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Effect of Human Breast Milk on Urinary 8-Hydroxy-2'-Deoxyguanosine Excretions in Infants

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ABSTRACT

During the perinatal period, oxidative stress is intimately involved in pathologic processes of serious diseases. Although breast milk contains many antioxidants, it is not clear whether breast milk can act as an antioxidant in infants *in vivo*. We compared the oxidative stress levels in total of 41 healthy 1-mo-old infants by measuring urinary 8-hydroxy-2'-deoxyguanosine, which is one of the biomarkers of oxidative DNA damage. These infants were divided into four groups according to the type of feeding. Urinary 8-hydroxy-2'-deoxyguanosine excretion of the breast-fed group was significantly lower than those of the artificial milk dominant mixed-fed group or the bottle-fed group. Our data suggest that breast milk, not artificial formula, acts as an antioxidant during infancy. **(*Pediatr Res* 53: 1–3, 2003)**

Abbreviations
8-OHdG, 8-hydroxy-2'-deoxyguanosine
ROS, reactive oxygen species

Reactive oxygen species (ROS) are known to be implicated in many pathologic processes such as aging, cancer development, ischemia-reperfusion injury to tissues, and others (1). At birth, the newborn encounters an environment much richer in oxygen than the intrauterine environment, and many asphyxiated or premature infants receive mechanical ventilation and supplemental oxygen. In addition, antioxidant defense mechanisms that are poorly developed in the neonatal period may be overcome by the generation of excessive ROS (2). Thus, ROS may be deeply involved in serious diseases in premature infants, including necrotizing enterocolitis (3, 4), chronic lung disease (5, 6), retinopathy of prematurity (6), and intraventricular hemorrhage (6).

Breast milk contains many antioxidants, such as catalase, superoxide dismutase, ascorbate, and vitamin E (7). It is conceivable that some antioxidants in breast milk may help newborn infants to eliminate ROS. However, it is not clear whether breast milk has antioxidant capacity in infants *in vivo*.

8-Hydroxy-2'-deoxyguanosine (8-OHdG) is produced by oxidative damage to the nucleoside deoxyguanosine and is subsequently excreted directly into urine. Recently, 8-OHdG has been used as a sensitive marker for oxidative DNA damage (8). In this study, we estimated oxidative stress levels by measuring urinary 8-OHdG excretions in both breast-fed and formula-fed infants to examine the effects of breast milk on the antioxidation *in vivo*.

METHODS

Subjects. Spot urine samples were collected from 41 healthy 1-mo-old infants, 23 boys and 18 girls, who attended the regular checkup clinic at Juntendo University affiliated hospitals from January to June 1999. This study was approved by our Institutional Review Board, and informed consent was obtained from their guardians before inclusion in the study. They all were born full term, appropriate for dates, from normal single pregnancies, and free from perinatal complications, including asphyxia, infections, and bleeding.

These infants were divided into four groups according to types of feeding. Group 1, the breast-fed group, was defined as receiving >90% of their intake as breast milk. Group 2, the breast milk dominant mixed-fed group, was defined as receiving 50% to 90% of their intake as breast milk. Group 3, the artificial milk dominant mixed-fed group, was defined as receiving >50% to 90% of their intake as formula. Group 4, the formula-fed group, was defined as receiving >90% of their intake as formula.

There were no significant differences in the mean birth weight, gestational age, or body weight at 1 mo among the four groups (Table 1). Physical examination revealed no abnormal findings in all infants.

8-OHdG measurements. Urine samples were stored at −20°C until assay. The concentration of 8-OHdG was deter-

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Table 1. *Profile of infants by the types of feeding*

Groups	1 Breast	2 Mixed	3 Mixed	4 Bottle
Male/female	$n = 10$ (4/6)	$n = 10$ (6/4)	$n = 11$ (5/6)	$n = 10$ (8/2)
Birth weight (g)	3197 ± 256	3020 ± 352	3218 ± 407	2983 ± 372
Gestational age (wk)	39.7 ± 1.1	39.8 ± 1.0	39.5 ± 1.2	38.9 ± 1.3
Body weight at 1-mo checkup (g)	4261 ± 295	4229 ± 353	4541 ± 415	4362 ± 321
Days of checkup	32.4 ± 3.4	32.2 ± 3.2	33.5 ± 3.8	30.4 ± 3.9

mined using a competitive ELISA kit (8-OHdG check; Japan Institute for the Control of Aging, Shizuoka, Japan). The specificity of the assay has been established (9), and the determination range was from 0.64 to 2000 ng/mL (10). Creatinine levels of the same samples were determined using a Jaffe's reaction measurement kit (RM119-K, JATRON, Tokyo, Japan). Urinary 8-OHdG excretions were expressed as creatinine ratio.

Data analysis. The results were expressed as the mean ± SD. The differences between the respective two groups were tested by ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Mean 8-OHdG concentrations in urine of each group were 3.3 ± 2.4, 10.1 ± 7.7, 17.8 ± 19.5, and 18.6 ± 14.3 ng/mL, respectively. 8-OHdG concentration of group 1 was significantly lower than those of group 3 or group 4. No difference in mean urinary creatinine concentrations was observed among the four groups (group 1, 8.2 ± 2.7 mg/dL; group 2, 8.6 ± 3.3 mg/dL; group 3, 10.4 ± 5.9 mg/dL; group 4, 8.5 ± 3.6 mg/dL).

Urinary 8-OHdG excretion (expressed as creatinine ratio) of groups 1 to 4 were 38.5 ± 22.7, 125 ± 99.7, 161.1 ± 109.4, and 204.4 ± 88.6 ng/mg creatinine, respectively. 8-OHdG excretion of group 1 was significantly lower than those of group 3 or group 4 (Fig. 1). There was no correlation between 8-OHdG excretions and either birth weight or body weight at the 1-mo examination or gestational age (data not shown).

