The Fatty Acid Composition of Maternal Diet Affects Lung Prostaglandin E2 Levels and Survival from Group B Streptococcal Sepsis in Neonatal Rat Pups¹,²,³

Jorge I. Rayon, Jane D. Carver,⁴ Lance E. Wyble, Doris Wiener, Sonja S. Dickey,* Valerie J. Benford, Li T. Chen² and Daniel V. Lim*

Department of Pediatrics, Division of Neonatology, University of South Florida College of Medicine, Tampa FL 33606; *Department of Biology, College of Arts and Sciences, University of South Florida, Tampa FL 33620; and ²Department of Anatomy, University of South Florida College of Medicine, Tampa FL 33612

ABSTRACT Dietary fatty acid effects upon the immune system may be mediated in part by effects upon the synthesis of proinflammatory mediators. The effects of maternal dietary fatty acid composition upon lung prostaglandin (PG) E₂ levels and survival from group B streptococcal (GBS) infection were investigated in neonatal rat pups. Beginning on d2 of gestation and throughout lactation, pregnant dams were fed a purified diet whose fat source (22% of energy) was either corn oil or menhaden fish oil. On postnatal d 3, pups were randomly cross-fostered to dams of the same diet group to minimize litter effects; litters were then culled to 10 pups per dam. On postnatal d 7, pups were either injected with 1 × 10⁷ GBS organisms or were killed for determination of lung tissue levels of PGE₂ and lung and erythrocyte fatty acid composition. Arachidonic acid and PGE₂ levels were significantly higher in the lungs of pups in the corn oil group compared with the fish oil group. Forty-nine percent of pups in the corn oil group survived the GBS challenge compared with 79% of pups in the fish oil group (P = 0.0005). These data suggest that the fatty acid composition of pre- and/or postnatal diet affects the neonatal response to immune challenge, which may be due in part to effects upon the synthesis of pro-inflammatory mediators. J. Nutr. 127: 1989–1992, 1997.

KEY WORDS: • group B streptococcus • sepsis • arachidonic acid • eicosapentanoic acid • eicosanoids • rats

Increased blood and tissue levels of eicosanoids and cytokines play an important role in mediating the hemodynamic and inflammatory sequelae of GBS sepsis (Baker and Edwards 1995, Gibson et al. 1995, Ling et al. 1995). Group B streptococcal organisms invade the lung microvascular endothelium and induce severe lung injury via the release of vasoactive arachidonic acid [20:4(n-6)]-derived eicosanoids such as prostaglandin (PG) I₂, PGE₂ and thromboxane (TX) A₂ (Baker and Edwards 1995, Gibson et al. 1995), whereas increased levels of TXA₂ are reportedly responsible for sepsis-induced pulmonary hypertension (Baker and Edwards 1995, Sandberg 1994, Truog et al. 1986). Proinflammatory cytokines, potent vasoactive substances with a wide range of physiologic effects, also play an important role in mediating tissue damage during GBS sepsis (Mancuso et al. 1994b, Teti et al. 1992, Williams et al. 1993). Administration of inhibitors of eicosanoid and cytokine synthesis and action can prolong survival from GBS sepsis (Del Moral et al. 1996, Givner et al. 1995, Mancuso et al. 1994a, Teti et al. 1993).

Diets supplemented with (n-3) fatty acids are used to attenuate eicosanoid and cytokine synthesis (Blok et al. 1996, Caughley et al. 1996, Gallai et al. 1995, Meydani et al. 1993). In the present study, survival from GBS sepsis was studied in 7-d-old rat pups whose dams were fed a diet enriched with either (n-6) or (n-3) fatty acids throughout pregnancy and...
lactation. This model of GBS infection has been demonstrated to have physiologic effects that parallel neonatal infection (Baker and Edwards 1995, Zeligs et al. 1982).

MATERIALS AND METHODS

Animals and diets

This protocol was approved by the University of South Florida Laboratory Animal Medicine Experimentation Committee. Beginning on d 2 of gestation and throughout lactation Sprague Dawley rats (Zivic Miller, Pittsburgh, PA) were fed a reference diet (Laboratory Rodent Diet 5001, Purina Mills, Richmond, Indiana) or a synthetic diet (Purina Basal Diet 5755) whose fat source was either corn oil or menhaden fish oil. Diets were stored at −20°C and contained 125 mg/kg ethoxyquin to minimize oxidation. All diets provided 17.4 kcal/g. Dams were caged individually and had free access to diets and water. Fresh diet was provided every 48 h.

On postnatal d 3, pups were cross-fostered to dams of the same diet groups to minimize litter effects. Litters were then culled to 10 per dam. Milk was removed from culled pup stomachs and stored at −70°C. Remaining dams were used for assessment of survival following GBS challenge or for analysis of lung PGE_2 levels and lung and erythrocyte fatty acid composition.

GBS challenge

**GBS inoculum.** GBS serotype I (strain USF 704, a clinical isolate from the placenta of a woman delivering a septic newborn) was streaked from a −70°C stock culture onto a sheep blood agar plate and incubated at 37°C in 5% CO_2 for 24 h. Isolated GBS colonies on the plate were resuspended in Todd-Hewitt broth to a concentration of −10^11 cells/L. The Todd-Hewitt broth suspension was used to charge 1-mL sterile syringes with the appropriate number of bacteria to inject into pups. Viable counts were taken from the Todd-Hewitt bacterial suspension to determine the actual number of GBS organisms injected into pups and to ensure there was no contamination of the suspensions.

