1\alpha}$, respectively (9). The average of duplicate assays was used to determine group means for the different experimental conditions. The limit of detection for TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ was <10 pg/0.1 mL. Matrix effects due to protein present in piglet plasma were measured in standard curves using eicosanoid-free piglet plasma prepared by charcoal stripping (9).

TNF_α ELISA assay. Two-mL arterial blood samples were obtained under each of the four experimental conditions for each piglet. The samples were stored on ice during the protocol and

then centrifuged at 15 000 rpm $\times$ 15 min. The decanted serum was frozen and stored at -70°C until a TNF_α ELISA assay was performed in duplicate. A human TNF_α serum ELISA kit was kindly provided by Genentech, Inc., San Francisco, CA. This "sandwich" ELISA assay used rabbit anti- TNF_α coat antibody (Genentech, Inc., Lot 4701-92) and horseradish peroxidase-conjugated mouse monoclonal anti- TNF_α (Genentech, Inc., Lot 4707-25), as described (8). A human recombinant TNF_α (Genentech, Inc., Lot TH151A) standard curve was prepared over the range of 35 to 2000 pg/mL in PBS containing BSA (5 mg/mL). A relationship between OD at 490 nm and TNF_α concentration is derived from the standard curve by linear regression. The piglet serum TNF_α levels were derived by interpolation from the average of the two assays. The limit of TNF_α detection by our assay was <35 pg/mL.

Experimental design. After instrumentation, each piglet received 10 mL/kg 0.9% saline i.v. to ensure a standardized euvoletic state. Data from three groups of piglets are reported: Group 1 (GBS alone), in which seven piglets received saline (2 mL/kg i.v. bolus, followed by saline infusion at 2 mL/kg/h) and 30 min later received i.v. GBS at 1.25×10^9 cfu/kg/h for 4 h; Group 2 (GBS+INDO), in which six piglets received INDO [3 mg/kg i.v. bolus (1 mL/kg), followed by 0.9% saline infusion at 2 mL/kg/h] and 30 min later received i.v. GBS at 1.25×10^9 cfu/kg/h for 4 h; and Group 3 (GBS+PTF+INDO), in which seven piglets received i.v. PTF (20 mg/kg i.v. bolus followed by 20 mg/kg/h infusion) and INDO (3 mg/kg i.v.) and 30 min later received i.v. GBS at 1.25×10^9 cfu/kg/h for 4 h. Piglets infused with 0.9% saline or PTF (20 mg/kg bolus, then 20 mg/kg/h) alone showed no significant physiologic abnormalities and no significant increases in TxB_2 , 6-keto- $\text{PGF}_{1\alpha}$, or TNF_α (8).

In each piglet, P_{pa} , P_{w} , P_{sa} , CO, arterial and mixed venous blood gas tensions, and arterial blood samples for measurement of TxB_2 , 6-keto- $\text{PGF}_{1\alpha}$, and TNF_α were obtained at baseline (PRE) and at 1, 2, and 4 h. PVR ($P_{\text{pa}} - P_{\text{w}}/\text{CO}$) and SVR (P_{sa}/CO) were calculated at the same time points for each piglet.

Statistical analysis. Analysis of variance with Student-Neuman-Keuls correction for multiple comparisons was used to compare values between the experimental groups; paired *t* tests were used to compare intragroup values (SPSS/PC+ v3.0, SPSS, Inc., Chicago, IL). A *p* value of <0.05 was considered significant.

RESULTS

Pulmonary hemodynamics. Figures 1 and 2 depict changes in P_{pa} and PVR, respectively, during the 4-h infusions for each group of piglets. INDO pretreatment inhibited GBS-induced

Fig. 1. PTF+INDO attenuates GBS-induced acute- and late-phase increases in P_{pa} . Mean P_{pa} values (kPa) are plotted versus time for the three groups of piglets. Values are expressed as mean $\pm$ SEM. * denotes $p < 0.05$ compared with the intragroup PRE value; # denotes $p < 0.05$ compared with the other two groups at the same time point.

