Zinc-binding ligands in milk and intestine: A role in neonatal nutrition?

(acrodermatitis enteropathica/zinc absorption)

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ABSTRACT The hypothesis that a zinc-binding ligand (ZBL) recently discovered in human milk but absent from cow’s milk might be related to zinc nutrition in the neonate was investigated. The zinc-binding characteristics of rat milk were examined to determine if the rat was a suitable model. By gel filtration, rat milk was found to contain a ZBL with characteristics similar to those of the ZBL found in human milk. A similar ZBL was identified in the intestinal mucosa of rats 16 days of age and older but was absent in rats from birth to 16 days. These results support the hypothesis that the ZBL of maternal milk may enhance zinc transport in the neonate period before the development of intestinal mechanisms for zinc absorption.

A recent report from this laboratory described the discovery in human milk of a low-molecular-weight zinc-binding ligand (ZBL) that is not present in cow’s milk (1). We have proposed (1) that this ZBL is the factor responsible for the therapeutic value of human milk in the treatment of a genetic disorder of zinc metabolism, acrodermatitis enteropathica (AE), and that the ZBL acts by enhancing the absorption of zinc in patients suffering from this condition.

AE is a hereditary disease in humans that appears in early infancy and is characterized by severe skin lesions, alopecia, and diarrhea (2). The onset of symptoms is frequently associated with the weaning of infants from breast milk to cow’s milk (2–4). Without treatment, there is a relentless progression leading to death within 1 to 3 years (4). Since 1953, AE has been treated by oral administration of diiodohydroxyquin (Diodo-quin) (5). Recently, Mynahan and Barnes (6) reported low plasma zinc levels in AE patients and successful treatment of the disorder with oral administration of zinc, the therapy resulting in an elevation of the plasma zinc and subsequent clearing of the epidermal lesions. The efficacy of zinc in the treatment of AE has been confirmed by other investigators (7, 8). These findings indicate that the basic metabolic defect in this disorder is closely, if not directly, related to zinc metabolism, possibly at the level of zinc absorption as we proposed.

We have also postulated that an intestinal ZBL necessary for zinc absorption is absent or immature at birth, even in normal individuals without AE, and that the ZBL of human milk may enable or enhance absorption of this element in the neonate until normal mechanisms of zinc absorption develop postnataally (9, 10). To test this hypothesis, we have used the rat as an experimental model. The present report describes the finding of a ZBL in rat milk similar to that present in human milk. In addition, the presence of a ZBL in the rat intestinal mucosa during the first few weeks of life was investigated.

MATERIALS AND METHODS

Animals. Normal Sprague–Dawley rats weighing 210 ± 10 g were mated overnight. Matings were confirmed by the presence of sperm in the vaginal smear. Sperm-positive females were kept in individual stainless steel cages and were fed a purified diet complete in all nutrients. The composition of the diet has been reported (11). On day 21 of gestation, the females were weighed, an aluminum screen was placed in the bottom of the cage, and the females were allowed to deliver naturally. After birth, the litters were used for one of two studies: (i) characterization of rat milk for ZBLs or (ii) determination of the presence of a ZBL in intestinal mucosa during postnatal development.

To study rat milk, the offspring were allowed to suckle for 10 days after birth and then were removed from the mother. The lactating females were anesthetized lightly with sodium pentobarbital (12 mg/rat) given intraperitoneally. After 30 min, 2 international units of oxytocin were injected intraperitoneally to enhance milk flow. The animals were hand-milked and milk samples were collected in micropipettes. The milk was centrifuged at 5000 × g for 5 min and the fat-free supernatant was withdrawn and diluted with 1 volume of 0.13 mM Tris-HCl buffer (pH 7.4) for further fractionation. Four samples were obtained in this manner.

To study the zinc-binding characteristics of intestinal mucosa, the suckling pups were killed at days 0 to 21 after birth. Four additional litters were weaned at day 21 and the animals were killed 28 days after birth. The small intestine from the pylorus to the cecum was removed and dissected free from any adhering tissue. After the contents had been removed by flushing with cold 0.9% saline, the intestine was cut longitudinally and the mucosal tissue was scraped off with a microscope slide. To obtain sufficient material from younger animals, it was necessary to combine the mucosal tissue from five newborn, three 7-day-old, and two 10-day-old animals for each sample. In all other groups, sufficient material was obtained from a single intestine. In this manner, four samples were obtained from newborn rats. All other groups contained three samples. The tissue was homogenized in 5 volumes of 0.13 mM Tris-HCl buffer (pH 7.4) and the homogenate was centrifuged at 5000 × g for 5 min. The fat-free supernatant was diluted with 1 volume of 0.13 mM Tris-HCl buffer (pH 7.4) for further fractionation.

