OBSTETRICS

Maternofetal pharmacokinetics and fetal lung responses in chronically catheterized sheep receiving constant, low-dose infusions of betamethasone phosphate

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BACKGROUND: Antenatal steroids are standard of care for cases of anticipated preterm labor to improve neonatal outcomes. However, steroids are potent drugs, and their use in pregnancy remains largely unoptimized.

OBJECTIVE: The objective of the study was to measure the maternofetal pharmacokinetics of constant, low-dose intravenous betamethasone phosphate infusions and correlate these data with the transcriptional effect exerted by subclinical betamethasone exposures on the ovine fetal lung.

STUDY DESIGN: Thirty-two ewes carrying a single fetus had surgery to catheterize fetal and maternal jugular veins at 116 days of gestation (term, 150 days). Animals were recovered for 2 days and then were randomized to receive 2 sequential maternal intravenous infusions of either (n = 4/group) of the following: 1) saline, 0.125, 0.04, or 0.0125 mg/kg betamethasone phosphate over 3 hours; or 2) saline, 0.25, 0.08, or 0.025 mg/kg betamethasone phosphate over 12 hours. Each infusion was separated by 2 days. Fetal lung tissue was collected for analysis using quantitative polymerase chain reaction and an ovine-specific microarray. Plasma betamethasone levels from time-course catheter samples were determined by mass spectrometry. Data were assessed for distribution, variance, and tested by an analysis of variance.

RESULTS: Betamethasone was detectable (>1 ng/mL) in fetal plasma only in animals randomized to 0.125 mg/kg 3 hour or 0.250 mg/kg 12 hour infusions. Fetal betamethasone half-lives were 1.7–2.8 times greater than maternal values. At maximum concentration, fetal plasma betamethasone levels were approximately 10% of maternal levels. Compared with saline control, all animals, other than those receiving 0.0125 mg/kg 3 hour betamethasone phosphate infusions, had evidence of dose-dependent glucocorticoid transcriptional responses in the fetal lung.

CONCLUSION: Constant maternal betamethasone infusions delivering substantially lower fetal and maternal betamethasone maximal concentrations than those achieved with current clinical treatment protocols were associated with dose-dependent changes in glucocorticoid-response markers in the fetal lung. Further studies to determine the minimally efficacious dose of steroids for improving outcomes in preterm infants should be viewed as a priority.

Key words: antenatal steroids, betamethasone, fetus, glucocorticoid, pharmacokinetics, preterm birth

The use of antenatal steroids to precociously mature the fetal lung in cases of expectant preterm birth (delivery before 37 weeks' gestation) is among the most important advances in perinatal medicine to date.[1] Worldwide, more than 15 million babies are born preterm every year, and, of those, more than 1 million will die.[2] First clinically studied in a trial in New Zealand,[3] following experimental studies with sheep,[4] antenatal steroid therapy was shown to reduce both neonatal death and a suite of comorbidities including respiratory distress syndrome and cerebral hemorrhage in premature babies delivered between 24 and 34 weeks of gestation.[5,6]

The following 2 drug formulations are in common clinical use in expectation of preterm delivery[7]: (1) a dual formulation of betamethasone phosphate and betamethasone acetate, administered as a course of 2, 12 mg intramuscular injections 24 hours apart; and (2) dexamethasone phosphate, administered as a course of 4, 6 mg intramuscular injections 12 hours apart. Betamethasone and dexamethasone have similar pharmacokinetic properties; the combined betamethasone phosphate and acetate dosing regimen used by Liggins and Howie[8] achieved a maximum fetal plasma betamethasone concentration of approximately 20 ng/mL 1–2 hours after treatment.

Steroids are both highly potent and broad-acting drugs that have regulatory effects on as much as 20% of the genome.[9] Antenatal steroids can perturb a wide range of homeostatic systems including insulin signalling,[10] and the catabolic effects of antenatal steroids are associated with increases in maternal and fetal plasma amino acid concentrations.[11] A number of investigators have expressed concern regarding aspects of antenatal steroid use in pregnancy, specifically with regard to a lack of dose optimization[12,13] and the use of repeated courses of antenatal steroids when delivery occurs more than 7 days after treatment.[14]

Importantly, the extent to which current antenatal steroid dosing regimens are optimal for safely inducing fetal lung maturation remains unclear.[7] Antenatal steroids are not Food and Drug Administration approved for this use in pregnancy,[1] and there has been comparatively little work undertaken to

Cite this article as: Kemp MW, Saito M, Usuda H, et al. Maternofetal pharmacokinetics and fetal lung responses in chronically catheterized sheep receiving constant, low-dose infusions of betamethasone phosphate. Am J Obstet Gynecol 2016;xxx:xx-xx.

0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2016.08.017

refine the optimal dosing. Moreover, there is no modification of antenatal steroid dosing to take into account pregnancy factors (ie, gestational age, maternal weight) that almost certainly have an impact on the magnitude and duration of fetal steroid exposure.

