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Betamethasone for Lung Maturation: Testing Dose and Formulation in Fetal Sheep

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Abstract

Objectives— We evaluated lung maturation responses to the mixture of betamethasone phosphate (Beta-PO₄) + betamethasone acetate (Beta-Ac) in comparison to Beta-PO₄ or Beta-Ac in fetal sheep.

Study Design— Ewes carrying singleton pregnancies at 122d gestation were randomized to: single doses of 0.5 mg/kg Beta-PO₄ + Beta-Ac, 0.25 mg/kg Beta-PO₄, 0.5 mg/kg Beta-PO₄ or 0.25 mg/kg Beta-Ac given 48h before delivery. These treatments were compared to saline placebo and 2 doses of 0.5 mg/kg Beta-PO₄ + Beta-Ac given 48 and 24h prior to delivery. Fetal lung maturation was evaluated.

Results— The 2 doses of the Beta-PO₄ + Beta-Ac mixture gave the best lung maturation. Single doses of the Beta-PO₄ + Beta-Ac mixture and Beta-Ac also induced lung maturation. There were no consistent responses to either dose of Beta-PO₄.

Conclusions— Beta-PO₄ alone is ineffective with the dosing schedule used, while Beta-Ac can induce lung maturation. However, the best responses result from the mixture of Beta-PO₄ + Beta-Ac.

Keywords

Respiratory Distress Syndrome; preterm; corticosteroids; fetal sheep; lung development

INTRODUCTION

Antenatal corticosteroid treatments are standard of care for women at risk of early preterm labor. However, there remain questions about the optimal corticosteroid, the dose and the formulation, as reviewed in the new meta-analysis of corticosteroids (1). The betamethasone preparation used clinically is a 1 to 1 mixture of soluble and rapid acting betamethasone-sodium-phosphate (Beta-PO₄) and the slow release betamethasone-acetate (Beta-Ac)

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CONDENSATION: The combined preparation of betamethasone phosphate and betamethasone acetate used clinically is more effective than either betamethasone component to induce lung maturation in fetal sheep.

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originally selected by Liggins and Howie (2). The Beta-PO₄ and Beta-Ac mixture is the primary corticosteroid preparation used world-wide because its use is associated with better neonatal outcomes than dexamethasone (3,4). Because there continue to be concerns about potential toxicity of corticosteroids in pregnancy, strategies to minimize maternal and fetal exposures are desirable. We proposed that the long half-life of Beta-Ac may not contribute to the beneficial fetal responses (3). We also suggested that two 12 mg doses may not be required because infants delivered 24h after the first dose have benefit (1,3). If true, maternal treatments with 6 mg Beta-PO₄ might be equivalent to the standard 24 mg dose given as two 12 mg doses. Ultimately it would be desirable to develop an optimal agent and dosing strategy for antenatal corticosteroids. We report a comparison of fetal lung responses in sheep to the clinical betamethasone preparation and its two components - Beta-PO₄ and Beta-Ac.

MATERIALS AND METHODS

Betamethasone treatments

The animal studies were approved by the animal ethics committees at Cincinnati Children's Hospital and by the Western Australian Department of Agriculture. Singleton pregnant Merino ewes were weighed and control ewes received IM saline injections 24h apart on days 122 and 123 of gestation. Other groups received: an IM dose of 0.5 mg/kg of the 1 to 1 clinical preparation of Beta-PO₄ and Beta-Ac on day 122 (0.5 Beta-Phos + Beta-Ac × 1), doses of 0.5 mg/kg with the same phosphate and acetate mixture on days 122 and 123 (0.5 Beta-Phos + Beta-Ac × 2), one dose of 0.25 mg/kg Beta-PO₄ on day 122 (0.25 Beta-Phos × 1), one dose of 0.5 mg/kg Beta-PO₄ on day 122 (0.5 Beta-Phos × 1), or 0.25 mg/kg Beta-Ac on day 122 (0.25 Beta-Ac × 1). The phosphate and acetate mixture was the Celestone Chronodose® (Schering Plough, Australia) routinely used clinically and given as the optimal dose to cause lung maturation in fetal sheep (5). The Beta-PO₄ (Sigma Chemical, St. Louis, MO) was solubilized in saline, filtered for sterility, and given in the same volume as the clinical drug. The Beta-Ac (Sigma Chemical) was solubilized in 70% ethanol and diluted into saline to generate a fine suspension. The fine suspension was visually similar to the suspension of the Beta-Ac in Celestone. This suspension also was given by IM injection in the same volume.

