0031-3998/94/3601-0055$03.00/0
PEDIATRIC RESEARCH

Vol. 36, No. 1, 1994
Printed in U.S.A.

Immaturity-Dependent Free Radical Activity in Premature Infants

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ABSTRACT

To examine the role of immaturity in the free radical–mediated rate of lipid peroxidation in premature infants, we studied 27 infants [gestational age, 27.1 (SD 2.4) wk; birth weight, 970 (SD 330) g]. Ethane and pentane were quantitated in expired air during the first 18 d of life. During the first 2 postnatal d ethane [24.1 (SEM 7.8) pmol × kg^{-1} × min^{-1}] and pentane [24.2 (SEM 4.1) pmol × kg^{-1} × min^{-1}] were stable but increased during d 5 to maxima of 79.1 (15.8) pmol × kg^{-1} × min^{-1} and 62.1 (8.1) pmol × kg^{-1} × min^{-1}, respectively. Maximum ethane and pentane correlated with gestational age ($r = -0.42$, $p = 0.03$ and $r = -0.52$, $p = 0.005$, respectively) and birth weight ($r = -0.38$, $p = 0.05$ and $r = -0.59$, $p = 0.001$, respectively). Infants with high maximum expired ethane and pentane (exceeding 40 pmol × kg^{-1} × min^{-1}) had higher odds of dying or having bronchopulmonary dysplasia than those with low ethane and pentane (odds ratio, 6.5; 95% confidence interval, 1.1 to 38.5; $p < 0.05$ for ethane and odds ratio, 5.6; 95% confidence interval, 1.1 to 29.3; $p < 0.05$ for pentane). We conclude that degree of prematurity is the single most important factor explaining free radical–mediated lipid peroxidation in premature infants. A therapeutic intervention to limit the effects of free radicals should be started during the 1st postnatal d in premature infants to be effective. (***Pediatr Res* 36: 55–59, 1994**)

Abbreviations
BPD, bronchopulmonary dysplasia
IVH, intraventricular hemorrhage
OR, odds ratio
PDA, patent ductus arteriosus
ROP, retinopathy of prematurity
CI, confidence interval

The newborn at term is better protected against the effects of free oxygen radicals than are prematurely born and adult individuals (1, 2). The reasons for such resistance are possibly the high activity of antioxidant enzymes and the capacity for induction of activity of these enzymes in response to oxidative challenge (1, 3, 4). Additionally, the concentrations of antioxidants are low in the premature infant (2, 5). Moreover, the prematurely born infant is frequently exposed to conditions associated with increased generation of free oxygen radicals, such as reperfusion and hyperoxia (6, 7). It has been proposed that free oxygen radicals participate in the pathogenesis of BPD and ROP (8–10).

In excess, free oxygen radicals may damage biologic macromolecules (11). Especially vulnerable are polyunsaturated fatty acids, in which free oxygen radicals may induce lipid peroxidation that causes disturbance of membrane function and eventual cell death (12).

Lipid peroxidation generates volatile products, *e.g.* ethane and pentane, the concentrations of which in expired air serve as indicators of the free radical activity (13, 14). The aim of the present investigation was to analyze the factors associated with free radical activity by quantifying ethane and pentane in expired air.

PATIENTS AND METHODS

Patients. A total of 27 infants were studied at the Children's Hospital of the Helsinki University Central Hospital (Table 1). All infants were born before 32 wk of gestation (range, 23 to 32 wk) and were admitted to the neonatal intensive care unit. Relative birth weight was estimated by standard Finnish intrauterine growth diagrams (birth weight of the infant minus mean weight at the infant's age divided by SD of birth weight at this gestational age).

The requirement of the fraction of inspiratory oxygen was adjusted to maintain arterial oxygen tension between

Received June 24, 1993; accepted February 1, 1994.
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Supported by the AGA AB Medical Research Fund, the Foundation for Pediatric Research, the Paulo Foundation, and the Finnish Academy.

Table 1. *Patient characteristics*

Number	27
Gestational age (wk)	27.1 ± 2.4*
Birth weight (g)	973 ± 330*
Relative birth weight	−0.5 ± 1.4*
Delivery (cesarean section/vaginal)	14/13
Preeclampsia	4
Chorioamnionitis	8
Apgar score—1 min	5 ± 2*
Apgar score—5 min	7 ± 2*
Apgar score—10 min	8 ± 1*
Respiratory distress syndrome	24
PDA	13
IVH	
Grade I	5
Grades II–IV	7
BPD	9
ROP	2
Death	7

* Mean ± SD.

