Osteocalcin and Human Milk

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Introduction

The serum concentration of osteocalcin [α -carboxyglutamic acid (GLA) protein] has been reported to be significantly greater in breastfed than in formula-fed infants [1,2]. Further, osteocalcin is a potent chemoattractant for peripheral blood monocytes, a major cell type found in human milk [3]. Therefore, to test the hypothesis that osteocalcin is present in human milk, the osteocalcin concentration was measured in blood and milk from 11 2-3 day postpartum lactating women.

Materials and Methodology

This investigation was approved by the Medical University of South Carolina Human Investigation Committee and written consent was obtained from each patient prior to initiating the study. Eleven 2-3 day postpartum lactating women were chosen randomly. Demographic data including age, obstetrical index, mode of delivery and medical complications were recorded for each mother, and birth weight, gestational age, and Apgar scores were recorded for the infants. Gestational age of infants at birth was initially determined from the history of the mother's last menstrual period and was confirmed with a Ballard examination within 36 h of delivery. Milk collection (20 ml) was performed by the women either manually or using an electric breast pump (Medela, Crystal Lake, Illinois), based on individual preference. Blood (10 ml) was obtained using routine methods of peripheral venipuncture. Both milk and blood specimens were collected between 11:00 a.m. and noon to minimize any effect of the previously reported diurnal variation in osteocalcin serum concentration [4].

Blood was divided into two aliquots. One aliquot was taken immediately after collection to the laboratory where it was centrifuged (200 g for 10 min) and the serum was separated and stored at -20 ° C until all specimens were ready for osteocalcin analysis. The second aliquot was placed in a tube containing ethylene-diamine-tetraacetic acid and taken to the hospital clinical laboratory for a total white blood cell count (via Coulter counter) and a routine differential cell count with Wright's staining.

Milk specimens were taken immediately to the laboratory after collection. The specimens were divided approximately in half using a sterile technique. The first half was used for total and differential cell counts of the milk. To determine the total cell count, centrifugation and a standard hemocytometer were used. The differential cell count was performed on a cytocentrifuged preparation following Wright's staining.

The second half of each milk specimen was centrifuged (200 g for 10 min). The cream was removed from the top and the serum separated from the cellular debris at the bottom. The milk serum was then stored at -20 ° C until all specimens were collected for osteocalcin analysis. Osteocalcin concentration was measured in both serum and milk using radioimmunoassay (RIA) methodology as previously described [5]. Both specimen collection and handling followed the procedure recommended by Incstar Corporation, Stillwater, Minn., USA. The lower limit of osteocalcin detection using this RIA methodology is 0.1 ng/ml. Pearson correlation coefficients were used to determine the association of variables.

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Results

The 11 study patients included 2 black and 9 white women. Their mean age $\pm$ SD was 30 ± 5 years. Four were delivered vaginally and 7 by Caesarean section. All women totally breast-fed their infants and were free from any significant medical complication or chronic disease. One woman was primiparous. The mean $\pm$ SD birth weight and gestational age for the 11 newborns was $3,589 \pm 703$ grams and 39 ± 2 weeks, respectively. Their median 1- and 5-min Apgar scores were 9 and 9, respectively.

The maternal blood serum contained a mean $\pm$ SD white cell count and GLA concentration of $11.2 \pm 2.6 \times 10^3$ cells/cc and 2.7 ± 1.4 ng/ml respectively. Similarly, the milk contained a mean $\pm$ SD $4.6 \pm 4.6 \times 10^3$ total cells/cc and < 0.1 ng/ml of osteocalcin in each specimen. These data and the milk and blood white cell differentials are shown in table 1.

Maternal serum GLA concentration did not significantly correlate with the total white blood cell count or the absolute number of peripheral blood monocytes per cubic centimeter. Serum GLA concentration was significantly ($p < 0.05$) correlated ($r = 0.606$) with the absolute blood neutrophil count. There was no significant correlation between the serum GLA concentration and the total milk cell count or absolute milk monocyte count.

The total milk cell count was significantly ($p < 0.001$) correlated ($r = 0.858$) with the absolute milk neutrophil but not the monocyte/macrophage count. Finally, none of the maternal demographic factors recorded significantly correlated with the serum GLA concentrations.

Discussion

Although osteocalcin has recently been extensively investigated, its sources and biologic functions continue to be elucidated. The 2-3 day postpartum period used in this study for the identification of osteocalcin in human milk was chosen because it is associated with the largest milk monocyte/macrophage concentrations of lactation [6]. Further, the 11 a.m.-noon time period used for specimen collection was chosen because it has been shown to be that portion of the day associated with the greatest serum concentration of osteocalcin [4]. This was thought to be important since postpartum serum osteocalcin concentrations are known to be low [7]. Despite these methodologic steps, osteocalcin was not detected in the 11 human milk specimens evaluated.

