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Oxidative Stress in Very Low Birth Weight Infants As Measured by Urinary 8-OHdG

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Very low birth weight (VLBW) infants can be subjected to oxidative stress in the course of intensive care. We measured 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative stress, and estimated the degree of oxidative stress in such infants. We also examined if the administered oxygen was related to oxidative stress.

Urine samples of 50 Japanese VLBW infants [birth weights: 956.3 ± 277.6 g, and gestational ages: 28.0 ± 2.6 weeks (mean ± SD)] were collected on various postnatal days and 8-OHdG levels were determined using an ELISA kit. Sixteen term infants served as normal controls.

As body weights at sampling increased, the average levels of urinary 8-OHdG decreased. 8-Hydroxydeoxyguanosine levels were: infants under 1000 g, 29.5 ± 16.4 μmol/mol creatinine ($n = 24$); 1000–1500 g, 23.8 ± 14.9 ($n = 12$); over 1500 g, 16.1 ± 8.5 ($n = 14$); and control, 10.9 ± 7.2 ($n = 16$). Significant differences were found between <1000 g group and ≧1500 g group ($p = 0.0030$), <1000 g group and control ($p < 0.0001$), and 1000–1500 g group and control ($p = 0.0108$).

Also as postconceptional age at sampling increased, the average levels of 8-OHdG decreased. 8-Hydroxydeoxyguanosine levels were: infants before 252 days (36 weeks) of postconception: 27.4 ± 15.5 μmol/mol creatinine ($n = 34$); after 252 days, 18.2 ± 12.5 ($n = 16$). Differences between <252 days group and control ($p < 0.0001$), and <252 days group and ≧252 days groups ($p = 0.0253$) were statistically significant.

Among the three groups based on ambient oxygen concentration (21%, 22–29%, and ≧30%) there was no significant difference ($p = 0.417$).

The more premature the infants were, the more intense was the oxidative stress, hence, it is the prematurity rather than the administered oxygen which causes oxidative stress in VLBW infants.

Drury *et al.* ["Urinary 8-hydroxydeoxyguanosine in infants and children" *Free Radic. Res.* 28 (1998) 423–428] measured urinary 8-OHdG of 28 infants (24–40 weeks gestation) and found no gestation or birthweight related differences. This discrepancy seemed to be because of difference in birth weights and sampling period of the subjects.

Keywords: VLBW infant; 8-OHdG; Oxidative stress; Neonatal intensive care

INTRODUCTION

The reactive oxygen species (ROS), by-products of oxidative metabolism, are harmful to living bodies and higher animals have defense systems against ROS, including superoxide dismutase (SOD). However, uncontrolled ROS can injure both cell membranes and DNA. Various conditions, including aging and carcinogenesis are probably related to ROS.[2]

In humans, defense systems against ROS during early life are less well developed,[3] and early exposure to oxidative stress may lead to deleterious effects. Chronic lung disease (CLD) and retinopathy of prematurity (ROP) are important complications of neonatal intensive care. Multifactorial events are involved, but ROS have often been referred to in discussing the etiology.[4,5] Thus it seemed important

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to evaluate the extent of possible oxidative stress in low birth weight infants.

As 8-hydroxydeoxyguanosine (8-OHdG) is an indicator of oxidative stress,[6–8] we measured 8-OHdG in the urine of very low birth weight (VLBW) infants of various body weights and postconceptional ages. Our data suggest that VLBW infants are exposed to intense oxidative stress, findings which may cast light on the various forms of events related to morbidity in VLBW infants.

METHODS

Urine

Urine samples were collected and stored at −20°C until measurement.

Urinary Levels of 8-OHdG

8-Hydroxydeoxyguanosine levels in urine were determined using an ELISA kit based on monoclonal antibody N45.1[9] (Japan Institute for the Control of Aging, Fukuroi City, Japan). Reported values are the average of triplicate determinations and urinary levels of 8-OHdG are expressed as μmol/mol creatinine.

