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ORIGINAL ARTICLE

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Dietary phosphorus reduction by pretreatment of human breast milk with sevelamer

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Abstract Hyperphosphatemia leading to hyperparathyroidism and ultimately renal osteodystrophy is a well-known complication of chronic renal failure. A new hydrogel binder, sevelamer, has recently become available for use in hyperphosphatemic patients with renal failure. We had previously mixed the capsule with pumped breast milk and formula, but discovered that the hydrogel formed a viscous solution that infants were unable or unwilling to swallow. We therefore evaluated the phosphorus content of fresh and frozen breast milk before and after treating with different doses of sevelamer at different temperatures and for varying lengths of time. The hydrogel bound promptly to phosphorus, reducing the phosphorus content 78% within 5 min. The viscous hydrogel settled to the bottom of the container within 10 min allowing the supernatant to be easily decanted. We also evaluated the breast milk for changes in other electrolytes, osmolality, pH, and macronutrient content. These results show that fresh or frozen breast milk can be safely pretreated with sevelamer without significantly changing its macronutrient or ionic content, with the exception of calcium and protein. The supernatant can be fed to infants or instilled through a gastrostomy tube without difficulty since the viscous hydrogel settles rapidly to the bottom of the container.

Keywords Sevelamer · RenaGel (Genzyme) · Hyperphosphatemia · Renal failure · Infants · Toddlers · Pretreatment

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Introduction

Chronic renal failure is associated with many electrolyte abnormalities, including hyperphosphatemia. Hyperphosphatemia leading to renal osteodystrophy occurs relatively early in the progression of renal failure, becoming apparent with as little as a 50% loss of glomerular filtration rate [1]. Prevention of renal osteodystrophy requires control of serum parathyroid hormone levels, which are increased in the face of hyperphosphatemia. Initial therapy for treating hyperphosphatemia in renal failure patients consists of dietary phosphorus restriction. For healthy infants less than 6 months of age, the recommended daily allowance for phosphorus intake is 300 mg. To limit their phosphorus intake, infants with renal failure are fed breast milk (approximately 141 mg/day of phosphorus) or low-phosphorus formula milk such as Similac PM 60/40 or Carnation Good Start (containing approximately 181 mg/day and 233 mg/day of phosphorus, respectively) [2, 3]. In spite of this significant restriction in dietary phosphorus intake, many infants with oliguric renal failure still develop hyperphosphatemia. Further reduction in their phosphorus intake requires the use of phosphorus binders. Historically, elevated serum phosphorus levels have been controlled with aluminum- or calcium-based binders. Aluminum-containing binders fell out of favor in the mid 1980s following reports of osteomalacia as well as the development of aluminum encephalopathy associated with mental retardation and seizures [4, 5]. Calcium binders then became the mainstay of therapy for control of hyperphosphatemia. It is not unusual for infants treated with calcium carbonate to develop hypercalcemia and/or elevated calcium phos-

phorus products. Additionally, in the last several years, numerous studies have looked at the supraphysiological dose of calcium required by patients with renal failure for phosphorus control and the significantly greater calcium load these patients carry. There is still much debate in the literature as to whether this large calcium burden alone is responsible for the widespread arterial calcification seen in young adults with end-stage renal disease [6, 7]. There is clearly, however, early and increased cardiovascular morbidity and mortality among end-stage renal disease patients, which may be associated with their increased coronary artery calcification.

In 1999, the FDA approved sevelamer for the treatment of hyperphosphatemia in hemodialysis patients [8, 9]. It is given in conjunction with meals, binding dietary phosphorus, and thereby preventing gastrointestinal absorption. Adult chronic renal failure and end-stage renal disease patients are now benefiting from this hydrogel binding treatment. To date infants and toddlers have not been routinely treated with sevelamer because it is only available in capsule and tablet form. We tried mixing the contents of the capsules into pumped human breast milk. The few infants with hyperphosphatemia and hypercalcemia already being treated with calcium carbonate suspension were then treated with this mixture and were either unable or unwilling to swallow the solution. Attempts at our institution to mix the contents of the capsules into formula or water and instilling through gastrostomy tubes also failed since the viscous mixture clogged the tubes. Mixing the capsule into greater volumes of water to make the mixture less viscous was not practical, since many patients with oliguric chronic renal failure or end-stage renal disease are on fluid restriction.

