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The Effect of Nutritional Additives on Anti-Infective Factors in Human Milk

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Summary: It has become a common practice to supplement human milk with a variety of additives to improve the nutritive content of the feeding for the premature infant. Twenty-two freshly frozen human milk samples were measured for lysozyme activity, total IgA, and specific IgA to *Escherichia coli* serotypes 01, 04, and 06. One mL aliquots were mixed with the following: 1 mL of Similac, Similac Special Care, Enfamil, Enfamil Premature Formula, and sterile water; 33 mL of Poly-Vi-Sol, 33 mg of Moducal, and 38 mg of breast-milk fortifier, and then reanalyzed. Significant decreases (41% to 74%) in lysozyme activity were seen with the addition of all formulas; breast-milk fortifier reduced activity by 19%, while no differences were seen with Moducal, sterile water, or Poly-Vi-Sol. No differences were seen in total IgA content, but some decreases were seen in specific IgA to *E. coli* serotypes 04 and 06. *E. coli* growth was determined after 3 1/2 hours of incubation at 37°C after mixing. All cow-milk formulas enhanced *E. coli* growth; soy formulas and other additives preserved inhibition of bacterial growth. Nutritional additives can impair anti-infective properties of human milk, and such interplay should be considered in the decision on the feeding regimen of premature infants.

Introduction

Human milk has been utilized in the nutritional support of premature infants based on its unique advantages:[1] the presence of anti-infective factors (protecting against infection);[2,3] enhanced absorption and utilization of fat, zinc, and iron (compared with premature formulas); low renal solute load; promotion of maternal-infant bonding; evidence of its protection against the development of necrotizing enterocolitis;[4] and its putative growth factors which may enhance intestinal maturation. However, nutritionally, human milk may be deficient in vitamins, minerals, and protein for the growing premature infant. Nutrient fortification of human milk can be accomplished by the addition of vitamin/mineral supplements, by the use of commercial human-milk fortifiers, or

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Table 1

LYSOZYME ANALYSIS RESULTS

	Lysozyme activity (μg/mL)
Control	23.8 ± 2.6
Similac	12.8 ± 4.2*
Similac Special Care	14.1 ± 3.7
Enfamil	6.3 ± 3.6*
Enfamil Premature	6.8 ± 6.5*
Breast-milk fortifier	19.2 ± 1.8
Moducal	22 ± 0.7
Poly-Vi-Sol	22.2 ± 4.4
Sterile water	

*$P < 05$
F = 14.2
mean ± SE, n = 22

by mixing human milk with premature-infant formulas. Such mixing has been shown to improve fat absorption (due to the presence of human milk lipase)[5] and to improve bone mineral status (due to the higher levels of calcium and phosphorus found in premature infant formulas).[6] In our previous studies, we examined the effect of microwave radiation on breast-milk protective factors, such as lysozyme and specific secretory IgA, and showed that microwave radiation decreased the effectiveness of these anti-infective factors.[7] While a variety of substances have been added to human milk in order to "improve the nutritive content" for the low-birth-weight infant,[6,8] there are few data regarding the impact of these additives on the beneficial effects of human milk. In this study, we have examined a number of common additives to breast milk to explore their effect on the anti-infective factors of human milk.

Materials and Methods

Milk Samples

Breast-milk samples (n = 22) were obtained from term and preterm lactating mothers after the first week of postnatal life, using sterile breast pumps or manual expression and collected, using proper collection technique,[9,10] into sterile glass jars. Sterility was checked on each specimen by culturing on trypticase soy media with 5% sheep's-blood agar plates. Breast-milk samples containing any of the *Enterobacteriaceae* were excluded from the study. Samples were frozen immediately at -18°C and analyzed within 2 to 7 days.

Milk Processing

Each frozen sample was allowed to thaw at room temperature. One mL of each human-milk sample was thoroughly mixed with 1 mL of the following: human milk, Similac (Ross), Similac Special Care (Ross), Enfamil (Mead Johnson), Enfamil Premature Formula (Mead Johnson), and sterile water. To other 1 mL aliquots, 33 mL of Poly-Vi-Sol (Mead Johnson), 33 mg of Moducal (Mead Johnson), and 38 mg of breast-milk fortifier (Mead Johnson) were added and vortexed. The mixtures were centrifuged at 7,000 rpm at 4°C for 60 minutes, sample volumes were recorded, and the fat and sediment were discarded. The supernatant was used for assay of lysozyme, total IgA, and specific IgA to *E. coli* serotypes. "Control" human-milk samples were handled as stated above but were not mixed with any additives.