Infants

Fifty VLBW infants, 27 male and 23 female, from two neonatal intensive care units (Kumamoto City Hospital, Kumamoto, Japan and Gunma Children's Medical Center, Gunma, Japan) were subjects of this study. Birth weights and number of infants were as follows: 500–1000 g, 31; 1000–1500 g, 19, and average weight 956.3 ± 277.6 g (mean ± SD). Gestational ages and number of infants were: 24–27 weeks, 26; 28–32 weeks, 21; and over 33 weeks, 3, and average age 28.0 ± 2.6 weeks (mean ± SD). Eleven out of 50 infants were treated with ventilator for at least 2 days before urine sampling. There were no infants with congenital malformation syndromes, renal diseases, or other congenital diseases and all the babies were of the Japanese race.

Urine samples were collected on the first to 276th postnatal day (average postconceptional age at sampling: 240.9 ± 51.8 days). Sixteen term infants from another hospital (Fukuda Hospital, Kumamoto, Japan) served as normal controls and here the gestational ages were 39.8 ± 1.5 weeks (ranged from 37 to 41 weeks) and birth weights were 3158.1 ± 370.5 g (ranged from 2568 to 3798 g). Urine samples were collected on the first to 11th postnatal days, the average being the 3.2th day.

Informed consent for the study was obtained from the parents.

Statistical Analysis

One-way Factorial ANOVA (Fisher's PLSD test) and Unpaired *t*-test were used for data analysis. A p value of <0.05 was considered to have statistical significance.

RESULTS

Urinary levels of 8-OHdG in control infants were 10.9 ± 7.2 μmol/mol creatinine (ranged from 3.9 to 31.1).

VLBW infants showed wider range of 8-OHdG levels. Figure 1 shows the relation between body

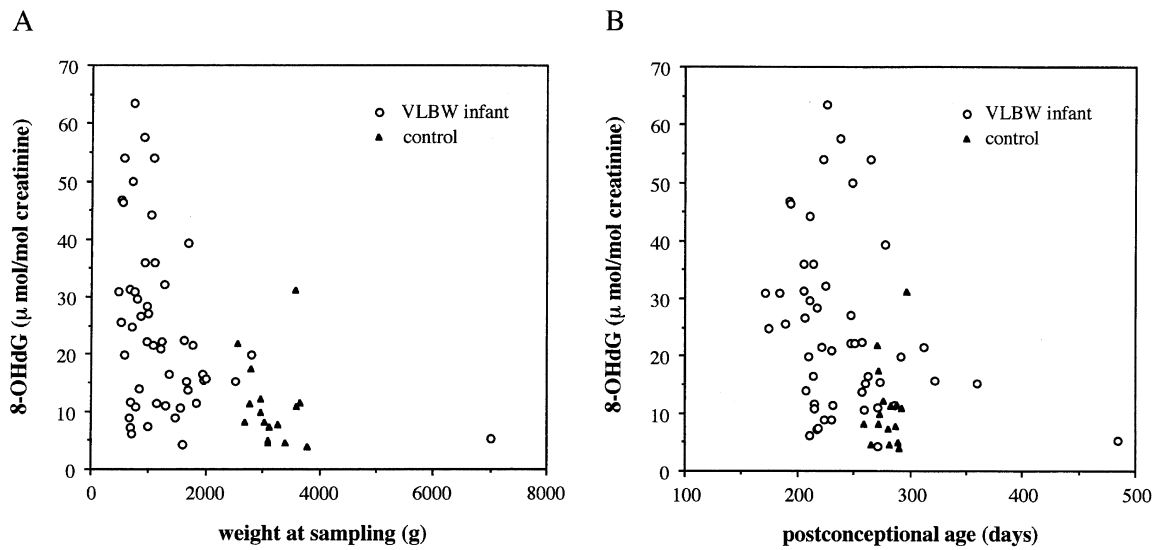

FIGURE 1 Relation between body weight at sampling and urinary 8-OHdG level (A), and postconceptional age and urinary 8-OHdG level (B). The average levels of 8-OHdG decreased as body weight and postconceptional age increased.

