Parathyroid Hormone-Related Protein in Preterm Human Milk

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Abstract

Background: Parathyroid hormone-related protein (PTHrP) has the ability to activate parathyroid hormone receptors and cause hypercalcemia. High concentrations of PTHrP are found in human breastmilk of mothers of term-infants. It is not known whether PTHrP is excreted in preterm human milk. This study tested the hypothesis that PTHrP concentrations in milk obtained from mothers of preterm infants are similar to those found in milk from mothers of term infants.

Methods: We collected samples of expressed human milk obtained from 27 mothers of preterm infants (27–34 weeks' gestation) and from 16 mothers of full-term infants. Samples were collected within the first 72 hours postpartum (colostrum) and again at 1 and 2 weeks postpartum. PTHrP concentrations in these samples were measured by two-site immunoradiometric assay.

Results: PTHrP concentrations were significantly higher in samples obtained after 1 week postpartum than in samples obtained during the first 72 hours of life in breastmilk obtained from mothers of both term and preterm infants (P < 0.0001). PTHrP concentrations were similar in colostrum and after 1 week in term and preterm milk. PTHrP concentrations after 2 weeks of lactation were significantly higher in samples obtained from mothers of term infants (P < 0.006).

Conclusion: Human milk expressed by mothers of preterm infants contains amounts of PTHrP similar to those measured in milk expressed by mothers of term infants.

Introduction

Parathyroid hormone-related protein (PTHrP), first described in the 1980s, has the ability to activate parathyroid hormone receptors and cause hypercalcemia.1-2 Both PTHrP and PTHrP mRNA have been localized in a wide range of normal and pathological tissues and are considered to be autocrine/paracrine or intracrine regulators of cellular growth and differentiation and of smooth muscle relaxation.2-5

During lactation, PTHrP is produced in significant amounts by breast tissues.6,7 Its concentrations in human milk are about 1,000–10,000 times higher than in maternal plasma7,8 and about 100 times higher than in the plasma of patients with hypercalcemia of malignancy.6,7 Several substances, such as progesterone or dexamethasone, have been found to influence the secretion of PTHrP by mammary cells.9 PTHrP in preterm human milk might be important in premature infants' calcium homeostasis. Human neonates, particularly preterm infants, experience a transient fall in serum calcium soon after birth,10,11 occurring to a considerable extent in formula-fed infants and less so in breastfed infants.11 Although the presence of high concentrations of PTHrP was demonstrated in breastmilk of term mothers,2-3 it was not investigated in preterm human milk, which is influenced by hormonal or pharmacological changes related to premature delivery.

We thus designed the current study in order to examine whether PTHrP is present in milk obtained from mothers of preterm infants. Specifically, we tested the hypothesis that PTHrP concentrations in milk obtained from mothers of preterm infants are similar to those found in milk from mothers of term infants.

Subjects and Methods

Participants and samples

The study received institutional ethics committee approval. Written informed consents were obtained from all participating mothers. We collected samples of expressed human milk obtained from 27 mothers of preterm infants, i.e., born at 27–34 weeks' gestation, who routinely expressed all their milk every 3 hours using a commercial breast pump (Medela AG, Baar, Switzerland). In addition, we collected samples of milk...
obtained from 16 mothers of full-term infants. Three samples were collected from each mother: one within the first 72 hours after delivery (colostrum) and one each at 1 and 2 weeks postpartum. All milk samples were taken during daylight hours and immediately placed at a home freezer at −20°C and stored later at −70°C, until analysis was performed. By design, all participating mothers were healthy and had not suffered from gestational diabetes or any metabolic disorder. We also measured PTHrP concentrations in two commercially available infant formulas (Similac Top 1 and Similac Special Care, Ross Laboratories, Columbus, OH).

**Laboratory methods**

Concentrations of PTHrP in human milk samples were measured by a two-site immunoradiometric assay (DSL-8100 Active Irmakit, Diagnostic Systems Laboratories Inc., Webster, TX). For the assay procedure, 0.2-mL frozen milk samples were thawed and diluted 1:80 with deionized water. We used a commercial kit developed primarily for measurements of PTHrP in plasma. We validated this assay for breastmilk whey. When 1,600 pg of human PTHrP was added to milk samples and assayed, the recovery was 98 ± 8% (n = 5). The sensitivity of the assay in breastmilk was 20 pg/mL. There is no cross-reactivity with PTH according to the manufacturer’s studies. The inter-assay coefficient of variation is 4.4%, and the intra-assay coefficient of variation is 4.7%.

