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Effects of Parenteral Lipid Infusion on DNA Damage in Very Low Birth Weight Infants

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Very low birth weight (VLBW) infants are known to have poorly developed antioxidant system and may be at increased risk for radical damage. Previous studies have reported higher levels of lipid peroxide products in lipid emulsion used for parenteral nutrition. To examine the direct effects of parenteral lipid infusion on DNA damage in VLBW infants, we measured urinary 8-hydroxydeoxyguanosine (8-OHdG) levels in VLBW infants before, during, and after the parenteral lipid infusion. In both the lipid-infused and lipid-free groups, urinary 8-OHdG excretion levels at 14 days old were significantly ($p < 0.01$) lower than those at 2 and 7 days old. However, there were no significant differences in urinary 8-OHdG excretion levels between the lipid-infused and lipid-free groups at 2, 7, and 14 days old. Our results suggest that parenteral lipid infusion does not cause oxidative DNA damage, but irrespective of the infusion DNA damage during the first week of life is enhanced when compared with 14 days after birth in VLBW infants.

Keywords: 8-Hydroxydeoxyguanosine; Lipid emulsion; Parenteral nutrition; Lipid peroxidation; DNA; Very low birth weight infant

INTRODUCTION

Lipid hydroperoxides are known to be cytotoxic and react to form organic free radicals, which can lead to structural alterations in protein and DNA.[1] Previous studies have reported higher levels of lipid peroxide products in lipid emulsion used for parenteral nutrition and have suggested that the adverse effects of parenteral lipid infusion are partially caused by oxygen free radicals generated by lipid peroxidation.[2–4] There is, however, only limited evidence that infused peroxides might have deleterious clinical consequences,[5,6] especially in very low birth weight (VLBW) infants who are known to have poorly developed antioxidant system and may be at increased risk for radical damage.[7]

8-Hydroxydeoxyguanosine (8-OHdG) has recently been accepted as a sensitive marker for reflecting oxidative DNA damage.[8–10] On hydroxylation of DNA caused due to oxidative stress, 8-OHdG is excised by constitutive enzymatic repair systems and subsequently excreted intact in the urine. In this manner urinary 8-OHdG excretion can serve as a non-invasive marker measuring *in vivo* oxidative DNA damage. There have been, however, no previous studies that have examined urinary 8-OHdG excretion and evaluated oxidative DNA damage in VLBW infants during parenteral lipid infusion.

Therefore, to examine the direct effects of parenteral lipid infusion on tissue damage in VLBW infants, we measured urinary 8-OHdG levels in VLBW infants before, during, and after the parenteral lipid infusion.

MATERIALS AND METHODS

Subjects

Infants were eligible for this study if they had a birth weight of less than 1500 g, were born in or were transferred to the neonatal intensive care unit at

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TABLE I Group characteristics of lipid-infused and lipid-free infants

	Lipid-infused group ($n = 12$)	Lipid-free group ($n = 11$)
Gestational age (mean weeks ± SD)	28.7 ± 1.9	29.2 ± 1.8
Birth weight (mean grams ± SD)	1073 ± 190	1168 ± 272
Sex (male:female)	7:5	6:5
Antenatal steroids	3/12	2/11
5-min Apgar < 6	4/12	2/11
Period of phototherapy (mean hours ± SD)	70.2 ± 67.6	70.9 ± 67.8
Period of ventilation (mean days ± SD)	16.6 ± 15.8	14.2 ± 15.3
Period of oxygen therapy (mean days ± SD)	52.3 ± 52.3	37.2 ± 36.2
Period of parenteral nutrition (mean days ± SD)	12.3 ± 3.2	11.4 ± 2.2
Period of parenteral lipid infusion (mean days ± SD)	5.8 ± 1.4	0

Juntendo Izu Nagaoka Hospital between January 2000 and September 2001, and had no major congenital abnormalities. This study was approved by our Institutional Review Board and informed consent from legal guardians, prior to the inclusion in this study, was obtained before any examination.

These infants were divided into two groups according to the introduction of parenteral lipid infusion. The lipid-infused group, composed of 12 infants (7 boys and 5 girls), defined as receiving parenteral lipid infusion, and the lipid-free group, composed of 11 infants (6 boys, 5 girls), defined as receiving no parenteral lipid infusion. All infants received more than 90% of their intake as breast milk. There were no significant differences in the mean gestational age, birth weight, periods of phototherapy, ventilation or oxygen therapies between the lipid-infused and lipid-free groups (Table I).

In the lipid-infused group, the administration of 0.5 g/kg/day of lipid emulsion (Intralipid 20%, Ohtsuka Pharmaceutical, Tokyo, Japan) started from 5 to 7 days after birth and continued for 4–9 days (mean ± SD, 5.8 ± 1.4 days) with the emulsion level being increased up to 2.0 g/kg. Parenteral nutrition with carbohydrates, amino acids and vitamins had started from 3 to 5 days after birth in all infants and there was no significant difference in the mean periods of parenteral nutrition between the lipid-infused and lipid-free groups (Table I).