TABLE I Comparison of 8-OHdG levels among control and three body weight groups (less than 1000 g, 1000–1500 g, more than 1500 g) at sampling

Body weight (g)	8-OHdG (μmol/mol creatinine) (mean ± SD)
<1000†	29.5 ± 16.4 ($n = 24$)*, ***
1000–1500‡	23.8 ± 14.9 ($n = 12$)**
≧1500¶	16.1 ± 8.5 ($n = 14$)*
Control	10.9 ± 7.2 ($n = 16$)**, ***

There were significant differences in urinary 8-OHdG levels among the four groups (p values were: *0.0030, **0.0108, *** < 0.0001).
† Body weight at sampling and postconceptional age were: 732.3 ± 153.5 g (mean ± SD) and 212.5 ± 21.6 days (mean ± SD), respectively.
‡ Body weight at sampling and postconceptional age were: 1188.9 ± 145.6 g (mean ± SD) and 230.5 ± 20.6 days (mean ± SD), respectively.
¶ Body weight at sampling and postconceptional age were: 2262.0 ± 1414.4 g (mean ± SD) and 298.4 ± 61.3 days (mean ± SD), respectively.

weight at sampling and 8-OHdG (Fig. 1A), and postconceptional age at sampling and 8-OHdG (Fig. 1B). As body weight and postconceptional age increased, the average levels of 8-OHdG decreased. Table I shows comparison of 8-OHdG levels among control and three groups of infant according to body weight at sampling (<1000 g, 1000–1500 g, and ≧1500 g). There were significant differences between <1000 g group and ≧1500 g group, <1000 g group and control, and 1000–1500 g group and control ($p = 0.0030$, <0.0001, and 0.0108, respectively).

Figure 2 shows the relation between postnatal age and urinary level of 8-OHdG. For the first month average level of 8-OHdG increased and then, it decreased. Table II shows comparison among control and two postconceptional age groups [<252 days (36 weeks) and ≧252 days]. Significant differences were found in urinary 8-OHdG levels between <252 days group and control, and <252 days group and ≧252 days group ($p < 0.0001$ and 0.0253, respectively).

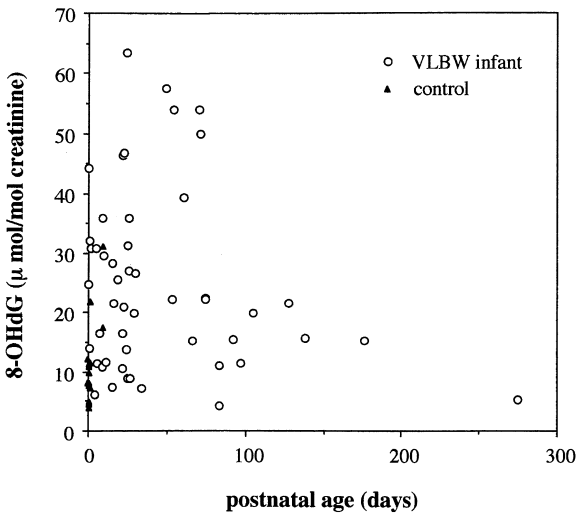

FIGURE 2 Relation between postnatal age and urinary level of 8-OHdG. For the first month average level of 8-OHdG increased and then it decreased.

TABLE II Comparison of 8-OHdG levels among control and two postconceptional age groups: before and after 252 days (36 weeks)

Postconceptional age	8-OHdG (μmol/mol creatinine (mean ± SD)
<252 days†	27.4 ± 15.5 ($n = 34$)*, **
≧252 days‡	18.2 ± 12.5 ($n = 16$)**
Control	10.9 ± 7.2 ($n = 16$)*

There were significant differences among control and two post-conceptional age groups (p values were: * < 0.0001, **0.0253).
† Postconceptional age and body weight at sampling were: 215.6 ± 19.7 days (mean ± SD) and 866.8 ± 259.7 g (mean ± SD), respectively.
‡ Postconceptional age and body weight at sampling were: 294.6 ± 58.0 days (mean ± SD) and 2027.5 ± 1367.8 g (mean ± SD), respectively.

The 50 infants were grouped into three according to ambient oxygen concentration at sampling, 21% ($n = 28$), 22–29% ($n = 14$), and over 30% ($n = 8$). There was no significant difference in urinary levels of 8-OHdG among these three groups ($p = 0.417$). Also no significant difference was found between male and female in urinary 8-OHdG level ($p = 0.961$).

Urinary creatinine levels of controls showed wider ranges compared with those of VLBW infants, but there were no postnatal age related or body weight related differences in urinary creatinine excretion.