Similar problems had been noted when sodium polystyrene sulfonate was used for the treatment of hyperkalemia in infants and toddlers with renal failure [10]. These problems were overcome by pretreating the breast milk and formula with sodium polystyrene sulfonate, allowing the potassium-bound resin to settle to the bottom, decanting, and then feeding the supernatant to the patient. We conducted a similar experiment with sevelamer to evaluate the dose required and appropriate length of pretreatment to decrease the phosphorus content of the breast milk. We then evaluated whether temperature and previous frozen storage affected the binding capacity of the hydrogel and the rate at which the hydrogel settled to the bottom of the container. The nutritional and electrolyte content of the breast milk was also studied to evaluate whether the hydrogel altered other components of the milk.

Materials and methods

The initial four experiments were conducted on a sample provided by a breast-pumping mother who was disposing of excess breast milk. The fifth experiment was conducted on breast milk donated by another mother. The Institutional Review Board of Naval Medical Center San Diego approved the study. Informed consent was obtained. An additional mother's milk was tested for clinical purposes; these data are included in this study. All mothers were greater than 4 days post partum at the time the specimens were obtained. Previous studies evaluating breast milk electrolytes showed a significant change in their concentrations during the first 4 days of lactation. Thereafter, breast milk constituents were relatively stable in their concentration, including phosphorus which averaged 5.09±1.14 mg/dl [2]. The sevelamer capsule was opened and the crystalline powder alone was poured into the breast milk specimen. The breast milk was then vigorously shaken by hand for 15 s, emulating how a parent might pretreat breast milk at home.

Initially previously frozen breast milk was divided into three 90-ml aliquots. Each aliquot was treated with an increasing dose of sevelamer, approximately 201.5 mg, 403 mg, and 806 mg, respectively. A fourth aliquot was not treated and used as a control to determine the phosphorus content of untreated breast milk. After 1 h at room temperature, the supernatant was collected and the phosphorus content of each aliquot was measured. The same dosing experiment was conducted at 3°C, but comparing pretreatment phosphorus levels with specimens that had been treated with sevelamer for 24 h. We did not evaluate milk treated for longer than 1 h at room temperature or 24 h at refrigerated temperature, since that is the longest time thawed breast milk should be stored before being fed to an infant.

Next, an aliquot to measure pretreatment phosphorus concentration was obtained from a previously frozen specimen and then 403 mg of sevelamer was mixed with 90 ml of remaining breast milk and a container similar in size and shape to a plastic baby bottle was placed in the refrigerator. Samples were obtained from the mid-center of the specimen container at 5-min intervals for the first 30 min and then every 15 min for the next 2 and 1.5 h. Each specimen was labeled with the time of collection and sent for phosphorus measurement. We chose to evaluate only the first 3 h post treatment since breast-fed infants tend to eat every 2–3 h. From a practical standpoint, pumping mothers who are bottle feeding pumped milk will pump at the time their babies are feeding, which would allow them no more than 2–3 h between feeding to treat the breast milk.

A previously frozen breast milk specimen was then divided into two 90-ml specimens. One specimen was treated with 403 mg of sevelamer at room temperature for 1 h and samples were sent for analysis of sodium, potassium, calcium, osmolality, pH, total protein, cholesterol, triglycerides, glucose, and IgA. The untreated specimen was used as a control.

Lastly, freshly pumped breast milk was separated into two aliquots. One of the aliquots (5 ml) was not treated and used as a control for the phosphorus content of that specimen. The remaining 90-ml specimen was immediately treated with the 403 mg of sevelamer and the supernatant sent for phosphorus analysis. The second aliquot was frozen and then defrosted 1 week later. Again, an initial 5 ml was decanted prior to treatment with sevelamer and used as a control for the phosphorus concentration of that specimen after freezing. The remaining 90 ml was treated with 403 mg of sevelamer at room temperature for 1 h. The supernatant's phosphorus content was measured and compared with the first aliquot to determine whether lack of freezing affected the phosphorus precipitation with sevelamer.