**GBS challenge.** On postnatal d 7, pups were injected intraperitoneally with a mean dose of 1 × 10^7 GBS colony-forming units. Mortality was assessed over the subsequent 48 h. spleens were removed from pups who died and cultured for the presence of GBS.

**Spleen cultures.** Spleen suspensions were prepared in cold 1X PBS (Gibco BRL, Grand Island, NY) using a Tekman stomacher 80 lab blender (Cincinnati, OH). Suspenders were aseptically streaked onto sheep blood agar plates, incubated at 37°C in 5% CO_2 for 24 to 48 h, and examined for the presence of GBS; β-hemolytic colonies were identified as GBS using the Phadebact Strep B Test (Boule Diagnostics AB, Huddinge, Sweden).

**Lung prostaglandin E_2 levels and lung and erythrocyte fatty acid composition**

**Lung and erythrocytes.** On postnatal d 7, pups were killed using a lethal dose of sodium pentobarbital (75 mg/kg, intraperitoneally). Lungs were quickly removed and snap-frozen in liquid nitrogen, and erythrocytes were enriched of (n-6) and (n-3) fatty acids in the corn oil and fish oil groups, respectively (Table 1). The fatty acid composition of maternal milk and pup lungs was determined and compared using cold dinitrophenylated blood was collected by cardiac puncture. Lungs and erythrocytes reflected the maternal diet, with significant enrichment of (n-6) and (n-3) fatty acids in the corn oil and fish oil groups, respectively (Table 2). Levels of 20:4(n-6) were 68% higher in lungs of pups of the corn oil group, whereas levels of eicosapentaenoic acid [20:5(n-3)] were 96% lower (P < 0.01).

**Lung prostaglandin E_2.** Mean recovery of [^3H]PGE_2 was approximately 80%. The PGE_2 levels in the lungs of pups in the fish oil and corn oil groups were 220 ± 106 and 760 ± 276 pg/mg protein, respectively (P = 0.004); levels in the reference group were 280 ± 122 pg/mg protein.

**GBS sepsis survival.** Mortality occurred within 48 h of GBS inoculation: 49% of pups in the corn oil group survived compared with 79% in the fish oil group (P = 0.0005); 67% of pups in the reference group survived.
DISCUSSION

During infection, increased blood and tissue levels of eicosanoids influence immune cell activity and modulate hemodynamic sequelae. GBS induce severe lung injury via the release of 20:4(n-6)–derived vasoactive and inflammatory eicosanoids such as PGE2, PGF2α and TXA2 (Baker and Edwards 1995, Gibson et al. 1995); interstitial inflammatory exudate is a consistent feature (Ablow et al. 1976). Thromboxane A2 metabolites are found in high concentrations in lung lymph during GBS sepsis, and TXA2 is reported to be responsible for sepsis–induced pulmonary hypertension (Baker and Edwards 1995, Sandberg et al. 1994). Treatment with inhibitors of eicosanoid synthesis can reduce the increase in pulmonary and systemic vascular resistance, improve cardiac output, stroke volume, lung compliance and oxygenation, and attenuate GBS sepsis–associated pulmonary hypertension (Baker and Edwards 1995, Gibson et al. 1995, Sandberg et al. 1994, Truog et al. 1986).

Increased synthesis of proinflammatory cytokines during infection can enhance host defense and modulate normal tissue remodeling. However, excessively high concentrations contribute to tissue injury and septic shock (Blok et al. 1996, Tracey et al. 1986). Tumor necrosis factor (TNF) α levels are particularly elevated during GBS sepsis (Baker and Edwards 1995, Ling et al. 1995, Tetri et al. 1993), and the administration of TNFα monoclonal antibodies or substances that inhibit TNFα release can attenuate the inflammatory and cardiovascular manifestations of GBS sepsis and reduce mortality (Del Moral et al. 1996, Givner et al. 1995, Mancuso et al. 1994a, Tetri et al. 1993).

Diet supplemented with fish oils is used to attenuate eicosanoid and cytokine synthesis because these diets result in replacement of 20:4(n-6) with 20:5(n-3) in membrane phospholipids (Blok et al. 1996, Caughey et al. 1996, Gallai et al. 1995, Meydani et al. 1993). Eicosanoids derived from 20:5(n-3) have less inflammatory activity and different vasoactive effects compared with those derived from 20:4(n-6) (Blok et al. 1996, Zurier 1993); 20:5(n-3) may also exhibit antiinflammatory effects by inhibiting the synthesis of leukotrienes (Lee et al., 1985). Although results vary depending upon species and tissue examined, fish oil–supplemented diets are also associated with lowered production of proinflammatory cytokines, particularly in humans (Blok et al. 1996, Gallai et al., 1995). These effects may be mediated through modulation of cytokine gene expression (Robinson et al. 1995), by alterations in eicosanoid regulation of cytokine synthesis, or by effects upon intracellular transduction pathways involved in cytokine synthesis (Blok et al. 1996). In the present study, lower mortality among pups of the fish oil–fed group may have been due in part to lower blood and tissue levels of eicosanoids derived from 20:4(n-6), as suggested by lower levels of PGE2 in pup lungs.

In summary, neonatal rat pups of dams fed corn oil–supplemented diet throughout pregnancy and lactation had higher erythrocyte and lung tissue levels of (n-6) fatty acids, higher mortality following GBS challenge and higher lung PGE2 levels compared with pups of dams fed fish oil–supplemented diet.
a fish oil–supplemented diet. These data suggest that the fatty acid composition of pre- and/or postnatal diet affects response to neonatal immune challenge and that this effect may be due in part to alterations in the synthesis of pro-inflammatory mediators.

LITERATURE CITED