Fig. 2. PTF+INDO attenuates GBS-induced acute- and late-phase increases in PVR. PVR values (kPa/L/min) are plotted versus time for the three groups of piglets. Values are expressed as mean $\pm$ SEM. * denotes $p < 0.05$ compared with intragroup PRE value; # denotes $p < 0.05$ compared with the other two groups at the same time point.

acute increases in P_{pa} and PVR, but resulted in no improvement in P_{pa} or PVR at 4 h in GBS+INDO compared with GBS alone piglets. PTF+INDO pretreatment prevented the GBS-induced acute increases in P_{pa} and PVR and markedly attenuated the increase in P_{pa} and PVR at 4 h compared with the other two groups of piglets ($p < 0.05$).

Systemic hemodynamics. Table 1 shows the changes in CO, P_{sa} , and SVR for each group of piglets during the 4-h infusions. INDO pretreatment did not improve CO at 1 h or 4 h in GBS+INDO compared with GBS alone piglets. PTF+INDO pretreatment prevented the acute fall in CO and significantly improved CO compared with the other two groups at 1 and 2 h; PTF+INDO pretreatment significantly improved CO at 4 h in GBS+PTF+INDO piglets compared with the other two groups ($p < 0.05$).

P_{sa} was not significantly reduced at 1 and 2 h of infusion in any of the groups; P_{sa} was significantly reduced at 4 h in GBS alone and GBS+PTF+INDO piglets. Piglets infused with GBS+INDO showed no significant fall in P_{sa} at 4 h. INDO treatment, but not combined PTF+INDO treatment, attenuated the GBS-induced decrease in P_{sa} at 4 h.

SVR increased significantly from baseline at 1, 2, and 4 h in GBS alone and at 2 and 4 h in GBS+INDO piglets; SVR did not significantly change from baseline at 1, 2, or 4 h in GBS+PTF+INDO piglets (Table 1).

Ratio of pulmonary vascular resistance to systemic vascular resistance. The PVR/SVR ratio was similar at baseline for the

three experimental groups (Table 2). The PVR/SVR ratio increased from baseline at 1, 2, and 4 h in the GBS alone piglets. INDO pretreatment prevented an increase in the PVR/SVR ratio at 1 h, but not at 2 or 4 h, where values were similar to those of GBS alone piglets. PTF+INDO treatment prevented a significant increase in the PVR/SVR ratio at 1 h and significantly attenuated the increase in PVR/SVR ratio at 4 h compared with GBS and GBS+INDO piglets.

Gas exchange. Table 3 shows the blood gas values for each group of piglets during the 4-h infusions. INDO treatment prevented the fall in P_{aO_2} at 1 and 2 h, and P_{aO_2} at 4 h was significantly improved in GBS+INDO compared with GBS alone piglets. However, P_{aO_2} at 4 h was significantly decreased from baseline values in GBS+INDO piglets. PTF+INDO treatment resulted in no significant decrease in P_{aO_2} at 1, 2, and 4 h. The P_{aO_2} at 4 h was significantly improved in GBS+PTF+INDO piglets compared with the 4-h P_{aO_2} in the other two groups.

In contrast to the other groups, piglets infused with GBS+PTF+INDO showed no significant decrease in mixed venous oxygen pressure during the 4-h infusion. All groups showed a statistically significant decrease in arterial pH by 4 h of infusion, but INDO and PTF+INDO treatment prevented the development of significant metabolic acidosis ($pH < 7.35$). The calculated base excess at baseline was similar for the three groups (GBS: 2 ± 0.8 , GBS+INDO: 2 ± 1.1 , GBS+PTF+INDO: 1.5 ± 0.9). However, both INDO and PTF+INDO treatment attenuated the GBS-induced decline in base excess at 4 h (GBS: 7 ± 1.1 , GBS+INDO: -1.8 ± 1.1 , GBS+PTF+INDO: -0.5 ± 0.8).

Eicosanoids. Figures 3A and 3B depict changes in serum TxB_2 and 6-keto-PGF $_{1\alpha}$ levels, respectively, for each group of piglets. INDO and PTF+INDO treatment prevented GBS-induced increases in serum TxB_2 or 6-keto-PGF $_{1\alpha}$ at 1, 2, and 4 h. Serum TxB_2 and 6-keto-PGF $_{1\alpha}$ levels were < 10 pg/0.1 mL at all time points for these two groups of piglets.