Gel Filtration. The diluted supernatant samples from rat milk and intestinal mucosa were subjected to gel filtration on a Sepharose 2B column (50 × 2.6 cm). Tris-HCl buffer (0.13 mM, pH 7.4) was used to elute the sample from the column.

Abbreviations: ZBL, zinc-binding ligand; AE, acrodermatitis enteropathica.
Fractions (2 ml) were collected and assayed for absorbance at 280 nm by using a Gilford model 2000 spectrophotometer and for zinc by using a Perkin-Elmer model 370 atomic absorption spectrophotometer. Low-molecular-weight fractions containing zinc (nonvoid volume) were pooled and rechromatographed on a Sephadex G-75 column (50 × 1.5 cm). Fractions were again assayed for absorbance at 280 nm and for zinc content.

**RESULTS**

**Rat Milk.** A representative elution pattern from gel filtration of rat milk on Sephadex G-75 is shown in Fig. 1. The zinc separated into three peaks, one of which (tubes 90–110) eluted with a pattern similar to that observed in earlier studies with human milk. The presence of a ZBL in rat milk with characteristics similar to those of the ZBL found in human milk, but absent from bovine milk, indicated that the rat was a suitable model for the second study.

**Rat Intestinal Mucosa.** Representative elution patterns from gel filtration of the supernatant fraction of rat intestinal mucosa on a Sephadex G-75 column are shown in Fig. 2. In newborn, 7-day-old, 10-day-old, and 14-day-old animals, the major zinc peak was associated with high-molecular-weight fractions (void volume). However, in 16-day-old rats, the zinc was evenly distributed between the high-molecular-weight materials and a lower-molecular-weight compound. By day 18, the major zinc peak was associated with the low-molecular-weight material. Samples of intestinal mucosa from rats at 21 and 28 days of age showed elution patterns similar to those of samples taken at day 18.

**DISCUSSION**

The results presented here demonstrate that rat milk contained a ZBL similar in size to that found previously by us in human milk (1) and validate the use of the rat as a model to test the hypothesis that this ZBL may be involved in absorption of zinc in the neonate. A ZBL similar to that found in rat milk was identified in the rat intestine of 16- to 28-day-old animals but was absent in younger animals.

Knowledge concerning the intestinal transport and absorption of zinc is limited at the present time, especially in regard to the neonatal period. Van Campen and Kowalski (12) have reported the presence of compounds of low molecular weight (10,000–15,000) in the rat intestinal mucosa that are associated with zinc and that may be involved in absorption of this element. Hahn and Evans (12) have also identified a zinc-containing compound of low molecular weight in the intestinal mucosa.
mucosa of rats and suggested that it may be important in zinc transport across the intestine. The identification of a low-molecular-weight ZBL from rat intestinal mucosa in the present study confirms the findings of Van Campen and Kowalski (12) and Hahn and Evans (13).

The presence of a ZBL in rat milk prior to the development of the intestinal ZBL supports our hypothesis that the milk ZBL may enable or enhance the intestinal absorption of zinc during the neonatal period, allowing for perhaps maximal absorption of this essential trace element before the appearance of the intestinal ZBL. The importance of adequate zinc absorption for growth and survival of suckling rats has been demonstrated by Mutch and Hurley (11) who found a high requirement for zinc during this period of development. A similar mechanism may be operative in normal human infants, for whom the importance of adequate zinc is emphasized by reports (14, 15) of suboptimal zinc nutrition in infants and young children. Anorexia, reduced zinc concentrations in hair, and impaired growth occurred in these children. In AE patients, the normal mechanisms of zinc absorption may be permanently immature or absent, and the ZBL in human milk may therefore enhance absorption of zinc even at later ages.

These studies suggest that the value of breast milk in infant nutrition may be greater than a simple numerical accounting of its nutrient composition might indicate. It is also possible that binding compounds for other nutrients may be present in breast milk and that these compounds may also be important for nutrition during the neonatal period. The value of early breast milk for immune responses of the infant is well recognized (16–18). An additional factor of importance in the neonatal period may be related to the presence of ZBL and perhaps other similar compounds in human milk.

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