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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:4 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...
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) and to improve bone mineral status (due to the higher levels of calcium and phosphorus found in premature infant formulas). 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. While a variety of substances have been added to human milk in order to “improve the nutritive content” for the low-birth-weight infant, 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, 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.

**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.

The milk samples were collated at ARIZONA STATE UNIV on January 4, 2011.
lected from mothers having pre-
term and term babies. These sam-
ple were previously studied for
total IgA and lysozyme; no signifi-
cant differences were found.

Bacterial Growth

Inhibition of E. coli growth was
studied as previously described. Ten
additional human milk sam-
ple 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 ran-
domized block design. Post hoc
comparison employed least
squares differences. Experimental
values were mathematically ad-
justed 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 signifi-
cant decreases in lysozyme activity
of 41% to 74% when either “regu-
lar” 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 antibod-
ies against E. coli 04 and 06 antigens
(Table 3) with the addition of Simi-
Table 3

<table>
<thead>
<tr>
<th>Antibody to E. coli</th>
<th>01</th>
<th>04</th>
<th>06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.9 ± .26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Similac</td>
<td>6.4 ± .44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Similac Special Care</td>
<td>5.0 ± .31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enfamil</td>
<td>7.9 ± .26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enfamil F1</td>
<td>10.2 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast-milk</td>
<td>10.6 ± .87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2O</td>
<td>10.2 ± .89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moducal</td>
<td>10.5 ± 1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly-Vi-Sol</td>
<td>12.7 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F Statistic</td>
<td>18.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05
n = 21, mc: = .05

lac Special Care, Enfamil Prela-
ture, 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 pre-
mature infants has been controver-
sial. Estimates of nutritional
requirements to mimic intrauterine
growth suggest that human milk is
inadequate in nutrient and mineral
content for low-birth-weight infants.

Investigations demonstrating clear
benefit to outcome by such addi-
tives has been limited. Our data
suggest that some of these addi-
tives 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 anti-
bodies 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 saw a
3.8% increase in infection rate in
high-risk neonates fed pasteurized
human milk compared to raw hu-
man milk. Use of partial formula
feedings between pasteurized hu-
man-milk feedings increased the
infection rate by 23%. Holder pas-
teurization (62.5°C for 30 min) of
human milk has been associated
with a 20% reduction in IgA titer and the substantial destruction of IgM and lactoferrin; but lysozyme was unchanged in these studies.\textsuperscript{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\textsuperscript{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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## REFERENCES