Tumor necrosis factor. Figure 4 depicts changes in serum TNF $_{\alpha}$ polypeptide levels in each group of piglets. INDO pretreatment did not significantly reduce GBS-induced serum TNF $_{\alpha}$ polypeptide levels at 1, 2 and 4 h in GBS+INDO compared with GBS alone piglets. PTF+INDO treatment attenuated GBS-induced serum TNF $_{\alpha}$ polypeptide levels at both 2 and 4 h in GBS+PTF+INDO compared with GBS alone piglets.

DISCUSSION

Pentoxifylline and indomethacin pretreatment of piglets infused with GBS caused significantly greater improvement in late-phase pulmonary hemodynamic and gas exchange indices compared with piglets treated with INDO alone. PTF+INDO treatment of GBS sepsis in piglets also resulted in greater hemodynamic and gas exchange improvement compared with historical

Table 1. Systemic hemodynamics*

	PRE†	1 h	2 h	4 h
CO (L/min)				
GBS	0.93 ± 0.07	$0.60 \pm 0.04‡$	$0.58 \pm 0.04‡$	$0.50 \pm 0.04‡$
GBS + INDO	0.85 ± 0.11	$0.69 \pm 0.11‡$	$0.60 \pm 0.07‡$	$0.38 \pm 0.06‡$
GBS + PTF + INDO	0.87 ± 0.07	$0.87 \pm 0.07§$	$0.83 \pm 0.06§$	$0.66 \pm 0.05‡§$
P_{sa} (kPa)				
GBS	10.9 ± 0.4	12.0 ± 0.4	12.4 ± 0.5	$8.0 \pm 0.5‡$
GBS + INDO	11.7 ± 0.7	11.5 ± 0.7	11.9 ± 0.9	9.6 ± 1.0
GBS + PTF + INDO	10.5 ± 0.3	10.0 ± 0.3	9.8 ± 0.4	$8.5 \pm 0.5‡$
SVR (kPa/L/min)				
GBS	11.7 ± 1.0	$20.1 \pm 2.9‡$	$21.5 \pm 4.5‡$	$16.9 \pm 3.0‡$
GBS + INDO	13.7 ± 3.2	16.7 ± 4.3	$19.9 \pm 2.0‡$	$25.2 \pm 7.9‡$
GBS + PTF + INDO	12.0 ± 1.9	$11.5 \pm 2.3§$	$11.8 \pm 2.2§$	12.9 ± 3.5

* Values are expressed as mean $\pm$ SEM. 1 mm Hg = 0.1333 kPa.

† PRE, baseline value.

‡ $p < 0.05$ compared with the intragroup PRE value.

§ $p < 0.05$ compared with the same time point for the other two groups.

Table 2. *PVR/SVR ratios**

	PRE†	1 h	2 h	4 h
GBS	0.131 ± 0.006	0.354 ± 0.019‡§	0.326 ± 0.010‡	0.425 ± 0.024‡
GBS + INDO	0.125 ± 0.007	0.132 ± 0.012	0.210 ± 0.031‡	0.364 ± 0.039‡
GBS + PTF + INDO	0.142 ± 0.008	0.157 ± 0.011	0.161 ± 0.009‡	0.201 ± 0.018‡§

* Values are expressed as mean ± SEM.

† PRE, baseline.

‡ $p < 0.05$ compared with the intragroup PRE value.

§ $p < 0.05$ compared with the same time point for the other two groups.