50 and 80 mm Hg (6.7 and 10.7 kPa). All infants had intubation and ventilation because they failed to establish sufficient spontaneous ventilation at birth. Twenty-four patients had respiratory distress syndrome. Two of these patients received exogenous surfactant (human surfactant isolated from amniotic fluid), one at the age of 2 h and one at the age of 5 h. Symptomatic PDA developed in 13 infants. The diagnosis of PDA was determined by echocardiography. Four doses of i.v. indomethacin (0.1 mg × kg^{-1}) at 8- to 12-h intervals was administered to these patients. All infants received i.v. glucose, ampicillin (200 mg × kg^{-1} × d^{-1}), and netilmycin (6 mg × kg^{-1} × d^{-1}) during the first 7 d. Seven of the patients had leukopenia, thrombocytopenia, and elevated C reactive protein indicative of infection, but the results of their blood cultures were negative. All the infants received i.v. fluconazole (6 mg × kg^{-1} × d^{-1}) as prophylaxis against fungal infections. I.v. lipid infusion (Intralipid, Kabi Ltd.) was started on d 3 to d 5 of life unless oral feeding had been established. Vitamins A (100 μg), D (1.5 μg), C (100 mg), and E (1 mg) were started i.v. at the beginning of lipid infusion or given perorally to patients receiving more than 10 mL × kg^{-1} × d^{-1} human milk. IVH was diagnosed by cranial ultrasonography, and the findings were classified according to Papile *et al.* (15). BPD was diagnosed according to the criteria of Bancalari and Gerhardt (16) when the infant was 28 d old. ROP was diagnosed and graded as described (17).

The study protocol was approved by the Ethics Committee of the Children's Hospital, and informed consent was obtained from the parents.

Collection and quantitation of alkanes. Single samples of expired air were collected daily for 10 d and then at 2- to 3-d intervals until the infants were 18 d of age. The patients breathed hydrocarbon-free air oxygen for 5 min, after which expired air was sampled during 2 min. Expired air was sampled through the intubation tube; if extubated, it was sampled through a gas-tight face mask (infant mask, Laerdal, Stavanger, Norway). With the face mask, the infant was breathing against a positive pressure at a constant flow of 2–4 L × min^{-1}. Ethane and pentane were analyzed by gas chromatography with a capillary column, with 2-pentene as an internal standard (14). Expired gas samples were additionally analyzed for carbon dioxide (Capnometer model CD 101, Datex Ltd.).

Statistical analysis. The data are expressed as mean ± SEM unless otherwise indicated. Logarithmic transformation of ethane and pentane values was performed to normalize the distribution. The unpaired two-sided t test was used to detect differences between the two groups. The postnatal profile of alkane excretion was examined with analysis of variance. Associations between prenatal and postnatal factors and expired gases were analyzed by multiple regression test. Association of high pentane excretion with BPD, neonatal death rate, IVH, and PDA is expressed with OR (odds of the specific complication to noncomplication), with 95% CI.

RESULTS

Between the 1st and 18th d of life, 27 premature infants were studied. All infants required ventilatory support immediately after birth. The collection of the first expired gas sample took place within the first 24 h. The expiration of ethane and pentane during the first 2 d was 24.1 ± 7.8 pmol × kg^{-1} × min^{-1} and 24.2 ± 4.1 pmol × kg^{-1} × min^{-1}, respectively. Both ethane and pentane increased to a peak, with ethane at 79.1 ± 15.8 pmol × kg^{-1} × min^{-1} and pentane at 62.1 ± 8.1 pmol × kg^{-1} × min^{-1} on d 5. Thereafter, between d 5 and 8, both ethane and pentane decreased ($p < 0.05$) (Fig. 1).

During the study period of up to 18 d, eight infants were fed enterally, starting with 10 mL/d of human milk. For 22 of the infants, i.v. amino acids and lipids were started at a mean age of 4.8 ± 1.1 d (5–10 mL × kg^{-1} amino acid infusion and 5 mL i.v. lipid infusion each). Infusion of lipids was stopped at least 3 h before collection of gas samples.

