Metabolism of Endogenous Surfactant in Premature Baboons and Effect of Prenatal Corticosteroids

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We studied the synthesis of surfactant and the effect of prenatal betamethasone treatment *in vivo* in very preterm baboons. Ten pregnant baboons were randomized to receive either betamethasone (beta) or saline (control) 48 and 24 h before preterm delivery. The newborn baboons were intubated, treated with surfactant, and ventilated for 6 d. They received a 24-h infusion with the stable isotope [U-^{13}C]glucose as precursor for the synthesis of palmitic acid in surfactant phosphatidylcholine (PC). Palmitic acid in surfactant PC became enriched 27 ± 2 h after the start of the isotope infusion and was maximally enriched at 100 ± 4 h. The fractional synthesis rate of PC palmitate in the beta group ($1.5 \pm 0.2\%$/d) was increased by 129% above control ($0.7 \pm 0.1\%$/d) ($p < 0.02$, Mann–Whitney U test). The absolute synthesis rate of PC in the beta group [1.6 ± 0.3 µmol/kg/d] was increased by 128% above controls [0.7 ± 0.2 µmol/kg/d] ($p < 0.02$). These data show that the synthesis of endogenous surfactant from plasma glucose as precursor is a slow process. It is shown, for the first time *in vivo*, that prenatal glucocorticosteroids stimulate the synthesis of surfactant PC in the very premature baboon. **Bunt JEH, Carnielli VP, Seidner SR, Ikegami M, Wattimena JLD, Sauer PJJ, Jobe AH, Zimmermann LJI. Metabolism of endogenous surfactant in premature baboons and effect of prenatal corticosteroids.** AM J RESPIR CRIT CARE MED 1999;160:1481–1485.

Little is known about surfactant metabolism after preterm delivery of humans or animals. In term and preterm monkeys and lambs with surfactant deficiency, the pool sizes of surfactant in the alveolus increase slowly postnatally (1, 2). Recovery from respiratory distress syndrome (RDS) in monkeys is associated with larger increases in lung pool sizes of disaturated phosphatidylcholine (DSPC) than is nonrecovery from RDS (3). In the preterm human, the accumulation of alveolar surfactant is slow, as shown by a slow postnatal increase in surfactant phosphatidylcholine (PC) concentration in tracheal aspirates (4), and low synthesis rates of surfactant PC (5).

The improved pulmonary function of previously unstressed preterm animals after treatment with prenatal glucocorticoids results primarily from structural changes of the lungs that include increased lung gas volume, decreased perilobular connective tissue, decreased alveolar wall thickness (6), and induction of antioxidant enzyme activities (7). Improved pulmonary function also can result from increased amounts of surfactant. *In vitro*, glucocorticoids increase the activity of a number of enzymes of the surfactant synthetic pathway and increase surfactant phospholipid and protein synthesis, and lamellar body appearance in human fetal lung explants (8–10). However, in most *in vivo* studies with preterm rabbits, preterm lambs, and very preterm baboons, treatment with betamethasone 48 h before delivery does not increase surfactant pool sizes of the alveolar lavage and total lung (2, 11, 12). We reported large increases in surfactant PC pools after birth in both the lung tissue and alveolar pools of preterm ventilated baboons during the first days of life, and pool sizes were not significantly increased by prenatal glucocorticoids (2). As pool size depends on both synthesis and clearance of surfactant, the measurement of the pool size is not equivalent to the measurement of the production rate. Therefore, it is currently unclear whether prenatal glucocorticoids increase surfactant production after preterm delivery.

We studied postnatal endogenous surfactant metabolism in the very premature baboon and tested the hypothesis that prenatal corticosteroids influence the synthesis of endogenous surfactant *in vivo*. These studies are comparable to our report of endogenous surfactant metabolism in preterm human infants (5) because we used the same stable nonradioactive isotope, [U-^{13}C]glucose, as a precursor for the synthesis of palmitic acid in surfactant PC.