DISCUSSION

Birth is a challenging event for mammals insofar as they begin to live in an aerobic environment. In humans the partial pressure of oxygen in the umbilical vein is approximately 27 mm Hg, which is far below the levels after birth.[10] Immediately after birth the partial pressure of oxygen can be five times as high. This is the initial step for humans to be exposed to aerobic oxygen, and oxidative metabolism is activated at the time of birth. On the other hand mRNA expressions of manganese superoxide dismutase (MnSOD), one of the most important antioxidant enzymes, in the lung and liver are very low in fetuses and increase toward adulthood.[3] Defense systems against ROS in premature infants, therefore, can be insufficient.

We evaluated urinary levels of 8-OHdG as a marker for oxidative stress in infants of various body weights or postconceptional ages and various concentrations of oxygen they inhaled.

Since urinary 8-OHdG levels were expressed as μmol/mol creatinine urinary creatinine levels of the subjects were crucial to this study. In human infants nephrogenesis is complete at 34 weeks of postconception and after that creatinine clearance increases abruptly.[11] In our study no significant differences were found in urinary creatinine levels between the two groups of postconception [creatinine levels: <36 weeks, 1.217 mmol/l ($n = 34$); ≧36 weeks, 1.709

($n = 16$), $p = 0.0644$], and among the three body weight groups [creatinine levels: <1000 g, 1.258 mmol/l ($n = 24$); 1000–1500 g, 1.103 ($n = 12$); $\geqq 1500$ g, 1.807 ($n = 14$), $p = 0.0816$].

We used ELISA kit for measurement of 8-OHdG in our study. 8-Hydroxydeoxyguanosine in biological materials including urine has been measured by HPLC.[1,7,12–14] Urinary levels of 8-OHdG measured by HPLC and ELISA kit based on monoclonal antibody N45.1 showed a strong correlation.[13] N45.1 has a high specificity for 8-OHdG, recognizing three sites of the molecule,[9] and ELISA kit based on N45.1 detects more 8-OHdG, about 1.7 times, than HPLC. It could be because the ELISA kit detects not only free 8-OHdG, but also 8-OHdG in undigested DNA fragments[13] and sulfate-conjugated- or glucuronidated-8-OHdG* [J. Kurashige (personal communication)].

In VLBW infants of our study, the lighter the body weights at sampling were and the earlier postconceptional ages at sampling were, the higher were average levels of urinary 8-OHdG. No significant difference was found among three groups with different oxygen concentrations. These findings suggest strongly that prematurity itself causes oxidative stress in VLBW infants.

Drury *et al.*[1] measured urinary 8-OHdG of 28 infants of 24–40 weeks gestation by HPLC and showed that 8-OHdG excretion increased linearly with time until 30 days after birth. In our study, 8-OHdG levels showed the same tendency until one month after birth, and then it changed to decrease (Fig. 2). The reason for this change is unclear but there was a sampling bias in the first month: all our control samples were collected in the first 10 days after birth and showed low level of 8-OHdG, while in the later period of the first month only the samples of infants under 1000 g showed high level of 8-OHdG.

Drury *et al.*[1] reported that there were no gestation or birthweight related differences in urinary 8-OHdG. Our data showed strong correlation between body weight at sampling and urinary 8-OHdG level, and between postconceptional age at sampling and urinary 8-OHdG level. This discrepancy seems to be because of differences in average birth weight of infants: Drury's study, 1469 g; this study, 956 g, and differences in sampling period: Drury's study, one month; this study, 276 days.

Within 3 days before urine sampling 10 received phototherapy, 10 were treated with diuretics, four were treated with catecholamines, and 15 were administered iron. No significant differences in 8-OHdG level were found between infants with and without these treatments ($p = 0.594$, 0.340, 0.512, and 0.844, respectively).

We compared urinary 8-OHdG levels of the control, infants treated with ($n = 11$) or without ventilator ($n = 39$) at sampling. There were significant differences between infants with and without ventilator, with ventilator and control, and without ventilator and control ($p = 0.0143$, <0.0001, and 0.0060, respectively). Mechanical ventilation is inseparable from prematurity, but mechanical stress itself may be an aggravating factor for oxidative stress.

In light of all this evidence oxidative stress in infants less than 1500 g seems to be evident. This early insult in VLBW infants may explain some of specificity of their morbidity in later life.[15,16]

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