Although the long-term effects on humans are unclear, fetal growth restriction from antenatal steroid exposure has been demonstrated in numerous animal models including sheep[15] and mice.[16] Sustained exposures to large quantities of antenatal steroids have been linked to changes in fetal fluid composition,[11] placental morphology, and alterations in brain development.[17] Kutzler et al[18] have reported that multiple courses of low-dose maternal dexamethasone (4 2 mg doses at 12 hour intervals) decreased birthweight and brain weight, although these changes did not persist at 2 weeks of postnatal life.

Data from animal studies suggest that efforts to optimize antenatal steroid dosing are warranted. Jobe et al,[19] for example, reported that fetal sheep have increased responsiveness to antenatal steroids with increasing gestational age and that multiple antenatal steroid doses may yield a decreased benefit with increasing gestation. Loehle et al[20] demonstrated that administering 50% of the clinical dose of betamethasone elicits maximal improvement in lung compliance (measured by pressure-volume curve) in fetal sheep at 0.75 gestation. Subsequent work, again in the sheep model, demonstrated that minimal fetal betamethasone exposures deriving from maternal betamethasone acetate injections were associated with lung maturation comparable with that with the clinical antenatal steroid dosing.[21]

Materials and Methods

The present study was designed to inform preclinical animal studies to allow the optimization of antenatal steroid dosing for expectant preterm deliveries. Aiming to lower peak fetal betamethasone exposure, we used an extended intravenous administration protocol to deliver set doses of betamethasone to achieve a lower maternal and fetal maximum concentration than with intramuscular administration.

The 2 primary objectives were to investigate the feasibility of delivering a weight-calibrated subclinical dose of antenatal steroids to the mother and fetus and to measure maternofetal betamethasone pharmacokinetics. A secondary objective was to correlate these data with evidence of fetal lung maturation as measured by transcriptional changes markers of glucocorticoid signaling response. These studies were undertaken using a well-validated sheep model of pregnancy.

Study design

Animal work

All protocols were reviewed and approved by the University of Western Australia's Animal Ethics Committee (RA/3/100/1378). All animal work was conducted in accordance with the guidelines set out in the National Health and Medical Research Council's Australian code for the care and use of animals for scientific purposes.

Thirty-two date-mated ewes carrying singleton fetuses at 116 days of gestational age (term, approximately 150 days) were acclimatized to a large animal surgical facility for 10 days before undergoing a recovery surgical procedure of 50 minutes in duration to place bilateral maternal jugular and unilateral fetal jugular catheters as previously described.[22] There were no interoperative fetal losses.

Animals were recovered for 2 days before being randomized to receive 2 sequential maternal intravenous infusions of either (n = 4/group) of the following: (1) saline, 0.125, 0.04, or 0.0125 mg/kg betamethasone phosphate over 3 hours; or (2) saline, 0.25, 0.08, or 0.025 mg/kg betamethasone phosphate over 12 hours. Each infusion was separated by 2 days. Infusions were delivered with an ambulatory CADD infusion pump (Smiths Medical, St Paul, MN) attached to the unrestrained ewe's back, with each infusion commencing at 8:00 AM. The total volume infused for all groups was 90 mL of betamethasone phosphate in sterile saline.

Betamethasone phosphate dosing was designed such that the highest dose, 0.25 mg/kg, was approximately equivalent to the dose received by a 50 kg woman receiving antenatal steroid therapy with a combined betamethasone phosphate and betamethasone acetate preparation, such as Celestone Chronodose (Merck & Co, Inc, Kenilworth, NJ); stepped-down doses were chosen to allow for the titration of a potential dose response in lung maturation. Two milliliters of maternal and fetal blood samples were collected into chilled K_3EDTA vacutainers at T-minus 10 minutes and then 1, 2, 3, 4, 6, 8, 10, 12, 14, 15, and 24 hours. Separate maternal catheters were used for infusions and sample collection. Samples were centrifuged at 3000 × *g*, and the cleared plasma frozen −80°C for the subsequent analysis.

All animals were euthanized with intravenous pentobarbitone (160 mg/kg) immediately after the conclusion of their second infusion, and the fetus delivered for necroscopy. Fetal lung tissue (right lower lobe) was dissected and snap frozen in liquid nitrogen before being stored at −80°C for quantitative polymerase chain reaction and microarray analysis.

Measurement of plasma betamethasone by mass spectrometry

Plasma samples and betamethasone standards (500, 250, 100, 50, 25, 12, and 0 ng/mL) in control fetal sheep plasma were extracted as follows: 50 μL sample or standard was added to 50 μL of internal standard (deuterated betamethasone, 50 ng/mL), vortexed in a sealed tube for 10 seconds and then incubated at room temperature for 5 minutes. One milliliter of methyl-tert-butyl ether was added before samples were vortexed in sealed tubes for 2 minutes and then centrifuged at 3000 rpm for 10 minutes at room temperature. A total of 850 μL of cleared sample was transferred to an autosampler vial, dried under vacuum at 3000 rpm for 40 minutes at 37°C, and reconstituted in 70 μL of a 1:1 solution of methanol plus 0.1% formic acid in H_2O. Samples were capped, incubated with gentle shaking for 10 minutes at 50°C, and then analyzed. Data were fitted to a

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