ORIGINAL ARTICLE

Suppressive effects of breast milk on oxidative DNA damage in very low birthweight infants

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Background: Human milk contains many kinds of antioxidant and is considered to prevent diseases mediated by oxygen free radicals in very low birthweight (VLBW) infants.

Aims: To examine the antioxidant effects of breast milk in VLBW infants by determining urinary 8-hydroxydeoxyguanosine (8-OHdG) excretion, which is known to be a non-invasive marker for in vivo oxidative DNA damage.

Methods: Urinary 8-OHdG concentrations were measured in 15 breast fed and 14 formula fed VLBW infants at 2, 7, 14, and 28 days of age.

Results: Urinary 8-OHdG excretion at 14 and 28 days of age was significantly lower than at 2 and 7 days of age in the breast fed group, and significantly lower than in the formula fed group.

Conclusion: This is the first direct evidence of the antioxidant action of human milk in VLBW infants.

Premature infants are exposed to many possible sources of oxygen free radical production including high concentrations of inspired oxygen, frequent alterations in blood flow to major organs, and inflammation with accumulation of neutrophils and macrophages.^{1,2} Moreover, premature infants are known to have a poorly developed antioxidant system and may be at increased risk of radical damage.^{3–5} An imbalance between oxidant generating systems and antioxidants in very low birthweight (VLBW) infants is implicated in the pathogenesis of the major complications of prematurity, including necrotising enterocolitis (NEC),⁶ chronic lung disease (CLD),⁷ retinopathy of prematurity (ROP),⁸ and intraventricular haemorrhage (IVH).⁹

Human milk has many enzymatic and non-enzymatic antioxidant constituents, including superoxide dismutase, glutathione peroxidase, catalase, vitamins E and A, and β -carotene, which may protect against the development of complications induced by oxygen free radical in infants.^{10–12} Breast feeding has been associated with low rates of a variety of illnesses in premature infants, including NEC,¹³ respiratory disease,¹⁴ and ROP,¹⁵ although there are no direct data establishing an antioxidant action of breast milk in infants.

Various methods have been established to evaluate oxidative stress and oxidative tissue damage, but accurate and reliable measurement of oxygen free radical activity is not easy. 8-Hydroxydeoxyguanosine (8-OHdG) is accepted as a sensitive marker for oxidative DNA damage.^{16,17} On hydroxylation, it is excised by constitutive enzymatic repair systems and subsequently excreted intact in the urine. Therefore, urinary 8-OHdG excretion can serve as a non-invasive marker for in vivo oxidative DNA damage.¹⁸ Recent studies have described the relation between prematurity and urinary 8-OHdG concentrations in low birthweight infants.^{19,20} However, there have been no previous studies to compare urinary 8-OHdG excretion between breast fed and formula fed VLBW infants. If breast milk has a suppressive effect on oxidative tissue damage in VLBW infants, breast feeding should improve the outcome of VLBW infants. Therefore, to examine the antioxidant effects of breast milk in VLBW infants, we measured urinary 8-OHdG concentrations in breast fed and formula fed VLBW infants at 2, 7, 14, and 28 days of age.

MATERIALS AND METHODS

Patients

Infants were eligible for this study if they had a birth weight less than 1500 g, were born in or transported to the neonatal intensive care unit at Juntendo Izu Nagaoka Hospital between April 2000 and August 2001, and had no major congenital abnormalities. This study was approved by the institutional review board, and written informed consent was obtained from all guardians before inclusion of all subjects in the study.

The breast fed group included 15 infants (eight boys and seven girls; mean (SD) gestational age and birth weight 29.2 (2.3) weeks and 1231 (298) g) who received more than 90% of their intake as breast milk. The formula fed group included 14 infants (eight boys, six girls; 28.7 (2.0) weeks and 1182 (281) g) who received more than 90% of their intake as commercial formula for premature infants. All of the subjects started feeding at 12–72 h after birth, and in most cases feeding started before the first sampling for urinary 8-OHdG measurement. There were no significant differences in the mean gestational age, birth weight, periods of oxygen therapy, phototherapy, and parenteral lipid infusion between the breast fed and formula fed groups (table 1).

Measurement of urinary 8-OHdG

Spot urine samples were collected in the morning at 2, 7, 14, and 28 days of age. They were then stored at -20°C until the assay. The concentration of 8-OHdG was determined using a commercially available competitive enzyme linked immunosorbent assay (ELISA) kit (8-OHdG check; Japan Institute for the Control of Aging, Shizuoka, Japan). The specificity of the assay was established,²¹ and the determination range was 0.64–2000 ng/ml. Urinary 8-OHdG excretion was expressed as a creatinine ratio.

Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; VLBW, very low birth weight; NEC, necrotising enterocolitis; CLD, chronic lung disease; ROP, retinopathy of prematurity; IVH, intraventricular haemorrhage; ELISA, enzyme linked immunosorbent assay

Table 1 Group characteristics of breast fed and formula fed infants

	Breast fed (n = 15)	Formula fed (n = 14)
Gestational age (weeks)	29.2 (2.3)	28.7 (2.0)
Birth weight (g)	1231 (298)	1182 (281)
Sex (male:female)	8:7	8:6
Antenatal steroids	3/15	2/14
Five minute Apgar <6	4/15	3/14
Period of phototherapy (hours)	68.5 (62.4)	72.5 (68.5)
Period of ventilation (days)	15.6 (14.7)	14.1 (12.5)
Period of oxygen therapy (days)	43.3 (40.4)	52.3 (50.9)
Period of parenteral nutrition (days)	12.3 (3.1)	14.4 (3.9)
Period of parenteral intralipid (days)	6.2 (2.1)	6.3 (2.6)
Retinopathy of prematurity	4/15	2/14
Intraventricular haemorrhage	1/15	2/14
Chronic lung disease	2/15	2/14
Necrotising enterocolitis	1/15	0/14

Where appropriate, values are mean (SD).

Statistical analysis

Differences between the different ages were tested by the Wilcoxon signed rank test, and those between the two groups by the Mann-Whitney U test and Fisher's exact test. $p < 0.05$ was considered significant.

RESULTS

In the breast fed group, urinary 8-OHdG excretion at 14 and 28 days of age was significantly lower ($p < 0.01$) than that at 2 and 7 days of age (fig 1). In the formula fed group, there were no significant differences in urinary 8-OHdG excretion observed at 2, 7, 14, and 28 days of age. Urinary 8-OHdG excretion at 14 and 28 days of age in the breast fed group was significantly lower ($p < 0.01$) than that in the formula fed group.

There were no significant differences in the incidence of ROP, IVH, CLD, and NEC between the two groups (table 1).

DISCUSSION

Our results suggest that oxidative DNA damage is considerably more suppressed in breast fed VLBW infants than in formula fed VLBW infants at 14 and 28 days of age. Although human milk contains many kinds of antioxidant and has long been thought to prevent diseases mediated by oxygen free radicals in VLBW infants,¹⁰⁻¹² our study is the first to present direct evidence of this antioxidant action by measuring urinary 8-OHdG excretion. Although the measurement of lipid peroxide concentrations using the malondialdehyde-thiobarbituric acid assay is the most widely used method of

measuring free radical activity,²²⁻²⁴ its accuracy in measuring radical activity and the resultant lipid peroxidation may be compromised by its indirectness and susceptibility to contamination by other thiobarbituric acid reactive species. Damaged DNA is repaired by non-specific endonucleases and specific glycosylases in vivo, and eliminated oxidised nucleotides are finally excreted into the urine as 8-OHdG. 8-OHdG concentrations have been measured by high performance liquid chromatography with electrochemical detection and gas chromatography-mass spectrometry, even though the complicated extraction procedures are known to cause recovery problems.²⁵ An ELISA based on monoclonal IgG has been developed for estimation of 8-OHdG in urine samples.²¹ This method has made it easier to measure urinary 8-OHdG.²⁶ Measurement of urinary 8-OHdG excretion is also considered to be a very sensitive biomarker of oxidative DNA damage in low birthweight infants.^{19, 20} It may also be useful as a true biomarker for free radical mediated diseases in VLBW infants, as well as a good index of the effects of antioxidant supplementation with agents such as selenium.

In this study, there were no significant differences in urinary 8-OHdG levels at 2 and 7 days of age between the breast fed and formula fed groups. There are many factors that can cause oxidative stress resulting in DNA damage in the early stages of life in VLBW infants, such as high concentrations of inspired oxygen, phototherapy, and intravenous lipid infusion.^{1, 2} Therefore, a possible explanation for the lack of antioxidant effects of breast milk during the first week of life is that the oxidative stress caused by these factors was too strong for the DNA damage to be suppressed. Another possible explanation for the lack of difference in urinary 8-OHdG concentrations between breast fed and formula fed infants at 2 days of age is that some of the infants had not yet started feeding at 2 days of age. It may also be related to the lack of any significant differences between the two groups with respect to the incidence of chronic complications such as ROP, IVH, CLD, and NEC. Inflammatory changes are probable precursors of these complications, and they are known to occur in the earliest stages of life.^{1, 27} A randomised controlled trial comparing the outcomes of breast fed and formula fed VLBW infants should help to explain this.

The exact antioxidative mechanisms of breast feeding in VLBW infants remain unclear. A possible mechanism is the effects of the antioxidants in breast milk on the various oxidative stresses and subsequent DNA damage. Human milk is known to contain many kinds of antioxidant,¹⁰⁻¹² but little is known about their stability in the gastrointestinal tract, their direct effects on the mucosa, their absorption from the intestine, and their effects after absorption into the blood. To elucidate the actual way in which antioxidants in breast milk protect against oxidative stress in VLBW infants, we need to examine their kinetics and effects. Another possible mechanism is promotion of the endogenous antioxidant activities of superoxide dismutase, glutathione peroxidase, and other enzymes by various substances in breast milk. These direct and indirect effects may explain the difference in urinary 8-OHdG excretion between breast fed and formula fed VLBW infants in this study.

In conclusion, measurement of urinary 8-OHdG excretion is useful for the assessment of oxidative DNA damage in VLBW infants. Human milk can suppress oxidative stress and oxidative DNA damage after 14 days of age.

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Figure 1 Change in urinary 8-hydroxydeoxyguanosine (8-OHdG) excretion at 2, 7, 14, and 28 days of age in breast fed and formula fed very low birthweight infants. Values are mean (SD). * $p < 0.01$ compared with 2 and 7 days.

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