Delay of Preterm Delivery in Sheep by Omega-3 Long-Chain Polyunsaturates

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ABSTRACT

A positive correlation has been shown between dietary intake of long-chain omega-3 fatty acids in late pregnancy and gestation length in pregnant women and experimental animals. To determine whether omega-3 fatty acids have an effect on preterm labor in sheep, a fish oil concentrate emulsion was continuously infused to six pregnant ewes from 124 days gestational age. At 125 days, betamethasone was administered to the fetus to produce preterm labor. Both the onset of labor and the time of delivery were delayed by the fish oil emulsion. Two of the omega-3-infused ewes reverted from contractions to nonlabor, an effect never previously observed for experimental glucocorticoid-induced preterm labor in sheep. Maternal plasma estradiol and maternal and fetal prostaglandin E2 rose in control ewes but not in those infused with omega-3 fatty acids. The ability of omega-3 fatty acids to delay premature delivery in sheep indicates their possible use as tocolytics in humans. Premature labor is the major cause of neonatal death and long-term disability, and these studies present information that may lead to a novel therapeutic regimen for the prevention of preterm delivery in human pregnancy.

INTRODUCTION

Over the last two decades, dietary n-3 long-chain polyunsaturated fatty acids of marine origin, particularly eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3), have gained increasing medical attention. It is known that the n-3 fatty acids (FAs) both inhibit the synthesis of arachidonic acid and compete with it for incorporation into the sn-2 position of glycerophospholipids and as a substrate for the cyclooxygenase, lipoxygenase, and epoxygenase enzymes, thus reducing the level of arachidonic acid in phospholipids and leading to the formation of eicosanoids (prostaglandins, leukotrienes, and thromboxanes) with lower biological activity [1, 2]. Through these effects, fish oils are anti-inflammatory, vasodilatory, antihypertensive, and antiatherothrombotic [3]. However, subsequent studies on their arrhythmogenic effects have revealed that EPA and DHA modulate ion currents through L-type calcium channels. This effect occurs within minutes and is reversible. In addition, infusing a fish oil emulsion i.v. just prior to the exercise-and-ischemia test decreases the heart rate and prevents ventricular fibrillation in susceptible dogs [4], indicating that the free FAs themselves may have direct effects on the structure and function of cardiac myocyte plasma membranes, independent of their role as eicosanoid precursors.

In pregnancy, a positive correlation has been shown between prolonged dietary intake of n-3 FAs in late pregnancy and gestation length and birth weight, possibly because these FAs alter the balance between stimulatory and inhibitory prostaglandins in the parturition process [5, 6]. The n-3 FAs may also increase the prostacyclin:thromboxane ratio, thereby promoting vasodilatation and reducing blood viscosity [3], both of which would facilitate placental blood flow and thus improve fetal growth.

Throughout pregnancy, uterine activity takes the form of low-amplitude, low-frequency contractures that last 3–15 min and occur every 40–60 min. At labor, contractures switch to expulsive contractions of higher amplitude occurring every 2–3 min [7, 8]. However, the effect of infusions of n-3 FAs on uterine contractility has not been reported. The purpose of this study was to evaluate the effects of n-3 FAs on prostaglandin synthesis and to determine whether n-3 FAs would prevent or delay the switch from myometrial contractures to contractions in a reproducible model of ovine premature labor [9].

MATERIALS AND METHODS

Instrumentation

Twelve Rambouillet-Columbia ewes, mated on a single occasion and of known gestational age, were instrumented at 116–117 days gestational age (dGA, term = 147 dGA) with stainless steel electrodes in the myometrium for recording electromyogram (EMG) activity, as described in detail previously [7]. Polyvinyl catheters were placed in the maternal uterine and jugular vein and carotid artery. Catheters were also placed in the fetal jugular vein, carotid artery, and the amniotic cavity. In twin pregnancies, the smaller fetus was removed and killed at surgery. All animals were allowed 5 days of recovery before any experiment began, during which period the ewes received a daily dose of 1 g ampicillin i.m. and 0.5 g ampicillin into the amniotic sac.