**Statistical analyses**

This is a pilot study designed in order to determine simply if this protein exists in preterm milk, and we decided arbitrarily to obtain between 15 and 25 milk samples in each group.

Results are expressed as mean ± SD. The distribution of the various parameters was examined for normality, and because it was skewed a square-root transformation was applied to the data. Comparison between groups and time points was by using a two-way analysis of variance with repeated measures using the Mixed Model (SAS for Windows, version 9.1.3, SAS Institute, Cary, NC). Linear regression analysis was used to study the correlation between PTHrP concentrations and gestational age or birth weight.

**Results**

Demographic and maternal characteristics of the participants in this study are presented in Table 1. On average, PTHrP concentrations were significantly higher in samples obtained after 1 week postpartum than in samples obtained during the first 72 hours of life (colostrum) in breastmilk obtained from mothers of both term and preterm infants (P < 0.0001). Specifically, PTHrP concentrations after 1 week exceeded colostrum values in milk obtained from mothers of preterm infants (in 24 out of 27 samples; 89%) and of term infants (in 15 out of 16 samples; 94%). Similarly, PTHrP concentrations after 2 weeks exceeded colostrum values in both preterm and term milk (Table 1). PTHrP concentrations after 2 weeks of lactation, however, were significantly higher in samples obtained from mothers of term infants than those of mothers of preterm infants (Table 1). PTHrP concentrations in two commercially available cow milk protein-based infant formulas were undetectable (data not shown). Finally, there was a significant correlation between PTHrP concentrations in colostrum and birth weight in preterm samples (R² = 0.26, P = 0.004) (Fig. 1).

**Discussion**

In the current study we found, as hypothesized, that human milk expressed by mothers of preterm infants contains amounts of PTHrP similar to those measured in milk expressed by mothers of term infants. This finding indicates that human breasts are ready to produce large amounts of PTHrP soon after premature delivery. Indeed, even colostrum samples of preterm human milk contained high concentrations of PTHrP.

To the best of our knowledge this is the first report to describe PTHrP in milk from mothers of preterm infants. The concentrations of PTHrP in the term milk group of our study are consistent with what has been previously reported in human7,8 and animal12,13 models. Lower concentrations of PTHrP in colostrum, with a gradual increase during the first and second week postpartum, was also observed by Cross et al.7 in women who were exclusively breastfeeding their

![FIG. 1. Correlation between PTHrP concentration (in pmol/L) and birth weight (in g) in colostrum samples of preterm milk. R² = 0.26, P < 0.004](Image 314x96 to 551x263)
term infants. These results are similar to ours in term and preterm milk, although the significance of the lower levels of PTHrP in preterm milk after 2 weeks is yet to be determined.

The physiological significance of the relatively high concentrations of PTHrP in term and preterm human milk has not been studied yet. During lactation, PTHrP is released on the stimulus of suckling\(^6,14,15\) and secreted into the milk. Because high concentrations of PTHrP can be found in the amniotic fluid\(^16,17\) that is swallowed by the fetus, the intestinal tract of fetuses has already been exposed to high concentrations of PTHrP.

PTHRP produces all of the major physiologic effects of parathyroid hormone, including parathyroid hormone receptor binding,\(^18\) accelerated bone resorption,\(^8\) phosphaturia,\(^8\) and hypocalciuria.\(^8\) Thus, PTHrP in preterm human milk might be important in premature infants’ calcium homeostasis. As mentioned before, human neonates, particularly preterm infants, experience a transient fall in serum calcium soon after birth\(^10,11\) occurring to a considerable extent in formula-fed infants and less so in breastfed infants.\(^11\) Although many variables can be attributed to explain this difference in calcium homeostasis between formula-fed and breast-fed infants, we suggest that PTHrP might be responsible, at least in part, for this difference. In support of our assumption is the finding of our current study and those of Khosla et al.,\(^19\) who demonstrated that PTHrP concentrations in term and preterm formulas were negligible, compared to the high concentrations measured in human milk samples.

In conclusion, we have demonstrated high concentrations of PTHrP in colostrum and mature preterm human milk. Clinical evidence of the beneficial effects of such high concentrations upon breast-fed preterm infants awaits future studies.

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Disclosure Statement

No competing financial interests exist.

References


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