Gestational age at birth and birth weight showed an inverse correlation with maximal alkane expiration. The peak of ethane expiration significantly correlated with gestational age ($r = -0.42$, $p = 0.03$) and birth weight ($r = -0.38$, $p = 0.05$). Corresponding correlations for the pentane peak were to gestational age ($r = -0.52$, $p < 0.005$) and birth weight ($r = -0.59$, $p < 0.001$). The following parameters had no detectable association with the peak alkane excretion: mean oxygen requirement before the peak for alkanes (ethane: $r = 0.22$, $p = 0.30$; pentane: $r = 0.32$, $p = 0.12$), daily mean airway pressures or arterial oxygen tensions before the peak, intubation time, or start of enteral or parenteral nutrition.

Multiple regression analyses were performed to distinguish which factors were associated with the peaks of ethane and pentane excretions (Table 2). The ethane and pentane peaks increased significantly with low gestational age regardless of the level of oxygen supplemen-

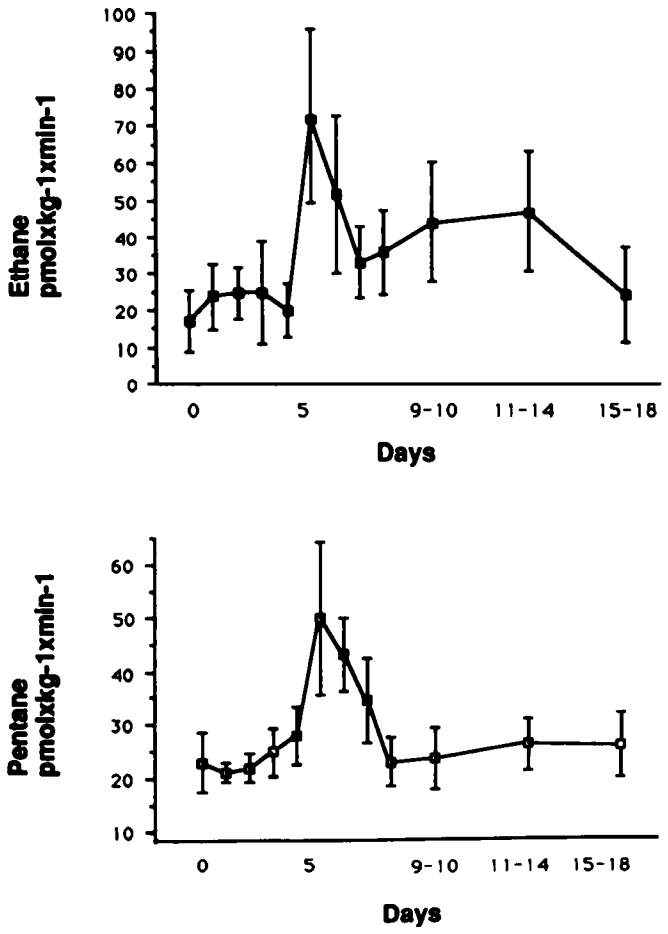

Figure 1. Expired ethane (*upper panel*) and pentane (*lower panel*) in 27 premature infants during the first 2 wk of life. Values are given as the mean ± SEM of 15 to 21 measurements/d on d 1 to 7 and of 7 to 19 measurements/d on d 7 to 18.

tation. However, both the actual fraction of inspiratory oxygen requirement and the cumulative fraction of inspiratory oxygen requirement had a nonsignificant association with the ethane and pentane peaks ($p \geq 0.10$).

High excretion of alkanes, *i.e.* maximum excretion exceeding 40 pmol × kg^{-1} × min^{-1}, was associated with BPD or neonatal death (ethane: OR, 6.5; CI, 1.1 to 38.5; $p = 0.03$; pentane: OR, 5.6; CI, 1.1 to 29.3; $p = 0.04$). BPD developed in nine patients according to the criteria of Bancalari and Gerhardt (16). Seven died (between 7 and 28 d). No higher odds of IVH were observed among the preterm infants with high alkane excretion. IVH was found in 12 patients, of whom five had grade I (detected on d 1–5) and seven had grades II–IV (detected on d 3–13).

Thirteen patients underwent treatment with indomethacin for PDA during the study period. High alkane excretion was not associated with the persistence of ductus. Two patients had stage 3 ROP. In one of these two infants, ethane was 19.6 and pentane was 65.4 pmol × kg^{-1} × min^{-1}, and in the other infant, ethane was 200.5 and pentane was 31.6 pmol × kg^{-1} × min^{-1}.