Assessments of lung function

The ewes were anesthetized with ketamine and xylazine and killed with a penetrating captive bolt just prior to delivery of the fetus. The fetal head was delivered through an abdominal incision and, following local injection of Lidocaine, a 4 mm endotracheal tube was secured by rapid tracheotomy (6). The fetus then was delivered, weighed, and mechanical ventilation was initiated using 100% heated and humidified oxygen. The initial ventilator settings were a rate of 40 breaths/min, a peak inspiratory pressure of 35 cmH₂O, a positive end expiratory pressure of 5 cmH₂O and an inspiratory time of 0.7 sec (7). An umbilical artery catheter was placed to permit blood sampling. The lambs were ventilated for 30 min with blood gas measurements at 10, 20, and 30 min. Tidal volume was measured continuously with a tidal volume monitor (Florian Infant Monitor, Acutronic Medical Systems, Switzerland) (8). Peak inspiratory pressure only was changed to target a Pco₂ of about 60 mmHg using a tidal volume of less than 10 ml/kg. Peak inspiratory pressures were limited to 40 cmH₂O pressure to avoid pneumothorax. Compliance/kg was calculated as tidal volume divided by peak minus end expiratory pressure and by body weight. Ventilation efficiency index is an integrated measure of ventilation that is calculated as:

Ventilation efficiency index = $3800/(P \bullet F \bullet PaCO_2)$, where 3800 is a CO₂ production constant, P is ventilatory pressure and F is ventilator rate for these lambs without spontaneous breathing (9). The investigators delivering the lambs, ventilating the lambs, and assessing the lungs were not aware of the Beta treatment that the ewe had received.

Assessments of lungs

After the collection of a final arterial blood sample at 30 min of age, the lambs were deeply anesthetized with pentobarbital and the tracheal tube was clamped for 3 min to achieve atelectasis by oxygen absorption. The lamb was then exsanguinated, the chest was opened, and the lungs were evaluated visually for pulmonary interstitial emphysema (10). A deflation pressure-volume curve then was measured after air inflation of the lungs to a pressure of 40 cmH₂O (11). After the removal of the lungs from the chest, each lung was weighed, and the left lung was lavaged 3 times by infusion and withdrawal of a sufficient volume of 4°C normal saline solution to fully fill the lung (7). The bronchoalveolar lavages (BAL) were pooled; the total volume was measured, and the aliquots were saved for later analyses.

Surfactant measurements

Saturated phosphatidylcholine (Sat PC) was isolated from chloroform-methanol (2:1) extracts of BAL fluid by neutral alumina column chromatography after exposure of lipid extracts to osmium tetroxide (12). Sat PC was quantified by phosphorus assay (13). The mRNAs for Surfactant Proteins-A, B, and C were measured using S1 nuclease protection assays, as described previously (14).

Statistical analysis

Data are presented as mean $\pm$ SEM, and statistical comparisons were performed with Instat software. A differences between groups was considered significant for a probability value of $<.05$. Data were compared between controls and each of the 5 corticosteroid treatment groups with one-way analysis of variance with the Tukey post hoc test for multiple comparisons. The pressure-volume curves were compared by a repeated measures ANOVA. Selected group comparisons to controls by two tailed t-tests are indicated in the text.

RESULTS

Description of animals

The numbers, birth weights, and sex of the lambs in each group are given in Table I. The birth weight of the 0.5 Beta-PO₄ + Beta-Ac $\times$ 2 group was lower than the control group by t-test ($p<.05$), but there were no other differences in birth weights in other groups. The animals had similar cord blood gas values at delivery (data not shown).

Ventilation outcomes

The newborn lambs were ventilated for 30 min in a standardized fashion to evaluate lung mechanisms and gas exchange (Table II). The 30 min blood gas pH values were higher for the two 0.5 Beta-PO₄ + Beta-Ac treated groups than controls and qualitatively better than for the other treatment groups. The Pco₂ values were lower for the 0.25 Beta-PO₄, 0.5 Beta-PO₄ and 0.25 Beta-Ac groups relative to control. Only the two dose 0.5 Beta-PO₄ + Beta-Ac group had a significant increase in Po₂, required less peak inspiratory pressure and achieved reasonable Pco₂ values. At autopsy this same group had a striking increase in pulmonary interstitial emphysema relative to control and the other groups.