Table 3. *Gas exchange data**

	pH	PaCO ₂ (kPa)	PaO ₂ (kPa)	PvO ₂ (kPa)
GBS				
PRE	7.45 ± 0.01	5.2 ± 0.1	11.3 ± 0.3	5.1 ± 0.1
1 h	7.43 ± 0.01	5.5 ± 0.3	8.0 ± 0.3†	4.3 ± 0.3†
2 h	7.37 ± 0.01†	5.7 ± 0.3	7.3 ± 0.4†	4.0 ± 0.3†
4 h	7.30 ± 0.02†	5.7 ± 0.3	7.1 ± 0.4†	3.7 ± 0.3†
GBS + INDO				
PRE	7.45 ± 0.02	5.2 ± 0.1	11.1 ± 0.3	4.9 ± 0.1
1 h	7.47 ± 0.01	5.3 ± 0.3	10.9 ± 0.3	4.5 ± 0.3
2 h	7.45 ± 0.02	5.2 ± 0.1	10.8 ± 0.4	4.5 ± 0.3
4 h	7.37 ± 0.03†	5.5 ± 0.3	9.9 ± 0.3†	3.9 ± 0.1†
GBS + PTF + INDO				
PRE	7.45 ± 0.01	5.1 ± 0.1	11.7 ± 0.4	5.3 ± 0.1
1 h	7.46 ± 0.02	4.9 ± 0.1	12.0 ± 0.4	5.1 ± 0.1
2 h	7.45 ± 0.01	4.8 ± 0.1	11.6 ± 0.5	5.2 ± 0.3
4 h	7.40 ± 0.02†	4.9 ± 0.1	11.5 ± 0.4‡	5.1 ± 0.3‡

* Values are expressed as mean ± SEM. 1 mm Hg = 0.1333 kPa. PaCO₂, arterial carbon dioxide pressure; PvO₂, mixed venous oxygen pressure; PRE, baseline value.

† $p < 0.05$ compared with the intragroup PRE value.

‡ $p < 0.05$ compared with the same time point for the other two groups.

controls treated with PTF alone (8). PTF+INDO and PTF pretreatment alone resulted in similar reductions in GBS-induced serum TNF α levels (8), but the combined treatment blocked GBS-induced thromboxane and prostacyclin production (8). PTF treatment of GBS sepsis caused augmentation of prostacyclin production (8). The inhibition of prostacyclin production by PTF+INDO may have improved both the late-phase gas exchange and hemodynamic responses compared with PTF treatment alone (8). In contrast to PTF+INDO treatment, INDO pretreatment alone did not significantly improve late-phase GBS-induced pulmonary hypertension, or late-phase decline in CO. Our findings are similar to those of Runkle *et al.* (5) in which piglets were treated with INDO (3 mg/kg) 15 min after the onset of the GBS infusion. INDO presumably has a beneficial effect in sepsis by inhibiting eicosanoid production. INDO does not affect endotoxin-induced TNF production in mice (17, 18) and did not significantly reduce GBS-induced TNF polypeptide levels in piglets. The beneficial effects of PTF+INDO pretreatment on GBS-induced late-phase features were greater than would be predicted from the effects of pretreatment with PTF or INDO alone. We speculate that the late phase of GBS sepsis in animal models is the most clinically relevant and the marked attenuation of the late-phase features by PTF+INDO is a new observation.

The presumed mechanisms for PTF+INDO treatment improving PaO₂ values during GBS sepsis include 1) attenuating the decline in CO with resultant increases in mixed venous oxygen pressure (6) and 2) reducing ventilation-perfusion mismatching due to inhibition of vasoactive mediators (6). PTF+INDO treatment caused a greater reduction in PVR than SVR during both the acute and late phases of GBS sepsis. PTF causes preferential vasodilation of either the pulmonary or systemic vascular bed, depending upon which is the most constricted before treatment (36). We speculate that the preferential pulmonary vasodilation by PTF+INDO is due to the relatively

Fig. 3. PTF+INDO and INDO inhibit GBS-induced increases in TxB₂ and 6-keto-PGF_{1α}. Panel A, Serum TxB₂ levels (pg/0.1 mL) are plotted versus time for the three groups of piglets. Panel B, Serum 6-keto-PGF_{1α} levels (pg/0.1 mL) are plotted versus time for the three groups of piglets. Values are expressed as mean ± SEM for both assays. * denotes $p < 0.05$ compared with intragroup PRE value; # denotes $p < 0.05$ compared with the other two groups at the same time point.

greater GBS-induced increase in PVR compared with SVR. PTF+INDO prevented the GBS-induced fall in CO at 1 and 2 h by attenuating the decline in stroke volume (data not shown); however, PTF+INDO did not prevent the decline in P_{sa} at 4 h and only attenuated the reduction in CO and stroke volume (data not shown) at 4 h. The three groups of piglets showed a similar degree of tachycardia at 4 h of GBS infusion (data not shown). The fall in CO at 4 h may in part reflect the untoward vasodilatory properties of PTF at a dose of 20 mg/kg/h or potential negative inotropic effects of GBS. However, PTF infusion (20 mg/kg/h) into control piglets caused no fall in CO or P_{sa} (8).