Lysozyme Activity

Lysozyme activity was measured from the rate of lysis of a suspension of lyophilized *Micrococcus Lysodeikticus* cells, using a minor modification of the procedure described by Litwack.[11] Egg-white lysozyme (Sigma) was used as the standard.

Estimation of Immunoglobulin

Total IgA was estimated by the "precision" single radial immunodiffusion technique. For the quantitation of IgA, the Sigma antibody was based on the use of 11S (not 7S or secretory component) human IgA as a standard. Specificity was tested by enzyme-linked immunosorbent assay against human IgG, human IgM, and human IgA; only the IgA showed reactivity.

Specific IgA to *E. coli* serotypes 01, 04, and 06 were assayed by enzyme-linked immunosorbent assay. Further details of these methods have been previously published.[7]

The milk samples were col-

Table 2

TOTAL IGA

	dL)
Control	± 1.7
Similac	± 1.7
Similac Spec	± 2.7
Enfamil	± 2.2
Enfamil Prem	± 2.0
Breast-milk f	± 4.2
Moducal	± 2.5
Poly-Vi-Sol	± 2.5
H2O	± 2.2

F = 1.0
mean ± SE, n =

Table 3

DIFFERENCES IN SPECIFIC IGA BINDING AGAINST *E. COLI* 01, 04, AND 06 ANTIGENS

Antibody to *E. coli*	01
Control	6.9 ± .28
Similac	6.4 ± .44
Similac Special Care	5.0 ± .31
Enfamil	7.9 ± .26
Enfamil Premature	10.2 ± 1.3
Breast-milk fortifier	10.6 ± .87
H_2O	10.2 ± .89
Moducal	10.5 ± 1.3
Poly-Vi-Sol	12.7 ± 1.2
F Statistic	*18.9*

*P ≤ .05
n = 21, mean ± SEM

lected from mothers having preterm and term babies. These samples were previously studied for total IgA and lysozyme; no significant differences were found.[7]

Bacterial Growth

Inhibition of *E. coli* growth was studied as previously described.[7,12] Ten additional human milk samples were utilized. In addition to the formulas and fortifier added previously, three soy formulas were also studied because the practice of using soy formulas as additives was discovered at the end of these studies.

Statistics

The statistical method used was analysis of variance for randomized block design. Post hoc comparison employed least squares differences. Experimental values were mathematically adjusted for the effect of dilution and expressed as units/mL. Mode of collection was recorded and did not appear to affect the results; thus, this factor was not considered in the analysis of the data.

Results

Results of lysozyme analysis are seen in Table 1. There are significant decreases in lysozyme activity of 41% to 74% when either "regular" or "premature" formulas are added. Breast-milk fortifier reduced activity by only 19%. No significant differences were seen with Moducal, sterile water, or Poly-Vi-Sol.

Total IgA was not statistically different after the additives (Table 2). Some differences in specific IgA binding were seen in antibodies against *E. coli* 04 and 06 antigens (Table 3) with the addition of Similac Special Care, Enfamil Premature, water, and Moducal.

The inhibition of bacteria growth was lessened significantly by all the cow's-milk-based formulas, though not by soy-based formulas or breast-milk fortifier (Figure 1).

Discussion

The suitability of human milk as the sole nutritional source for premature infants has been controversial. Estimates of nutritional requirements to mimic intrauterine growth suggest that human milk is inadequate in nutrient and mineral content for low-birth-weight infants. Human-milk protein supplementation has been advocated as a way of supplying extra protein and preventing signs of early malnutrition.[8] However, this technique has not been widely used.