METHODS

Animal Treatment and Postnatal Care

Animal care, fetal treatments, deliveries, and the postnatal studies were performed at the Southwest Foundation for Biomedical Research (San Antonio, TX) as reported previously (2). All procedures were reviewed and conformed with AAALAC guidelines. Pregnancies were dated on the basis of cycle dates and growth parameters determined by prenatal ultrasounds performed at estimated fetal gestational ages of 70 and 100 d. At 123 ± 2 d of gestation (term is 185 d) the pregnant baboons were randomly assigned to receive 6 mg of betamethasone (Celestone Soluspan; Schering Pharmaceuticals, Kenilworth, NJ) or saline by intramuscular injection 48 and 24 h before delivery. At delivery, the pregnant baboons were sedated with ketamine (10 mg/kg, intramuscular), intubated, and anesthetized with 1.5% halothane. The preterm fetuses were delivered by cesarean section at 125 ± 2 d gestation and intubated. The newborns received sufactant (100 mg/kg) by tracheal instillation (Survanta; donated by Ross Products, Columbus, OH). At birth, animals received parenteral fluids containing glucose, amino acids, multivitamins, and appropriate electrolytes intravenously; they were not fed, and did not receive lipids.

To study the endogenous surfactant PC palmitate production, all newborn baboons received a constant intravenous infusion of the stable isotope [U-^{13}C]glucose for 24 h, starting immediately after birth (t = 0), at a rate of 0.17 mg/kg/min. Arterial blood (0.5 ml) was drawn at 0, 12, 18, and 24 h, for determination of plasma glucose enrichment. Tracheal aspirates were obtained every 12 h during the study period of 6 d. After instillation of 0.5 ml of saline into the endotracheal tube, aspiration was performed with a 5F catheter, and the aspirate was frozen.

At 144 h (6 d), the animals were killed with pentobarbital. Alveolar wash was performed *in situ* by filling the lungs with 0.9% NaCl at 4° C and recovering the fluid by syringe (2). The lavage procedure was repeated five times. The lungs were removed, weighed, and homogenized and aliquots were frozen.

Analytical Procedures

The tracheal aspirates, alveolar washes, lung homogenates, and plasma glucose samples were processed as described previously (5). In brief, blood was collected and directly centrifuged to separate plasma and cells. Plasma was delipidated with chloroform and methanol (13). The water fraction was passed over anion- and cation-exchange resin. The eluate containing the glucose was derivatized to an aldonitril pentacetate derivative (14). After thawing, the tracheal aspirate and alveolar wash were vortexed and centrifuged at $450 \times g$ for 10 min at 4° C, and the pellet was discarded. From the tracheal aspirate, alveolar wash, and lung homogenate, lipids were extracted (15). PC was isolated from the lipid extract by thin-layer chromatography (16). The PC was transmethylated to form fatty acid methyl esters (17). The saturated (Sat) PC pool size was measured in the alveolar wash and lung homogenate by treatment of the lipid fractions with osmium tetroxide (18). Saturated PC was isolated by column chromatography using alumina, and quantified by phosphorus assay (19).

Determination of ^{13}C Enrichment

The ^{13}C enrichment of plasma glucose and of palmitate in surfactant PC was measured by gas chromatography–combustion interface–isotope ratio mass spectrometry (GC–CI–IRMS) (VG Isotech, Middlewich, Cheshire, UK) as previously described (5). For glucose, 1 μl was injected onto a 25 m × 0.25 mm, 0.11 μm HT-5 capillary column (Scientific Glass Engineering, Victoria, Australia), with a split ratio of 30:1. The oven temperature was isothermal at 220° C. For PC palmitate analysis, 1 μl was injected on a 30-m Omega wax column (Supelco, Zwijndrecht, The Netherlands). The injection and oven temperature were 45° C for 2 min and raised at 15° C/min to 175° C, held for 15 min at 175° C, and subsequently increased by 2° C/min to 240° C. The enrichment was expressed in atom percent excess (APE), which represents the increase in the percentage of ^{13}C atoms in total carbon dioxide from the combusted compounds above baseline enrichment (before isotope infusion). Enrichments were corrected for the contribution of unlabeled carbon atoms added during derivatization. Calculations were performed as described previously (5) for palmitic acid