Experimental Procedures

Beginning at 1200 h on 123 dGA, 8-ml blood samples were taken daily under sterile conditions from the fetal and maternal carotid arteries and the uterine vein on the pregnant side, with return of the blood cells to the respective jugular veins after centrifugation and plasma removal. From 124 dGA, a 20% emulsion of either intralipid (Baxter, Deerfield, IL; control, n = 6) or omega-3 (n = 6) was continuously infused (3 ml/kg per day) i.v. to the ewes until the end of the study. Doses of omega-3 were chosen to correspond to a moderate to high infusion rate of intralipid known from our previous studies to be well tolerated by pregnant sheep. At 125 dGA, betamethasone (Celestone, Deerfield, IL; control, n = 6) was administered into the fetal jugular vein at a rate of 10 μg/h over 48 h to induce premature labor, as described in detail elsewhere [9]. Labor was defined as having occurred when the EMG record showed a clear switch from myometrial contractures to labor-type contractions followed by contraction activity for at least 5 h. Necropsy was performed after delivery or, if delivery did not occur within 5 days from the end of the
Betamethasone infusion (post-beta), after cesarean section. Fetal body weight and the weight of the fetal liver, lungs, and brain were recorded at necropsy. All surgeries and necropsies were conducted under halothane general anesthesia. Studies were approved by the Cornell University Institutional Animal Care and Use Committee. All facilities were approved by the American Association for the Accreditation of Laboratory Animal Care.

Oil Emulsions

Intralipid is a 20% lipid emulsion containing soybean oil with only 7% of the total lipid as n-3 FA, a-linolenic acid (C18:3n-3), and trace amounts of long-chain fatty acids added incidentally as a component of egg lecithin emulsifier. Omega-3 is a 20% n-3 fatty acid emulsion made using a modification of the method of Billman et al. [10]. To make 1000 ml emulsion, 200 ml of fish liver oil 30/20 concentrate (Arista, Darien, CT; total n-3 FA content 61% with EPA and DHA, as ethyl ester, composing 31% and 20% of the total, respectively), 15 g purified egg lecithin, 200 IU a-tocopherol, and 500 mg butylated hydroxytoluene were added to 800 ml of water containing 12.5 g glycerol, pH adjusted to 7.4 with NaOH; and the mixture was sonicated to a milky white stable emulsion. It was stored at 4°C for 8 h with TxB2, 0.8%, and PGF2a, 2.2%, and the solvent blank was negligible. Assay values were not corrected for recovery. Plasma pools were made by adding 0.5, 1, 2, and 8 ng/ml exogenous PGE2 to charcoal-extracted pooled sheep plasma. When aliquots of 100 μl of these levels were extracted and assayed, 0.58, 0.74, 1.61, and 5.69 ng/ml were measured, respectively (n = 10). The sensitivity of the assay was 3.13 pg/tube. The intraassay CV was 8.7% and the interassay CV 14.9%.

Hormone Assays

Blood samples were drawn anaerobically and collected in chilled heparinized tubes. The tubes were then spun at 3000 × g for 5 min. The plasma was removed, immediately flash frozen in liquid nitrogen, and stored at −80°C until analysis. Tubes for the storage of samples for prostaglandin assay were pre-rinsed with a preservative aspirin-EDTA solution.

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\text{Prostaglandin E}_2
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Prostaglandin (PG)E2 was quantified in maternal uterine vein and fetal carotid plasma by RIA [12]. The assay uses a polyclonal antibody, kindly provided by Dr. L. Myatt, generated in rabbits to a PG E2-BSA conjugate. The cross-reactivity of the antibody with PGF2a was 100%, with PGD2 1.8%, with PGF2α 1.5%, with 6-keto-PGE2 2.2%, and with TxB2 < 1%. Tritiated PG E2 (DuPont NEN, Boston, MA) served as the labeled ligand, and dextran-coated charcoal PBS solution was used to separate bound from free ligand. PG E2 standards (Cayman Chemical Co., Ann Arbor, MI) used at 3.13–400 pg per tube were diluted in the assay buffer (0.1% gelatin in 0.1 M PBS). Aliquots (100 μl) of diethyl ether-extracted and bovine gamma globulin-reconstituted sample were assayed in duplicate. Tritiated PG E2 at 5000 cpm and PGF2a antisemur diluted 1:15,000 were added in 100-μl aliquots each to the standards and extracted samples, and incubation was performed at 4°C overnight. A 15-min incubation with 1 ml dextran-charcoal solution at 4°C was followed with centrifugation at 2400 × g for 15 min at 4°C. The supernatant containing the bound ligand was decanted into scintillation vials, scintillation fluid was added, and beta counting was performed. Extraction recovery of tritiated PGE2 added to plasma was 79.5 ± 2.1%, and the solvent blank was negligible. Assay values were not corrected for recovery. Plasma pools were made by adding 0.5, 1, 2, and 8 ng/ml exogenous PGE2 to charcoal-extracted pooled sheep plasma. When aliquots of 100 μl of these levels were extracted and assayed, 0.58, 0.74, 1.61, and 5.69 ng/ml were measured, respectively (n = 10). The sensitivity of the assay was 3.13 pg/tube. The intraassay CV was 8.7% and the interassay CV 14.9%.