Some nurseries have added cow's-milk formulas, vitamins, and other specific fortifiers to human milk to enhance nutritive content. Investigations demonstrating clear benefit to outcome by such additives has been limited.[6] Our data suggest that some of these additives may adversely affect the anti-infective properties of human milk. The exact mechanism of this effect is unclear; possible causes may involve binding of either antibodies or lysozyme to substances (such as proteins) contained within the formula.

The clinical significance of these in vitro findings is difficult to quantitate. Narayanan et al[3] saw a 3.8% increase in infection rate in high-risk neonates fed pasteurized human milk compared to raw human milk. Use of partial formula feedings between pasteurized human-milk feedings increased the infection rate by 23%. Holder pasteurization (62.5°C for 30 min) of human milk has been associated

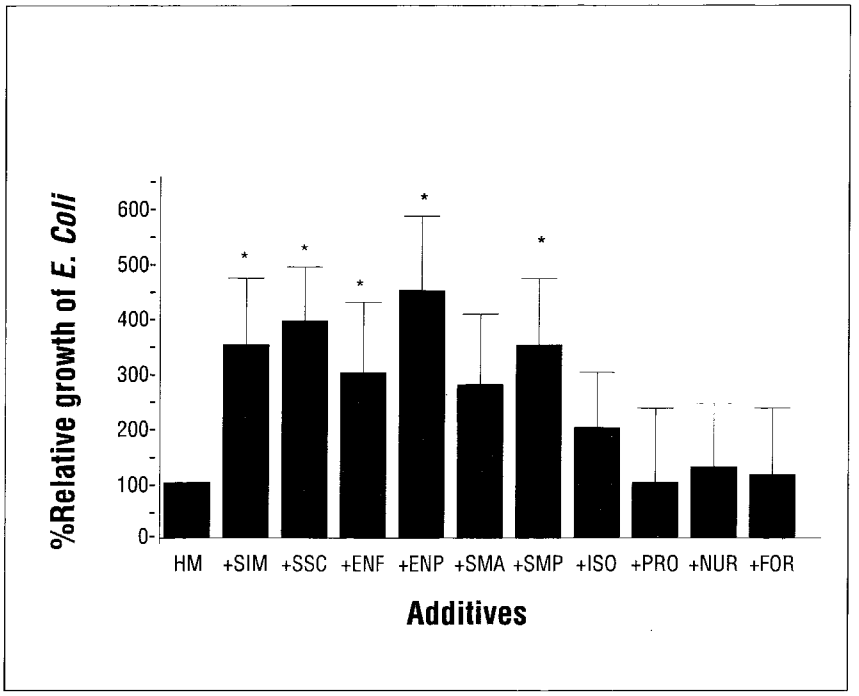

Figure 1. Effect of nutritional additives on *E. coli* growth. Growth of *E. coli* is expressed as percent relative to control ± SEM. HM = breast milk alone, +SIM = Similac, +SSC = Similac Special Care, +ENF = Enfamil, +ENP = Enfamil Premature, +SMA = SMA, +SMP = SMA-Preemie, +ISO = Isomil, +PRO = Prosobee, +NUR = Nursoy, +FOR = breast-milk fortifier. $^{*}P \leq .05$

with a 20% reduction in IgA titer and the substantial destruction of IgM and lactoferrin; but lysozyme was unchanged in these studies.[13,14] In the present study, comparable decreases in IgA titer (total and specific) were seen, while lysozyme activity decreased by 41% to 74%. Thus, our data would support the idea that the infection rate among low-birth-weight infants might similarly be affected, and that a clinical study in this area is warranted.

It is possible that nutrient and mineral supplementation could afford significant advantages to the low-birth-weight infant. The advantages might justify the decrease in anti-infective factors in human milk. If it were possible to precisely quantify the immunologic advantage of human milk in premature infants, then the importance of the preservation of anti-infective factors could be weighed against other potential benefits of supplements. Further investigations are needed to examine the effects of the addition of a variety of other substances (e.g., zinc, calcium, phosphorus, carnitine) on the beneficial effects of breast milk. To avoid the potential for impairment of anti-infective factors in human milk, premature formula could be alternated with human milk in successive feedings, rather than mixing the formula with human milk, although data by Narayanan et al[3] suggest there is still some loss of protection. The effect of this regimen on anti-infective properties of human milk would need to be studied in a classical trial.

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