only, as this is by far the most abundant fatty acid in PC. Tissue-bound and alveolar surfactant were regarded as one pool because studies in newborn rabbits showed that recycling is ~ 16 times faster than the *de novo* synthesis and clearance (20). The first appearance of enrichment was defined as the time delay between the start of the [U-^{13}C]glucose infusion and the detection of enriched palmitic acid in surfactant PC. The first appearance of enrichment was calculated by plotting the regression line for the linear increase of the enrichment-versus-time curve, and extrapolating it to baseline enrichment. The fractional synthesis rate (FSR) of palmitic acid was expressed as the percentage of the total surfactant PC palmitate pool synthesized from glucose per day. It was calculated by dividing the slope of the linear increase in enrichment of PC palmitate by the steady state enrichment of plasma glucose (5, 21). The absolute synthesis rate (ASR) of surfactant PC from glucose only, was calculated by multiplying the FSR by the total lung Sat PC pool size. The value for the total lung Sat PC pool was the Sat PC pool size measured at birth in seven similar baboons (~ 38 μmol of Sat PC per kilogram) plus the amount of Sat PC in the exogenous surfactant given to the animals at birth (~ 68 μmol of Sat PC per kilogram) (2).

Data Analysis

Data are presented as means $\pm$ standard error. The nonparametric Mann–Whitney U test was applied to compare groups. A value of $p < 0.05$ was accepted as significant.

RESULTS

The six control baboons and the four baboons exposed prenatally to betamethasone (beta group) had similar birth weights: 394 ± 65 and 340 ± 27 g, respectively. The two groups had similar P_{O_2} and P_{CO_2} values while on similar ventilatory settings as reported previously (2).

The ^{13}C enrichment of plasma glucose reached similar steady states in all baboons during the period of isotope infusion (3.29 ± 0.38 in controls and 3.29 ± 0.16 APE in the beta group). The mean curves for ^{13}C enrichment of palmitic acid in surfactant PC recovered from tracheal aspirates for both groups are shown in Figure 1. The first appearance of enrichment in surfactant PC was similar at 27.6 ± 2.4 h for controls and 26.0 ± 4.5 h for the beta group (Table 1). The ^{13}C enrichment of PC palmitate increased linearly in both groups and the enrichment was significantly higher in the beta group as compared with the control group during the interval from 48 to 96 h ($p < 0.05$). The FSR was significantly increased in
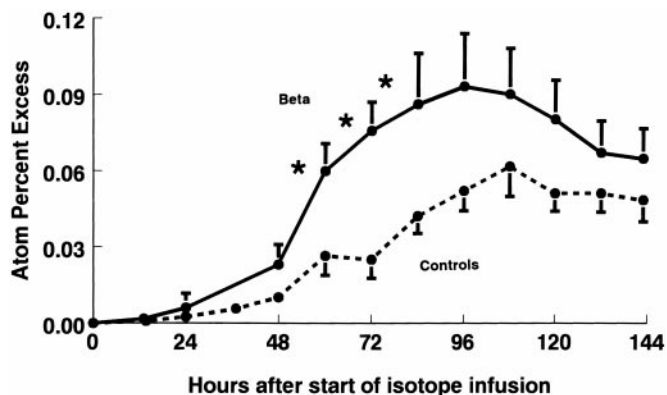

Figure 1. ^{13}C labeling of palmitic acid of surfactant phosphatidylcholine (PC) (expressed in atom percent excess, APE) after a 24-h [U-^{13}C]glucose infusion. Pregnant baboons received either prenatal betamethasone or saline. When error bars are not visible, they coincide with the markers. The increased synthesis of PC palmitate after betamethasone is shown by the increased incorporation of ^{13}C during the first days of life (*p < 0.05).