Statistical Analyses

Student’s two-sample t-tests were used to compare the two groups’ onset of labor, the fetal body, liver, brain, and lung weights, and daily hormone levels. Times of delivery or cesarean section were compared using the Mann-Whitney Rank Sum test. Within each group, hormone levels at Day 0 (baseline samples just before commencement of lipid infusion) were compared with levels at Day 3 (at the end of betamethasone infusion) and Day 4 using a one-way ANOVA with Student-Newman-Keuls procedure. Differences of p < 0.05 were considered significant. Data are presented as mean ± SEM.

RESULTS

Labor and Delivery

Three outcomes were observed in the six omega-3-infused ewes: two ewes went into labor 19–44 h post-beta and delivered 4–15 h later, giving results comparable to those of the control group; two ewes went into labor at 102 h post-beta and delivered 5–11 h later; two ewes went into labor at 25–26 h post-beta, remained in labor for 26–27 h, and then resumed the contractures mode until elective cesarean section at 120–180 h post-beta (Fig. 1). The cervix was found closed in these two pregnant ewes.

Both the onset of labor (53 ± 16 h post-beta in omega-3-infused ewes compared with 20 ± 7 post-beta in controls) and time of delivery, whether spontaneous or by cesarean section (68 ± 14 h post-beta in omega-3-infused ewes compared with 30 ± 11 h in control ewes), were significantly different.
different from Day 0 of the same group; b: different from control (p < 0.05).

Body and Organ Weights

All fetuses were healthy at delivery. The weights of the fetal body, liver, lungs, and brain were, respectively, 3.5 ± 0.2 kg, 109.1 ± 4.1 g, 101.5 ± 4.9 g, and 49.5 ± 1.9 g for the control group and 3.4 ± 0.1 kg, 95.2 ± 3.5 g, 103.2 ± 5.6 g, and 43.9 ± 0.7 g for the omega-3 group. These weights were related neither to the sex of the fetus nor to the presence of a twin at surgery, and, relative to body weight, were similar in the two groups.

Plasma Hormone Levels

Maternal estradiol. Baseline (Day 0) maternal plasma estradiol concentrations were similar between control and omega-3 groups (33.7 ± 2.6 and 34.5 ± 2.7 pg/ml, mean ± SEM, respectively, Fig. 2A). After betamethasone infusion, there was a rise in estradiol levels in control ewes (to 158.9 ± 39.5 pg/ml on Day 4) but not in the omega-3 group.

Maternal progesterone. Baseline maternal plasma progesterone levels were similar between control and omega-3 groups (25.5 ± 2.8 and 25.8 ± 2.7 ng/ml, respectively, Fig. 2B). After betamethasone treatment, there was an equal decline in maternal progesterone in the two groups (to 10.7 ± 2.1 and 9.6 ± 2.1 ng/ml, respectively, on Day 4).

Maternal uterine vein PGE 2. Baseline maternal PGE 2 levels were similar in the two groups (1.2 ± 0.2 and 0.9 ± 0.2 ng/ml for control and omega-3 groups, respectively, Fig. 2C). PGE 2 levels were elevated in control ewes after betamethasone infusion (to 2.1 ± 0.3 ng/ml) but not in omega-3-infused ewes.

Fetal PGE 2. Baseline PGE 2 in fetal plasma was similar in the two groups (0.8 ± 0.1 and 0.6 ± 0.1 ng/ml for control and omega-3 groups, respectively, Fig. 3). Fetal plasma PGE 2 levels were elevated in control ewes after betamethasone infusion (to 1.4 ± 0.1 ng/ml) but not in omega-3-infused ewes.