Lung function was evaluated at 30 min by measuring total thoracic compliance, ventilation efficiency index as a measure of gas exchange, and the lung gas volumes on the deflation limbs of the pressure-volume curves. Thoracic compliance was increased by the 2 dose 0.5 Beta-PO₄ + Beta-Ac treatment relative to all groups and the single dose of 0.5 Beta-PO₄ + Beta-Ac increased compliance relative to the control by t-test ($p=0.04$) (Fig. 1). The two 0.5 Beta-PO₄ + Beta-Ac groups increased ventilation efficiency relative to control and the two Beta-PO₄ groups. The 0.5 Beta-PO₄ + Beta-Ac $\times$ 2 group had a higher ventilation efficiency index

than all other groups. The 0.25 Beta-Ac group had increased ventilation efficiency relative to control by t-test ($p=0.012$). The maximal lung volumes measured at 40 cmH₂O airway pressure were increased similarly and significantly by the treatments with the Beta mixture and the Beta-Ac alone relative to control and Beta-PO₄. The only treatments that improved the deflation limbs of the pressure-volume curves were the two 0.5 Beta-PO₄ + Beta-Ac groups and the 0.25 Beta-Ac group (Fig. 2). The lack of much increase in volumes at 0 and 10 cmH₂O pressure suggest little effect of surfactant to stabilize deflation for any of the treatment groups. The patterns of physiologic responses for blood gas values and lung mechanics indicate that the single dose of 0.25 Beta-Ac improved lung function but not as much as either a single or two doses of the mixture of 0.5 Beta-PO₄ + Beta-Ac. There was no consistent improvement in lung function in response to either dose of Beta-PO₄ for the dosing schedule used for this experiment.

Surfactant components

The amounts of Sat PC recovered by BAL were similar across all groups (Table III), as has been reported previously 48h after antenatal corticosteroid treatment (7). The mixture of 0.5 Beta-PO₄ + Beta-Ac increased the mRNA levels for SP-A, SP-B, and SP-C by approximately 2-fold relative to control values. The 0.5 Beta-PO₄ and the 0.25 Beta-Ac also significantly increased the mRNA levels for all three surfactant proteins. The 0.25 Beta-PO₄ did not increase these mRNA levels.

COMMENT

Although corticosteroids for antenatal treatments of women at risk of preterm delivery are standard of care (1), there is minimal clinical information to guide the choice of corticosteroid or dose. Also, the formulation and dose of the corticosteroid have not been developed and tested for this unique indication in pregnant women. Our hypothesis about the betamethasone mixture used clinically was that Beta-Ac did not contribute to the clinical response because clinical effects occur within 24h and the half life of Beta-Ac is reported to be 7d (3,16). Our second hypothesis was that two doses separated by 24h is unnecessary because 24h is enough to detect benefit in women and lung responses in fetal sheep are apparent by 15h (1,17). Both hypotheses were not supported by these results in preterm sheep. The two doses of Beta-PO₄ + Beta-Ac separated by 24h improved lung maturation more than did the single dose 48h prior to delivery. Furthermore, the 0.25 mg/kg Beta-PO₄ given at an equivalent Beta-PO₄ content as the 0.5 mg Beta-PO₄ + Beta-Ac mixture had essentially no effects on lung maturation and twice the dose had minimal effects. Therefore, Beta-PO₄ by itself does not effectively induce lung maturation as dosed in this study. The surprise was that Beta-Ac seemed to be effective.

These results need to be considered within the context of several aspects of antenatal corticosteroid treatments that remain to be explained. In sheep fetal injections with the Beta-PO₄ + Beta-Ac mixture using ultrasound guidance causes some lung maturation but no fetal growth restriction (11). In contrast, maternal treatments result in a better lung maturation response, but cause fetal growth restriction despite exposure of the fetus to much lower peak levels of betamethasone (11,18). A large dose of cortisol given IV to the fetus does not cause lung maturation (5) but, repetitive doses of cortisol given over 6h to the fetal sheep do cause lung maturation (6). When taken together, these observations suggest that the lung maturational effects of betamethasone depend on a continuous exposure over hours, but that maternal or fetal plasma levels of betamethasone may not need to be very high. The differences in fetal growth restriction responses may result from unidentified effects of the corticosteroid on the mother and/or the placenta.