TABLE 1

MEASUREMENT OF ^{13}C ENRICHMENT AND KINETICS OF PC PALMITATE IN
BABOONS EXPOSED OR NOT EXPOSED TO PRENATAL BETAMETHASONE

	First Appearance of Enrichment (h)	Maximal Enrichment Time (h)	Fractional Synthesis Rate (%/d)	Absolute Synthesis Rate (μmol/kg/d)	Lung Sat PC Pool on Day 6 (μmol/kg)
Control group	27.6 ± 2.4	107 ± 3	0.7 ± 0.1	0.7 ± 0.1	186 ± 32
Beta group	26.0 ± 4.5	90 ± 8	1.5 ± 0.2	1.6 ± 0.3	199 ± 13
p Value	NS	0.08	0.02	0.02	NS

* Ten pregnant baboons were randomized to prenatal betamethasone or saline. After preterm delivery, baboons received [U-^{13}C]glucose for 24 h and ^{13}C enrichment of palmitic acid in surfactant phosphatidylcholine (PC) was measured for 6 d. Kinetic parameters were calculated using enrichment-versus-time curves from plasma glucose and surfactant PC palmitate, as described in Methods and Results. Saturated PC pool size was measured after killing on Day 6. Statistical analysis to compare groups was performed with the Mann–Whitney U test.

the beta group (1.5 ± 0.2%/d) compared with controls (0.7 ± 0.1%/d, p < 0.02, Table 1) as shown by the increased slopes of the enrichment-versus-time curves of PC palmitate in the beta group compared with the slopes in the control group (Figure 2). The ASR of Sat PC from glucose was higher in the beta group (1.6 ± 0.3 μmol/kg/d) compared with the control group (0.7 ± 0.1 μmol/kg/d, p < 0.02). On Day 6, the total lung Sat PC pool size (alveolar wash plus lung tissue) was similar for the control and beta groups (Table 1). The time of maximal enrichment tended to be earlier in the beta group (90 ± 8 h) than in the control group (107 ± 3 h, p = 0.08). The enrichments at 6 d in tracheal aspirates, alveolar washes, and lung homogenates were similar for the individual animals, and the mean values for the control and beta groups also were similar (tracheal aspirates, 0.052 ± 0.030; alveolar washes, 0.050 ± 0.023; and lung homogenates, 0.056 ± 0.026 APE). This implies that the enrichment of surfactant PC palmitate measured from tracheal aspirates is a good reflection of surfactant PC in the alveoli and tissue and is in agreement with a fast recycling of surfactant phospholipids (20).

DISCUSSION

An understanding of surfactant metabolism is necessary to optimize treatment of the very premature human infant with sur-

factant deficiency and possibly other diseases with decreased surfactant function. We infused the stable isotope [U-^{13}C]glucose in very premature baboons for 24 h and measured ^{13}C incorporation into palmitic acid in PC in the alveolar compartment. The ^{13}C-labeled PC increased slowly, reached the maximum value more than 4 d after the start of the isotope infusion, and remained high at Day 6. These data show that the endogenous synthesis and secretion of PC is a slow process and that endogenously synthesized surfactant remains in the lung for a long time in the preterm primate. Five days after birth, the baboons also received an intravenous bolus of radioactive palmitate labeled with ^{3}H and the specific activity in surfactant PC was measured at killing 24 h later, as described (2). The percentage of surfactant PC secreted to the alveoli was only ~ 7.5% in 24 h. This slow rate of secretion is compatible with the kinetics calculated from the stable isotope data. A slow metabolism of surfactant has been described in nonprimate animals. In studies of newborn rabbits and lambs, maximal alveolar enrichments were found 35 to 60 h after a single injection of radiolabeled palmitic acid (20, 22, 23). In term newborn sheep and term newborn rabbits, half-lives of [^{3}H]palmitate in surfactant PC were ~ 11.6 and 2–4.5 d, respectively (20, 24, 25).