Results

Pretreatment of 90 ml of breast milk with one-half capsule of sevelamer (approximately 201.5 mg) at room temperature for 1 h or refrigerated for 24 h decreased the breast milk phosphorus content by 46% and 53%, respectively. Room temperature treated breast milk showed a 62% decline in phosphorus content when pretreated with one capsule (403 mg) and an 81% decline when pretreated with two capsules (806 mg, Table 1). A similar decline in phosphorus concentration of 78% and 84% was

Table 1 Phosphorus content of breast milk treated with varying doses of sevelamer at different temperatures

Aliquot	Dose of sevelamer	Phosphorus content	
		1 h at room temperature	24 h at 3°C
1	None	7.3 mg/dl	6.8 mg/dl
2	~201.5 mg	3.9 mg/dl	3.2 mg/dl
3	403 mg	2.8 mg/dl	1.5 mg/dl
4	806 mg	1.4 mg/dl	1.1 mg/dl

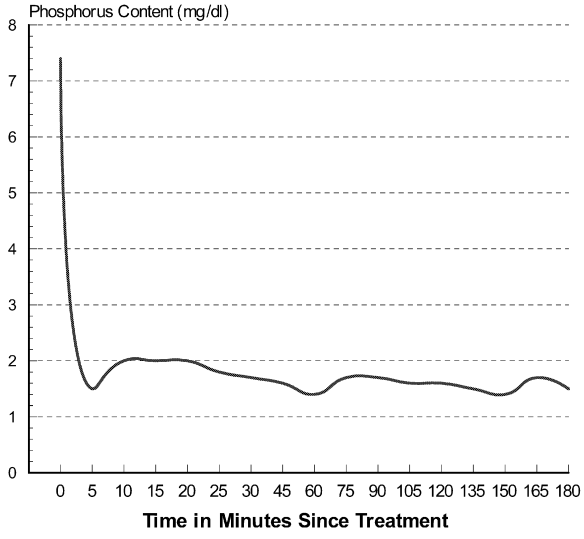

Fig. 1 Length of pretreatment at 3°C with sevelamer 403 mg/90 ml of breast milk

obtained with increasing doses of sevelamer when the breast milk was refrigerated for 24 h.

Based on the significant decrease in breast milk phosphorus with any sevelamer pretreatment, it was decided to continue the study using the dose of 403 mg (one capsule) per 90 ml of breast milk. This greatly improves the ease of administration by not requiring parents to divide one capsule into two doses and it allows for an increase in dose if needed based on persistently elevated serum phosphorus levels.

The length of pretreatment study demonstrated an immediate decline in phosphorus content (Fig. 1) and a very small continued decline throughout the time period during which measurements were taken [not readily apparent in Fig. 1, but visible in a statistically significant (P=0.024) regression slope of –0.002 for the measurements from 5 through 180 min]. The apparent increase in phosphorus at 10 min compared with the 5-min specimen is likely an artifact of laboratory measurement variation between the 0-, 5-, and 10-min specimens. Since the viscosity and taste of pretreated breast milk were other issues affecting patient treatment, we also evaluated how rapidly the hydrogel settled to the bottom of the container. Whether it was maintained at room temperature or refrigerated, the hydrogel maximally settled to the bottom of the container by 10 min post treatment. The supernatant was easily decanted after pretreatment at either temperature, but the hydrogel from the refrigerated specimen was slightly more solid, limiting the chance that a parent would mix the precipitant into the decanted supernatant.

Table 2 Analysis of other breast milk components after pretreatment with sevelamer 403 mg/90 ml of breast milk at room temperature for 1 h

Other breast milk components	Concentration (units expressed individually)	
	Pretreatment	Post-treatment
Sodium	7.6 mEq/l	7.8 mEq/l
Potassium	13.4 mEq/l	13.6 mEq/l
Calcium	13.6 mg/dl	8.3 mg/dl
Total protein	1.4 g/dl	1.2 g/dl
IgA	13 mg/dl	13 mg/dl
Glucose	30 mg/dl	30 mg/dl
Triglycerides	1,629 mg/dl	1,606 mg/dl
Cholesterol	15 mg/dl	19 mg/dl
Osmolality	284 mosmol	290 mosmol
pH	6.52	6.48

The electrolyte and macronutrient study revealed no significant change in sodium, potassium, glucose, triglycerides, osmolality, IgA, or pH between pre- and post-treatment specimens (Table 2). There was a 26% increase in cholesterol even though there are no steroid esterol components in sevelamer. The measured increase in cholesterol is likely a consequence of laboratory measurement error based on the minimal concentration of cholesterol in breast milk and the expected assay variance.