DISCUSSION

We evaluated the antioxidative effects of human breast milk by measuring urinary 8-OHdG excretions in 1-mo-old infants who were recipients of four different feeding regimens. Our results demonstrated that urinary 8-OHdG excretions in the breast-fed group was lower than those of artificial formula dominant groups. Moreover, urinary 8-OHdG excretions seemed to be correlated with the percentage of formula intake. These results indicate that oxidative DNA damage of breast-fed infants is significantly lower than that of formula-fed infants.

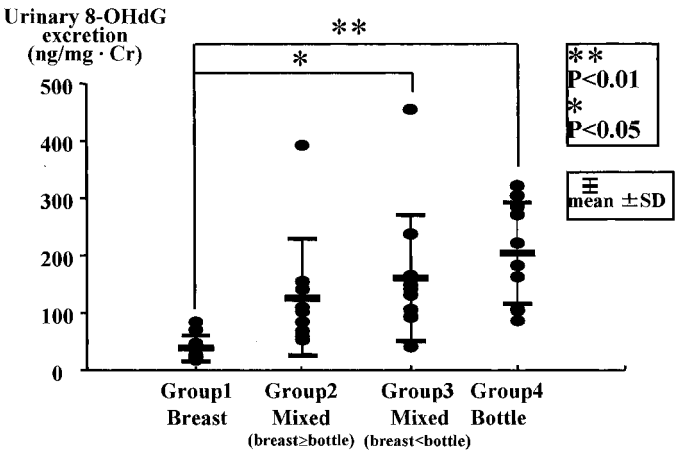

Figure 1. Urinary 8-OHdG excretions of the four groups. Mean ± SD, *$p < 0.05$, **$p < 0.01$.

ROS, such as superoxide anions ($\cdot O_2^-$) and hydroxyl (·OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are continuously produced in living organisms (11). For instance, activated phagocytes, such as neutrophils, monocytes, and macrophages, generate large amounts of superoxide as a part of the mechanism by which foreign organisms are killed. Oxidative stress occurs when there is an imbalance between the concentrations of ROS and the intra- and extracellular antioxidant systems. Overproduced ROS is known to be involved in many diseases.

Various methods have been established to evaluate the levels of oxidative stress or oxidative tissue damage. Determination of lipid peroxidation, such as malondialdehyde in plasma and urine, are the most widely used methods (12). However, ROS react not only with the lipids but also with proteins and nucleic acids. In this study, we evaluated the oxidative stress levels by measuring free radical damage to DNA. ROS may cause specific chemical modifications of DNA bases, and eliminated oxidized nucleotides are finally excreted into urine (13). 8-OHdG is formed by oxidative damage to the nucleoside deoxyguanosine, and 8-OHdG is the most commonly studied altered base. 8-OHdG has been shown to be a useful biomarker of oxidative DNA damage. Damaged DNA is repaired by nonspecific endonucleases and specific glycosylases *in vivo* (14).

The presence of 8-OHdG in biologic samples has been measured by HPLC with electrochemical detection and gas chromatography-mass spectrometry, but the complicated extraction procedures cause recovery problems in HPLC–electrochemical detection (15). Recently, an ELISA based on monoclonal IgG (N45.1 clone) was developed for estimation of 8-OHdG in urine samples (9, 10). This method has made it easier to measure the urinary 8-OHdG (16).

In adults, 8-OHdG increases in the urine of people who have been exposed benzene (17), smokers (18), patients with cancer (10), and those with atopic dermatitis (19). Conversely, it decreases in people who receive dietary intervention that increase consumption of vegetables and fruit (20).

8-OHdG is also detected in breast milk and formula. We have preliminarily measured 8-OHdG concentrations of five different human milk samples (two colostrums, three mature milks) and three commercially available Japanese formulas for

normal infants. Because the 8-OHdG concentration of breast milk (4.0 to 9.1 ng/mL, mean 6.2 ng/mL) was lower than that of formula (18.1 to 43.3 ng/mL, mean 29.5 ng/mL), we speculate that exogenous 8-OHdG intake may affect the urinary excretion of 8-OHdG in infants. However, Shigenaga *et al.* (21) reported that the intact recovery of intragastrically administered [^{3}H]8-OHdG in the urine of rats was 1% of the administered dose and that the estimated contribution of dietary 8-OHdG represented $<2\%$ of the urinary 8-OHdG. Accordingly, we concluded that the difference in urinary 8-OHdG excretions between the study groups resulted from the amount of breast milk intake and was the antioxidative properties of breast milk.

Breast-feeding has been associated with lower rates of a variety of infant illness, such as necrotizing enterocolitis (22), sepsis (23), and respiratory illness (24). In fact, breast milk contains a number of bioactive substances, including enzymes, growth factors, and hormones (25). Consumption of breast milk has many advantages over formula, including the potential ability to provide antioxidant protection to infants (26). Antioxidants such as enzymic antioxidants (*e.g.* catalase, superoxide dismutase), scavengers (*e.g.* ascorbate, vitamin E), metal-binding compounds (*e.g.* lactoferrin), and constituents of antioxidative enzymes (*e.g.* Cu, Zn) have been reported to be present in breast milk (27–29). Buescher *et al.* (26) showed the antioxidant effects of human colostrums on the oxidant products of human neutrophils *in vitro*.

Our data demonstrated that urinary 8-OHdG excretion is significantly lower in breast-fed infants compared with formula-fed groups at 1 mo of age, which means that oxidative DNA damage is lowest in breast-fed infants. The result of this study suggests that breast milk, not artificial formula, acts as an antioxidant during infancy, although it remains unclear which antioxidants in breast milk are responsible for this beneficial action to the infants.

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