In comparison with human preterm infants, the first appearance of enrichment in the alveolar compartment was significantly later in the baboons (~ 27 versus ~ 19 h) (5). The
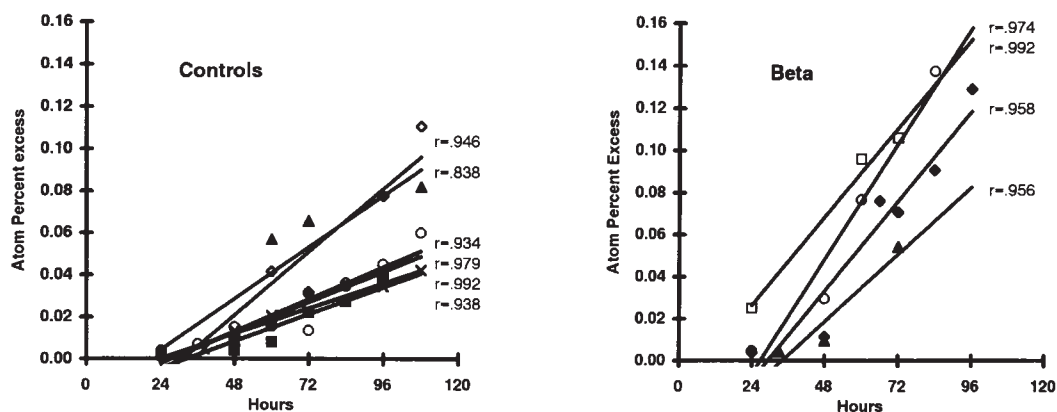

Figure 2. Linear increase in ^{13}C enrichment of palmitic acid in surfactant phosphatidylcholine (PC). Pregnant baboons received either prenatal betamethasone (beta) or saline (controls). The regression line for each preterm baboon is shown. For calculation of the time delay of first appearance of enrichment, the regression line was extrapolated to zero enrichment. The fractional synthesis rate (FSR) was calculated by dividing the slope of the regression line by steady state enrichment of plasma glucose. As plasma glucose enrichment was identical in both groups, the increased FSR is directly represented by the increased slopes in the betamethasone group compared with the control group.

time of maximal enrichment in steroid-treated baboons ($\sim$ 90 h) was comparable to the time of maximal enrichment in steroid-treated human preterm infants ($\sim$ 70 h). In the present study, the absolute production of Sat PC from glucose in the beta group of $\sim$ 1.6 μmol/kg/d, was lower than $\sim$ 4.3 μmol/kg/d in preterm human infants treated with prenatal corticosteroids and exogenous surfactant (5). These comparisons suggest a slower surfactant synthesis from glucose in the preterm baboon as compared with the human preterm infant, but do not show the contributions of other precursors. These baboons were more immature than the preterm human infants, which may explain the lower synthesis rates. The effects of gestational age or degree of lung immaturity have not been evaluated by these techniques in either preterm humans or baboons.

We also found that prenatal corticosteroids significantly stimulated the incorporation of palmitate derived from glucose into PC: the FSR in the beta group was increased by $\sim$ 129% relative to controls and the absolute production rate was increased in the beta group by $\sim$ 128% relative to controls (Table 1). The PC palmitate tended to be maximally enriched earlier in the beta group ($\sim$ 90 h) than in the controls ($\sim$ 107 h, p < 0.08, Table 1, Figure 1), which is also compatible with stimulated ^{13}C incorporation from plasma glucose into PC palmitate by betamethasone.