Breast milk has several major proteins: α-lactalbumin, lactoferrin, casein, and secretory IgA [11]. The IgA concentration did not change following treatment, but the total protein content decreased by 14% following treatment with sevelamer. The casein concentration in mature breast milk is approximately 0.2 g/dl and is mostly bound in micellar form. The casein micelle contains the majority of the calcium and phosphorus in the breast milk [11]. Although we were unable to measure casein directly, this decrease in total protein is probably a consequence of the entire micelle binding to sevelamer and precipitating out with the hydrogel. Similarly, the calcium concentration decreased by 39%–51%. The experiment was run twice to confirm the decrease in calcium content. This drop in calcium is probably a consequence of the fact that a majority of the calcium in breast milk is contained in the casein micelle with the phosphorus and precipitates out with the sevelamer. This hypothesis could be more rigorously tested by evaluating the ionized calcium content of pre- and post-treatment specimens, but we were unable to find a laboratory that was able to measure ionized calcium concentrations of breast milk

Freezing of breast milk is known to destroy the leukocytes expressed in breast milk and since phosphorus is largely an intracellular anion, the final stage of this experiment looked at the handling of breast milk prior to treatment with sevelamer (Table 3). This breast milk was obtained from a different mother than the breast milk used for the first four stages of this study. The concentration of phosphorus in her freshly pumped milk was not different

Table 3 Comparison of fresh and previously frozen thawed breast milk treated with sevelamer 403 mg/90 ml of breast milk at room temperature for 1 h

Specimen handling	Phosphorus content
Fresh, no treatment	7.5 mg/dl
Fresh, immediate treatment	3.5 mg/dl
Frozen, no treatment	7.3 mg/dl
Frozen, delayed treatment	3.5 mg/dl

from that measured in the first specimen analyzed after 1 week of being maintained at −18°C. Immediate pretreatment and pretreatment after 1 week at −18°C decreased the phosphorus content by 53% and 52%, respectively.

For clinical purposes, the breast milk from the mother of an infant with congenital obstructive uropathy and hyperphosphatemia was evaluated for its change in phosphorus concentration following pretreatment with sevelamer. Based on the preliminary results of this study her breast milk was treated with 403 mg of sevelamer per 90 ml of breast milk for 1 h at room temperature. The initial breast milk phosphorus content was 4.5 mg/dl. After treatment, the breast milk phosphorus content decreased to 1.6 mg/dl, which is a decrease of 64%. Although her initial breast milk phosphorus was lower than the other two women studied, the percentage change in phosphorus concentration was comparable to the other milk specimens.

Discussion

Our study shows a significant decrease in the phosphorus content of breast milk following pretreatment with sevelamer. The use of one capsule per 90 ml of breast milk led to an average decrease in the available phosphorous by 65% (range 52%–81%) within 1 h. Although a 10-min post-treatment concentration was only measured once, the phosphorous content dropped by 72% in that short interval. Although only two women contributed breast milk to this particular study, there is no reason to believe that the percentage change in phosphorous would be different among breast milk samples. Human breast milk is relatively comparable between women and sevelamer binds phosphorus in the form of mostly ionic bonds and some hydrogen bonds [12]. This hypothesis is borne out by a third woman's breast milk that was studied for clinical purposes. In this case the phosphorus content decreased by 64%.

There does not appear to be a significant difference as a result of pretreatment at room temperature or after the specimen is refrigerated, although the refrigerated specimen does allow a slightly more semi-solid layer of precipitant to form (Fig. 2). This greater distinction in the layering may improve a parent's ability to decant the supernatant alone. The ability of the sevelamer to bind phosphorus also does not appear to be affected by freezing the breast milk prior to treatment. Since the viscosity of the mixture may have been one of the reasons infants had refused to drink the treated breast milk, it does appear

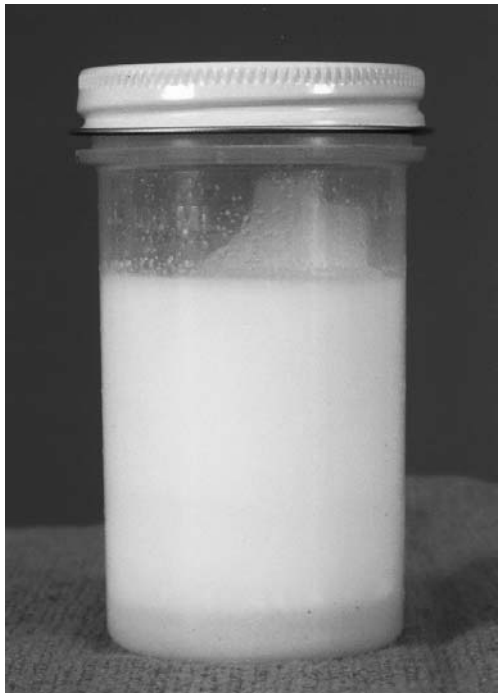

Fig. 2 Visible layering of sevelamer resin in human breast milk after as little as 10 min of pretreatment at 3°C

that waiting 10 min while allowing the precipitant to settle and decanting the breast milk will minimize the thickening effect of the hydrogel. We did not attempt to feed the pretreated breast milk to any infant so there may still be a change in taste that will limit an infant's acceptance of the supernatant.