The kinetics of Beta appearance and clearance in the maternal and fetal compartments are best modeled as polyexperimental curves with terminal half-lives used to describe clearance (19). Betamethasone given to the fetus is cleared from the fetus 4 times faster than it lost from the

fetus when the ewe is treated (19). The terminal half-life of Beta-PO₄ in the ewe is about 4h while the value recently reported for the Beta-PO₄ - Beta-Ac mixture is 14h, long relative to Beta-PO₄ but much shorter than reported in standard texts (20). While the different preparations of Beta will have effects while the drug is in the circulation, this corticosteroid binds tightly to receptors and can have prolonged effects that extend well beyond plasma availability. A further complication for the interpretation of plasma levels of Beta is the presence of pro-drug (Beta-PO₄ or Beta-Ac) in plasma that may be hydrolysed prior to assay, which will artificially elevate plasma Beta levels (21). The link between plasma corticosteroid levels and lung maturational responses in the fetus remains poorly characterized. Based on fetal cardiovascular responses, Schwab suggested that the clinical dose may be excessive (21).

Although the 2 dose Beta-PO₄ + Beta-Ac treatment caused the largest improvement in lung function, the lungs also had more ventilation mediated injury as detected by the pulmonary interstitial emphysema. Fetal sheep at 124d gestation are profoundly surfactant deficient and corticosteroids require 4–7 days to increase surfactant pool sizes (7). The primary effect of the corticosteroid on the fetal lung is to decrease the mesenchyme and increase potential volume for gas (22). The physiologic result is a lung that contains more gas at high pressures on the pressure-volume curve but does not have increased stability at low transpulmonary pressures. This more distensible lung with immature support structures is prone to rupture when ventilated (10).

A challenge for the field is to develop a corticosteroid preparation that is better suited to the indication of corticosteroid treatment for women at risk of preterm delivery. The Beta-PO₄ in the currently preferred mixture of Beta-PO₄ and Beta-Ac may not be helpful to the fetus. The high maternal plasma levels of Beta also may add risk. The Beta-Ac dose and formulation also did not achieve good lung maturation. There are other chemical formulations of betamethasone that might be better suited to this clinical indication. However, as shown by this experiment and as indicated by the recent review of the available clinical information (1), the mixture of Beta-PO₄ and Beta-Ac is the corticosteroid that is presently indicated for women at risk of preterm delivery.

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Fig. 1.

Compliance and ventilation efficiency index at 30 min of age. **A.** The 2 dose 0.5 mg/kg Beta-PO₄ + Beta-Ac treatment increased compliance relative to control and all other groups. The single dose 0.5 mg/kg Beta-PO₄ + Beta-Ac increased compliance relative to control by t-test. **B.** The ventilation efficiency index was increased by the single and two dose 0.5 mg/kg Beta-PO₄ + Beta-Ac mixture relative to control, and the two dose treatment was different from all other treatments. The Beta-PO₄ treatments had no effect on compliance or ventilation efficiency index, while the 0.25 mg/kg Beta-Ac increased ventilation efficiency index relative to control by t-test. * - different from control by ANOVA, 1 - different from all other groups by ANOVA, t - different from control by t-test.

Fig. 2.

Deflation limbs of pressure-volume curves measured after 30 min of ventilation. The curves for the 2 doses of 0.5 mg/kg Beta-PO₄ + Beta-Ac (△), 1 dose of 0.5 mg/kg Beta-PO₄ + Beta-Ac (▼), and 1 dose of 0.25 mg/kg Beta-Ac (▽) were different from the control curve (○), but not from each other. The 0.25 mg/kg Beta-PO₄ (●) and 0.5 mg/kg Beta-PO₄ (▲) curves were not different from control. Pressure-volume curves were measured for all control and Beta-PO₄ treated animals, for 8 Beta-Ac treated animals, for 10 0.5 mg/kg Beta-PO₄ + Beta-Ac × 1 treated animals, and for 7 0.5 mg/kg Beta-PO₄ + Beta-Ac × 2 treated animals because some animals had air leaks caused by pulmonary interstitial emphysema. * - different from control by repeated measures ANOVA for the curves.