Spot urine samples were collected from the subjects in the morning using a urine collecting bag at 2, 7, and 14 days old. Urine samples were then stored at −20°C until the assay.

8-OHdG Measurements

The concentration of 8-OHdG was determined using a 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,[11] and the determination range was from 0.64 to 2000 ng/ml. Urinary 8-OHdG excretions were expressed as a creatinine ratio.

Data Analysis

The results were expressed as the mean ± SD. Differences were tested between the different ages by the Wilcoxon signed-rank test and between the two groups by the Mann–Whitney's *U*-test. A p value of less than 0.05 was considered statistically significant.

RESULTS

In both the lipid-infused and lipid-free groups, urinary 8-OHdG excretion levels at 14 days old were significantly ($p < 0.01$) lower than those at 2 and 7 days (Fig. 1). There were no significant differences in urinary 8-OHdG excretion between the lipid-infused and lipid-free groups at 2, 7, and 14 days.

DISCUSSION

This is the first report that directly examines the effects of parenteral lipid infusion on oxidative DNA damage in VLBW infants by measuring urinary 8-OHdG excretion levels. Although many previous studies have reported increased lipid peroxidation in parenteral lipid emulsion,[2–4] *in vivo* production of

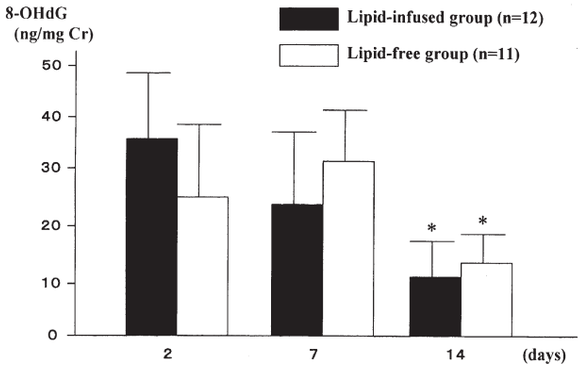

FIGURE 1 Changes in urinary 8-OHdG excretion 2, 7, and 14 days after birth in lipid-infused (closed bar, $n = 12$) and lipid-free (open bar, $n = 11$) groups of very low birth weight (VLBW) infants (mean ± SD). *$p < 0.01$ vs. 2 and 7 days.

oxygen free radicals by lipid peroxidation during parenteral lipid infusion is still somewhat controversial.[4,12–14] Moreover, there has been no data that directly demonstrates the radicals as causing the DNA damage during parenteral lipid treatments in VLBW infants.

The assaying of lipid peroxide concentrations by measuring blood and urine malondialdehyde–thiobarbituric acid (MDA–TBA) or exhalation levels of ethane, has been the most widely recognized assay of *in vivo* lipid peroxidation used in current clinical research.[4,12–14] However, these assay methods have been criticized as a measurement of lipid peroxidation and resultant tissue injury due to the fact that it is indirect. Although the measurement of hydrogen peroxide in the urine is available to confirm oxidative stress,[15] no study has been performed in patients receiving parenteral lipid infusion. On the other hand, damaged DNA is repaired by non-specific endonucleases and specific glycosylases *in vivo*, and the eliminated oxidized nucleotides are ultimately excreted into the urine as 8-OHdG. Therefore, the measurement of urinary 8-OHdG excretion levels is considered to be a very sensitive biomarker of oxidative DNA damage.[8–10]

Our results showed no significant differences in urinary 8-OHdG excretion levels between the lipid-infused and lipid-free VLBW infants before (2 days old), during (7 days old), or after (14 days old) parenteral lipid infusion. These results may be explained by three possible reasons: First, the lipid emulsion used did not undergo any oxidation. Second, lipid peroxidation was not enhanced in infants with parenteral lipid infusion. Third, oxidative DNA damage was not caused by lipid peroxidation. Although we did not measure peroxide levels in the lipid preparation used in this study, parenteral lipid infusion does not affect urinary 8-OHdG excretion levels, which indicates that peroxides either found in lipid preparations or produced endogenously in the infant do not result in detectable oxidative DNA damage in VLBW infants.

The differences in lipid peroxidation, which depend on the age of VLBW infants during parenteral lipid infusion, has yet to be fully examined. In accordance with a previous report by Inder *et al.*[16] that demonstrated elevated levels of plasma MDA–TBA during the first week of life in VLBW infants, our results showed higher urinary 8-OHdG excretion levels at 2 and 7 days old than at 14 days old in infants both with and without parenteral lipid infusion. These results may be due to many factors, including high concentrations of inspired oxygen, phototherapy, frequent alterations of blood flow to major organs, and inflammation with the accumulation of neutrophils and macrophages, which can cause oxidative stress and are often observed in the earlier period of life in VLBW infants.[1,17]

In summary, the measurement of urinary 8-OHdG excretion levels may be useful for the assessment of oxidative DNA damage in VLBW infants. Parenteral lipid infusion dose not cause oxidative DNA damage, but irrespective of the infusion, DNA damage during the first week of life is enhanced when compared with 14 days after birth in VLBW infants.

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