In 1991, Bunchman et al. [10] reported the pretreatment of commercially available formulas and supplements with sodium polystyrene sulfonate to reduce the dietary intake of potassium. Although they were able to lower the potassium content of the formula by approximately 60%, the sodium content increased dramatically. Schroder et al. [13] studied the pretreatment of liquid supplements with calcium polystyrene sulfonate several years later. They demonstrated a 50% drop in potassium content without any increase in the sodium content. At the time, they suggested there might be some benefit to any increased dietary calcium exposure. In 1997, Bonnet et al. [14] and in 1998, Fassinger et al. [15] reported comparison trials of pretreatment of infant formulas and milk with calcium versus sodium polystyrene sulfonate. Bonnet et al. [14] noted significant variations in the final electrolyte composition of the supplements based on the type of resin used and formula studied. Fassinger et al. [15] demonstrated a more significant dietary reduction in potassium with sodium polystyrene sulfonate, but reported increases in both dietary sodium and calcium availability following decanting of the supernatant.

Since sevelamer is a hydrogel binder, this present study also addressed the issue of changes in electrolyte composition, osmolar load, pH, and macronutrient composition of the pretreated breast milk. There were no changes

in the pH or sodium and potassium concentrations following pretreatment. Although lactose and not glucose is the major carbohydrate in breast milk, we were unable to measure lactose directly. Importantly, neither the glucose concentration nor the osmolality were altered by the sevelamer. Since lactose is the major osmole in breast milk and lactose is hydrolyzed to glucose and galactose, it can be inferred from the stable concentration of glucose and unchanged osmolality that the lactose concentration was not significantly altered.

The majority of lipids in breast milk are triglycerides with phospholipids and cholesterol making up the remainder of the breast milk fat. There was no appreciable change in triglyceride concentration following treatment with sevelamer and the increase in cholesterol most likely represents laboratory variation and measurement error.

The issue of changes in immunoglobulin concentration is particularly important since the immuno-protective benefit of breast milk is one of the principal reasons to encourage breast feeding of all infants. Secretory IgA is one of the four major proteins found in breast milk and is unchanged by pretreatment with sevelamer. The total protein concentration decreased by 14% upon treatment with sevelamer. Casein exists in breast milk in micellar form at a concentration of approximately 0.2 g/dl, which is the same amount by which the total protein content of the post-treatment breast milk decreased. Because the casein micelle contains the majority of the phosphorus and calcium in breast milk, it is possible that all of the casein was removed by the sevelamer.

The most notable change in the breast milk constituents following treatment with the hydrogel was the decrease in calcium concentration. As with the casein, the calcium was probably extracted in its micelle form with the phosphorus-bound sevelamer. This decreased dietary calcium does have physiological implications for infants fed pretreated breast milk since adequate bone growth in infants and young children requires maintenance of a positive calcium balance. Any infants treated with sevelamer-exposed breast milk will need routine monitoring of their serum calcium and alkaline phosphatase levels and may need some calcium supplementation.

This study has shown that breast milk can successfully be pretreated with sevelamer to markedly decrease the dietary phosphorus load. This pretreatment can be performed in as little as 10 min, which is important based on the frequency and sometimes the urgency of infant feedings. The breast milk does not need any special handling during pretreatment allowing parents to treat the breast milk even if they are not at home. Treatment with the hydrogel does not appear to alter the other components of breast milk, with the exception of protein and calcium; serum calcium should be routinely measured in infants being fed sevelamer-treated breast milk.

Finally, although this study did not address the willingness of infants to drink pretreated breast milk, we did show that the hydrogel maximally settles to the bottom of the container by 10 min, minimizing the viscosity problem, and at least allowing administration through gastrostomy tubes. Based on the studies of Bonnet et al. [14], these results should not be applied to commercially available formulas. Commercially available infant and pediatric formulas that provide greater dietary exposure to phosphorus should be studied for their change in phosphorus as well as other nutritional and electrolyte components following pretreatment with sevelamer for those children with oliguric renal failure who can not or do not have access to breast milk.

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