Further, because bone formation, as reflected by the longitudinal change in neonatal bone width, has been shown not to be greater in breast-fed than formula-fed infants [8], in-

creasing bone formation may not explain the increased serum osteocalcin concentration reported in breast-fed infants either. Thus, a less clearly defined osteocalcin source may be active in breast-fed infants.

A relevant observation in both laboratory animals and adult humans is that 1,25-dihydroxyvitamin D induces the synthesis of osteocalcin [9, 10]. Further, a significant correlation between serum 1,25(OH)₂D and osteocalcin concentration has been reported in both low birth-weight [11] and very low birth-weight [5] infants during the first week of life. Therefore, since some breast-fed infants with low calcium and phosphorus intake have increased serum concentrations of this vitamin D metabolite, a secondary increase in serum osteocalcin among breast-fed infants may be a

physiologic response [12]. This, however, is at best only a partial explanation for the increased serum GLA concentrations of breastfed infants, since serum calcitriol concentrations are not consistently greater in breast-fed than in formula-fed babies [13].

In summary, although elevated serum osteocalcin concentrations are described in breast-fed babies, the etiology for this observation is not certain. GLA protein was not present in measurable concentration in the human milk specimens in this study. These data suggest a metabolic rather than an en-teral origin for the reported increased serum osteocalcin concentrations of breast-fed infants. Further study, however, will be required to clarify the origin and significance of this unique biologic observation.

References

- Pettifor JM, Stein H, Herman A, Ross FP, Blumenfeld T, Moodley GP: Mineral homeostasis in very low birthweight infants fed either own mother's milk or pooled pasteurized preterm milk. *J Pediatr Gastroenterol Nutr* 1986;5:248-253.
- Lichtenstein P, Gormley C, Martinez R, Poser J, Specker B, Tsang R: Elevated serum GLA-protein in infancy: Higher values in breast milk versus cow-milk formula feeding. *Pediatr Res* 1983; 17:292.
- Malone JD, Teitelbaum SL, Griffin GL, Senoir RM, Kahn AJ: Recruitment of osteoclast precursors by purified bone matrix constituents. *J Cell Biol* 1982;92:227-230.
- Gundberg CM, Markowitz ME, Mizruchi M, Rosen JF: Osteocalcin in human serum: A circadian rhythm. *J Clin Endocrinol Metab* 1985;60:736-739.
- Pittard WB, Geddes KM, Hulsey TC, Hollis BW: Osteocalcin, skeletal alkaline phosphatase and bone mineral content in very low birth weight infants: A longitudinal assessment. *Pediatr Res* 1992;31:181-185.
- Pittard WB: Breast milk immunology: A frontier in infant nutrition. *AJDC* 1979;133:83-88.
- Rodin A, Duncan A, Ovartero H, Pistofidis G, Whitaker K, Crook D, Stevenson J, Chapman M, Fogel-man I: Serum concentrations of alkaline phosphatase isoenzymes and osteocalcin in normal pregnancy. *J Clin Endocrinol Metab* 1989;68: 1123-1127.
- Greer F, Marshall S: Bone mineral content, serum vitamin D metabolite concentrations and ultraviolet B light exposure in infants fed human milk with and without vitamin D₂ supplements. *J Pediatr* 1989; 114: 204-212.
- Rader J, Baylink D, Hughes M, Sa-filian E, Haussler M: Calcium and phosphorus deficiency in rats: Effects on PTH and 1,25 dihydroxyvitamin D₃. *Am J Physiol* 1979;236: E118-E122.
- 10 Mahonen A, Pirskanen A, Keina-nen R, Maenpaa P: Effect of 1,25(OH)₂D₃ on its receptor mRNA levels and osteocalcin synthesis in human osteosarcoma cells. *Biochim Biophys Acta* 1990;1048:30-37.

Delmas P, Glorieux F, Delvin E, Salle B, Melki I: Perinatal serum bone GLA-protein and vitamin D metabolites in preterm and full-term neonates. *J Clin Endocrinol Metab* 1987;65:588-591.

Steichen J, Tsang R, Greer F, Ho M , Hug G: Elevated 1,25-dihydroxyvitamin D concentrations in rickets of very low birth weight infants. *J Pediatr* 1981;99:293-298.

Hillman L, Chow W, Salmons S, Weaver E, Erickson M, Hansen J: Vitamin D metabolism, mineral homeostasis and bone mineralization in term infants fed human milk, low-milk-based formula or soy-based formula. *J Pediatr* 1988; 112: 864-874.