Vasoactive arachidonic acid metabolites are important mediators in models of gram-negative and GBS sepsis (4–9, 19–21)

Fig. 4. PTF+INDO attenuates GBS-induced TNF α production. Serum TNF α polypeptide levels (pg/mL) are plotted versus time for the three groups of piglets. Values are expressed as mean $\pm$ SEM. * denotes $p < 0.05$ compared with the intragroup PRE value; # denotes $p < 0.05$ compared with the other two groups at the same time point.

and in some models of TNF α -induced shock (22, 23). INDO, ibuprofen, and combined lipoxygenase and cyclooxygenase inhibitors ameliorate endotoxin-induced shock (17, 18, 21). INDO treatment blocks the GBS-induced late-phase decline in P_{sa} , but not late-phase GBS-induced pulmonary hypertension (5). Dazmegrel, a thromboxane synthase inhibitor, reverses GBS-induced pulmonary hypertension up to 2 h after the onset of infusion, but was not tested at 4 h into GBS infusion when there were greater increases in serum TNF α levels (9). There are discrepant reports on the effect of these drugs on the attenuation of TNF α -induced shock (18, 23, 24). Some investigators report that INDO only attenuates endotoxin effects and has no influence on combined endotoxin- and TNF α -induced shock (24). There is less information available on the role of eicosanoids in TNF α -induced pulmonary hypertension (22, 25). Recombinant human-TNF α infusion in piglets induced sustained pulmonary hypertension; pretreatment with dazmegrel inhibited the rise in P_{pa} up to 30 min after the onset of TNF α infusion, but did not blunt a later phase of TNF α -induced pulmonary hypertension (25). Bacterial- or TNF α -induced eicosanoids are important mediators of the hemodynamic and gas exchange abnormalities of sepsis, but inhibition of arachidonic acid metabolites alone is not sufficient to attenuate all the effects of TNF α or sepsis.

TNF α is an important mediator of gram-negative and gram-positive septic shock (8, 10, 22, 26–28). Pretreatment with anti-TNF α antibody prevented septic shock in a primate model of lethal gram-negative bacteremia (26); inhibition of serum TNF α production by pentoxifylline significantly improved survival in a murine model of endotoxic shock (15). However, in a model of gram-negative pneumonia, anti-TNF α antibody neutralized lung lavage TNF α levels and blunted pulmonary antibacterial defenses (29). Therefore, although the attenuation of the systemic TNF α response may be beneficial, altering local TNF α production at inflammatory sites may have some deleterious effects (29, 30). However, other studies suggest that TNF α alone cannot mimic the features of septic shock (24). TNF α may need to interact with bacterial products or other vasoactive mediators to cause untoward effects (17, 22–24, 31, 32). TNF α can act synergistically with IL-1 (31) or with gram-negative or gram-positive bacterial products in the induction of shock in animal models (24, 32). There is no direct evidence that TNF α and eicosanoids act synergistically in the pathophysiology of sepsis, but this possibility seems reasonable based on our observations that PTF+INDO pretreatment caused marked attenuation of GBS-induced late features compared with the modest effects of INDO or PTF alone (8).

The production and/or activity of TNF α and arachidonic acid

metabolites is interrelated in adult animal models of sepsis (17, 18, 22, 23, 25, 31). TNF α infusion can induce the synthesis of TxA $_2$, PGI $_2$, PGE $_2$, and leukotrienes (22, 27); leukotriene B $_4$ can augment alveolar macrophage production of TNF α *in vitro* (33). Inhibition of thromboxane and prostaglandin production by ibuprofen or indomethacin ameliorates endotoxin- and TNF α -induced shock and lung injury in some adult animal models (17, 21, 23, 31). In addition, dual inhibition of both cyclooxygenase and 5-lipoxygenase markedly attenuates serum TNF α levels and lethality in a murine model of endotoxic shock (18). These observations also support the speculation for an additive or synergistic interaction between TNF α and arachidonic acid metabolites.