Table 1

Description of Animals

Treatment Group and Dose	N	Weight (kg)	Sex (M/F)
Saline Control	12	2.48 $\pm$ 0.09	7/5
0.5 mg/kg Beta-PO ₄ + Beta-Ac $\times$ 1	13	2.44 $\pm$ 0.10	7/6
0.5 mg/kg Beta-PO ₄ + Beta-Ac $\times$ 2	14	2.22 $\pm$ 0.08*	6/8
0.25 mg/kg Beta-PO ₄ $\times$ 1	15	2.33 $\pm$ 0.06	6/9
0.5 mg/kg Beta-PO ₄ $\times$ 1	12	2.48 $\pm$ 0.10	6/6
0.25 mg/kg Beta-Ac $\times$ 1	9	2.34 $\pm$ 0.13	4/5

* p<0.05 vs. control by t-test

Table II

Values after 30 min. of Ventilation

Groups	pH	Pco ₂ (mmHg)	PO ₂ (mmHg)	Peak Inspiratory Pressure (cmH ₂ O)	Tidal Volume (ml/kg)	Pulmonary Interstitial Emphysema (N/Total)
A Saline Control	6.96±0.03	116±8	86±22	39.3±0.5	7.4±0.5	1/12
B 0.5 mg/kg Beta-PO ₄ + Beta-Ac × 1	7.11±0.02*	76±3*	86±13	37.3±0.9	8.9±0.5	3/13
C 0.5 mg/kg Beta-PO ₄ + Beta-Ac × 2	7.23±0.02* [‡]	61±3, * [‡]	188±36 [§]	33.2±1.1* [‡]	9.3±0.2*	9/14
D 0.25 mg/kg Beta-PO ₄ × 1	7.03±0.02	98±?*	72±6	38.1±0.9	8.5±0.4	3/15
E 0.5 mg/kg Beta-PO ₄ × 1	7.00±?0.02	92±6*	139±14	39.1±0.6	7.1±0.2	1/12
F 0.25 mg/kg Beta-Ac × 1	7.03±0.03	85±4*	100±34	37.0±1.1	7.8±0.3	2/9

* Different from control by ANOVA

[‡] Different from all groups except B by ANOVA

[‡] Different from all other groups by ANOVA

[§] Different from A, B, and D by ANOVA

Table III

Surfactant Sat PC and Surfactant Protein mRNA

Group	Sat PC In BALF ($\mu\text{mol/kg}$)	SP-A mRNA	SP-B mRNA	SP-C mRNA
A Saline Control	0.10 $\pm$ 0.01	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
B 0.5 mg/kg Beta-PO ₄ +Ac $\times$ 1	0.14 $\pm$ 0.03	1.9 $\pm$ 0.1 [*] [†]	1.9 $\pm$ 0.1 [*] [†]	2.1 $\pm$ 0.1 [*]
C 0.5 mg/kg Beta-PO ₄ +Ac $\times$ 2	0.11 $\pm$ 0.01	1.8 $\pm$ 0.1 [*] [†]	2.3 $\pm$ 0.2 [*] [†]	1.9 $\pm$ 0.2 [*]
D 0.25 mg/kg Beta-PO ₄ $\times$ 1	0.18 $\pm$ 0.03	1.3 $\pm$ 0.2	1.2 $\pm$ 0.2	1.3 $\pm$ 0.2
E 0.5 mg/kg Beta-PO ₄ $\times$ 1	0.12 $\pm$ 0.02	1.4 $\pm$ 0.1 [‡]	1.3 $\pm$ 0.1 [‡]	1.6 $\pm$ 0.2 [‡]
F 0.25 mg/kg Beta-Ac $\times$ 1	0.11 $\pm$ 0.03	1.6 $\pm$ 0.2 [‡]	1.4 $\pm$ 0.1 [‡]	1.6 $\pm$ 0.1 [‡]

^{*} Different from control by ANOVA[†] Different from D,E, F by ANOVA[‡] Different from control by t-test