Fetal ACTH. ACTH levels were similar in fetuses of the two groups during the baseline period (14.2 ± 1.4 and 16 ± 2.5 pg/ml for control and omega-3 groups, respectively, Fig. 3), and were equally elevated after betamethasone infusion (to 57.3 ± 24 and 52 ± 20.1 ng/ml on Day 4 in fetuses of control and omega-3-infused ewes, respectively).

Fetal cortisol. Both groups’ fetal cortisol levels were below the assay’s detection limit during the baseline period but showed a small rise after betamethasone treatment (to 19.1 ± 13.4 ng/ml and 16.8 ± 8.3 ng/ml on Day 4 in control and omega-3 groups, respectively).

DISCUSSION

Omega-3 long-chain polyunsaturates (LCPs) increased the time interval from fetal betamethasone infusion to the onset of contractions and labor and delivery. One previous study on women indicated that dietary intake of fish oil did not affect the duration of labor or the need for operative delivery [6]. However, in our study, precise control of delivery of omega-3 LCPs was possible in a manner not achievable in the human study. Other studies on the prolongation of gestation by fatty acids [5, 13], however, do not comment on the time interval between labor onset and delivery, so it is difficult to evaluate the present study in sheep in relation to available reports in human pregnancy. Neither is it possible to speculate on the effect of n-3 FAs on the length of gestation, since all our experiments were terminated before normal term (147 dGA). The heterogeneous response of the six omega-3 ewes in our study indicates that the dosage of betamethasone used is sufficiently powerful to overcome the effects of n-3 LCPS, which is not surprising in view of the fact that betamethasone is a very effective inducer of labor in pregnant sheep [9]. If so, less powerful premature delivery may be blocked more effectively by n-3 LCPS. It should be noted, however, that this study presents the first reported instance of sheep switching...
into contractile labor, as indicated by myometrial EMG recordings, and then returning to the contractures mode. We have extensive experience with this model in over a hundred animals and have never before seen this pattern of reversal of the mode of uterine activity. A similar irreversibility of the switch from contractures to contractions has been demonstrated in a wide variety of laboratories throughout the world since Liggins [14] first demonstrated in the late 1960s that glucocorticoids administered to fetal sheep, at the doses we have used, invariability and inexorably lead to premature delivery [14].

The observed changes in maternal hormone levels suggest that a possible mechanism of action of n-3 LCP is to decrease the activity of placental 17α-hydroxylase (P45017α-OH), with a resultant decrease in conversion of progesterone to estradiol. We have demonstrated that estradiol up-regulates both prostaglandin endoperoxide synthase-2 mRNA and protein in ovariectomized nonpregnant sheep myometrium [15]. Thus, decreased estrogen production would result in decreased PGE2 synthesis as we have demonstrated and delay or prevent the switch of the myometrial contractility pattern to labor-type contractions. In addition to the observed decreased production of 2-series prostaglandin, n-3 LCP may lead to production of 3-series prostaglandins. Because of the lack of a selective PGE2 antibody, we were unable to measure PGE2 levels in order to determine whether there might have been competitive inhibition of 2-series prostaglandin with accompanying increased 3-series prostaglandin production in the omega-3-infused ewes.

Concerns have been expressed regarding side effects, such as anti-inflammatory responses and prolongation of bleeding time, that might accompany the therapeutic use of fish oil-supplemented diets [16]. It is doubtful, however, that relatively short-term administrations would have deleterious effects, especially if appropriate dosages were established. In this experiment, we infused the fatty acid emulsions at a rate that has previously been used without harm to the ewes’ health [17]. If further clinical trials did prove that n-3 FAs could prevent premature delivery in human preterm labor, similar uses of these agents in suitable doses would represent a very benign intervention and potentially reduce the effective dose of tocolytic drugs required.

In conclusion, the results show that betamethasone-induced premature delivery is prevented or delayed by the action of omega-3 long-chain polyunsaturated fatty acids. Premature labor is the major cause of neonatal death and long-term disability, and these studies present information that can be used to design therapeutic regimens for the prevention of preterm delivery in human pregnancy. However, although these preliminary results are encouraging, further work is required to determine the mechanism of action of n-3 FAs on myometrial activity and evaluate the effects of different dosage levels.

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REFERENCES