Serum TNF α polypeptide levels may overestimate *in vivo* TNF α bioactivity in piglets treated with PTF+INDO. GBS infusion in piglets induces TNF α bioactivity as assayed by the L-929 cytotoxicity assay (8). Piglets infused with GBS+PTF+INDO had significant attenuation of serum TNF α polypeptide levels at 4 h compared with GBS alone piglets, but it is uncertain how much residual TNF α bioactivity remained. To measure *in vivo* TNF α bioactivity, serum TNF α bioassays would need to be done *in vitro* at the same concentrations of PTF present in the plasma of treated piglets, and these were not performed in this study. PTF inhibits TNF α production *in vitro* by 50% at a concentration of 5 μ M (10), but it required approximately 100-fold greater PTF concentrations to inhibit TNF α -induced L-929 cytotoxicity (11). PTF blood levels have not been reported in animal models of sepsis, and the PTF concentration necessary to inhibit TNF α -induced cardiopulmonary responses is uncertain. We speculate that PTF inhibits serum TNF α bioactivity in the GBS+PTF+INDO group to a greater degree than suggested by the serum TNF α polypeptide levels (11, 15), and this could partially explain the beneficial effects of PTF treatment of GBS sepsis.

The beneficial effects of PTF+INDO treatment in the piglet model of GBS sepsis may be due to factors other than the inhibition of thromboxane, prostacyclin, and TNF α . PTF attenuates TNF α production and TNF α -induced lung injury (8, 10–15), but has multiple additional effects in adult animals that could improve the untoward effects of GBS sepsis. These include 1) decreased sepsis-induced lung injury (12), 2) attenuation of TNF α - and endotoxin-induced endothelial cell injury *in vitro* (34, 35), 3) decreased neutrophil sequestration in the lung (13), 4) vasodilatory properties, including the attenuation of hypoxic pulmonary vasoconstriction (36, 37), and 5) reduced blood viscosity (37). It is uncertain if all of these PTF effects are due to the inhibition of TNF α . There are no published reports on the effect of PTF treatment on either TNF α - or TxA $_2$ -induced pulmonary vasoconstriction. However, preliminary data from our laboratory show that PTF attenuates acute (<30 min) pulmonary hypertension in piglets infused with human recombinant TNF α (38). INDO inhibits thromboxane and prostaglandin synthesis by inhibition of cyclooxygenase, but in some species, or at high concentrations, also inhibits phospholipase A $_2$, the initial enzymatic step in eicosanoid metabolism (39). Additional eicosanoids, such as leukotrienes, not measured in this study, but potentially inhibited by INDO, may contribute to the untoward effects of GBS sepsis. There are indirect data that leukotrienes are not important in the acute phase of GBS sepsis (20), but there are no data on the role of leukotrienes in the late phase of GBS sepsis. Finally, GBS sepsis probably stimulates additional proinflammatory and vasoactive mediators, not measured in this study, that may be inhibited by PTF or INDO treatment. For example, GBS induces the production of additional cytokines *in vitro*, such as IL-1 β (40), which if induced *in vivo* could act synergistically with TNF α to cause hemodynamic alterations and lung injury (31). GBS may also cause hypoxemia and pulmonary hypertension by granulocyte- or complement-mediated pulmonary vascular injury (41, 42), or GBS toxins may act alone or synergistically with proinflammatory mediators to cause unto-

ward effects (43). PTF and/or INDO may ameliorate these potential GBS-induced effects in addition to inhibiting the vasoactive mediators tested in this study.

We conclude that in this piglet model of GBS sepsis, combined PTF+INDO pretreatment is superior to INDO or PTF pretreatment alone in improving the hemodynamic and gas exchange alterations. This beneficial effect of combined PTF+INDO is in part due to inhibition of thromboxane, prostacyclin, and TNF α production and presumably attenuation of *in vivo* TNF α activity. Inhibition of both serum eicosanoid and TNF α production may provide adjunctive therapy for human newborns with sepsis and pulmonary hypertension.

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