In vitro studies support the idea that glucocorticoids enhance surfactant synthesis (8–10). Glucose incorporation into surfactant PC in perfused rat lungs is increased by betamethasone (26). However, data on pool size measurements as an indication of surfactant synthesis *in vivo* are conflicting. In some studies with newborn rabbits, preterm monkeys, and lambs chronically catheterized *in utero*, corticosteroid treatment 2–3 d before delivery increased surfactant PC pool sizes in alveolar lavage and lung tissue significantly after birth (27–30). In contrast, other studies in large animals show no prenatal steroid-induced increase in the alveolar (9, 31–34) or total lung Sat PC pool size after birth (9, 31–33). Ballard and coworkers and Ikegami and colleagues did not find an increase in total lung Sat PC pool size at birth in preterm lambs when glucocorticoids were administered 2 to 4 d before preterm delivery (11, 31). However, when steroids were administered 1 wk before delivery, the total lung Sat PC pool sizes at birth increased significantly (11, 31). In the present study, we did not measure surfactant PC pool sizes at birth. We demonstrated that the processes of synthesis and secretion are slow and that the production rate of Sat PC from glucose in preterm newborns is low compared with the total Sat PC pool on Day 6. Therefore, the pool size will not increase significantly until several days after exposure to betamethasone. This low synthesis rate relative to the pool size may explain why most studies find long delays between prenatal glucocorticoid treatment and increased surfactant PC pools (11, 31).

The dosage and exposure time to steroids, the species (48 h of exposure in a rat is relatively long compared with lambs or baboons), and the experimental conditions that could cause secondary stress to the fetus also may contribute to discrepancies in the various reports of steroids on surfactant metabolism.

In the present study, there was no effect of prenatal betamethasone treatment on Sat PC pool size in total lung at Day 6 (Table 1). Probably physiological changes after birth and therapeutic interventions such as ventilation have larger effects on surfactant metabolism than do prenatal glucocorticoids. Such effects are consistent with the findings by Seidner and coworkers, who showed that the lung tissue Sat PC pool size was larger in the very preterm baboon after 6 d of ventilation (125 d of gestation plus 6 d of ventilation) than in either the near term baboon (175 d of gestation) or the adult baboon

(2). A theoretical explanation for the fact that we did not find increased pool sizes on Day 6 could be that prenatal betamethasone stimulates both synthesis and clearance of surfactant as shown by Fiascione and colleagues in preterm rabbits (35). However, in the present study the clearance of exogenous labeled surfactant was not increased (2).

In the type II pneumocyte, glucose is a primary precursor for fatty acids used for the synthesis of surfactant PC. This is especially true in a state of relative fatty acid deficiency, as in the present study (36, 37). The baboons did not receive any lipids during the entire study period, and the fat stores in these very premature baboons are minimal. Sanders and coworkers showed that the conversion of glucose to palmitic acid is a fast process (38). Other substrates for surfactant PC are present as well, such as intracellular glycogen, fatty acids, lactate, pyruvate, and ketone bodies. The overall kinetics for surfactant PC presented, and the increased synthesis rates after glucocorticoids administration, are measured from glucose only and may not represent total surfactant synthesis. The synthesis rates are valid from this precursor but are probably underestimations; a more direct precursor of PC palmitate such as palmitic acid may yield more accurate estimations of absolute synthesis rates but ignores the importance of lipogenesis in the type II cell. It is, in the present study, unclear whether prenatal steroid stimulates the use of other precursors and how they influence glucose enrichment in the type II cell. *In vitro*, steroids increase glycogenolysis in the type II cell, which is associated with synthesis of surfactant PC; whether this occurs in the living primate has not been studied. If prenatal steroids increased glycogenolysis in the type II cell during the period of labeled glucose incorporation into surfactant PC palmitate, then the glucose as a precursor would be diluted, resulting in a lower enrichment of surfactant PC palmitate after steroids. We found, however, increased ^{13}C enrichment of surfactant PC palmitate. It is unlikely that glycogen depletion had already occurred before birth, as in a few baboons no glycogen depletion was found by electron microscopy after 6 d of ventilation (2). Glucose can be used to describe surfactant metabolism, to evaluate differences between groups, and to study effects of interventions and clinical conditions. In addition, glucose can be used safely and easily in critically ill preterm human infants.

In conclusion, the data show that endogenous surfactant synthesis and turnover are slow processes. The endogenous synthesis of surfactant PC from glucose is stimulated within 48 h by prenatal betamethasone after delivery of the very premature baboon.

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