Carbon dioxide remained stable in expired samples [3.5 ± 1.7 (SD) mL × kg^{-1} × min^{-1}], indicating good recovery in different specimens.

DISCUSSION

The present data show a significant correlation between immaturity and postnatal free radical–mediated lipid peroxidation in preterm infants. A high level of lipid peroxidation in turn was associated with high neonatal mortality and increased incidence of BPD.

To some extent, free radical–mediated lipid peroxidation seems to be part of the normal metabolism (11). In 19 healthy term newborns, ethane and pentane excretion (6.2 ± 1.6 pmol × kg^{-1} × min^{-1} and 7.4 ± 1.7 pmol × kg^{-1} × min^{-1}, respectively) remained low and remarkably stable during the 1st wk of life (unpublished data). However, in situations with an excess of free radicals, the lipid peroxidation cascade increases and becomes autocatalytic, resulting in pertubation of the membrane function and eventual cell death (12, 14, 18). In premature infants, tissue damage caused by free radicals has been suggested as important in the development of several chronic complications, such as ROP, BPD, and IVH (8–10). The present findings support a role for free radicals in the pathogenesis of chronic sequelae of the preterm infant.

Several explanations exist for high free radical activity in the preterm infants. The deleterious effects of hyperoxia and its association with increased production of free oxygen radicals is well known (6, 10). However, in the present patients both the birth weight and the length of gestation showed a significant negative association with level of lipid peroxidation, which hyperoxia failed to

Table 2. *Factors associated with maximum alkane expiration: a multiple regression analysis*

Dependent variable	Independent variable	Regression coefficient	SEM	*p* value
Log ethane (pmol × kg^{-1} × min^{-1})	Gestation at birth	−0.090	0.033	0.015
	Actual inspiratory O_2	0.004	0.003	0.20
	Relative birth weight*	0.01	0.06	0.82
	Sex (female = 1; male = 2)	0.23	0.15	0.15
		Constant term = 3.57		
Log pentane (pmol × kg^{-1} × min^{-1})	Gestation at birth	−0.048	0.017	0.013
	Actual inspiratory O_2	0.0020	0.0019	0.32
	Relative birth weight*	−0.044	0.034	0.21
	Sex (female = 1; male = 2)	−0.026	0.084	0.76
		Constant term = 2.95		

* (Mean birth weight of infant − mean birth weight for infant's gestational age)/SD of mean birth weight at this gestational age).

show. This finding differed from earlier report, where the oxygen requirement during the alkane peak correlated with alkane excretion (10). One reason for this discrepancy might be the larger number and wider range in the length of gestation of patients in the present study. Infants at a lower gestational age and with more severe conditions were included, which might also explain the overall higher content of alkanes excreted.

In the premature infants, a steep increase in lipid peroxidation took place at the end of the 1st wk of life. This pattern of free radical activity in preterm infants is in accordance with previous findings (10). The reason for the peak activity during this period is enigmatic, however. When ethane and pentane are measured, potential contamination (*e.g.* from intestinal bacterial flora) has to be excluded (19). However, in our patients this possibility is unlikely because of the broad-spectrum antibiotics given to the premature infants and because of the remarkably stable levels of ethane and pentane in term infants receiving breast milk and therefore establishing an intestinal bacterial flora during the 1st wk of life (20).

An event of potential importance for the increase in lipid peroxidation is the recruitment of phagocytizing cells and increased inflammation in the lungs observed in preterm infants with respiratory distress syndrome at the end of the 1st postnatal wk (21). During this period, decreased concentrations of the antioxidant lactoferrin are also found in patients who later have BPD (22). In premature infants, levels of vitamin E (23) and of antioxidant scavengers such as transferrin and ceruloplasmin are low (24, 25). The protective antioxidant enzymes have a low activity and a poor inducibility in hyperoxia (3, 4). Therefore, toward the end of the 1st wk of life, the antioxidant defense system of the premature infant may be overwhelmed by free radicals generated by hyperoxia, inflammatory responses, or, possibly, reperfusion events (7, 26).

Ethane and pentane are formed in free radical–mediated lipid peroxidation from ω-3 and ω-6 polyunsaturated fatty acids, respectively (13, 14). Although analogous in principle, the two alkanes show some differences. First, in *in vitro* lipid peroxidation the stoichimetric yield of ethane from its parent compounds is somewhat higher than that of pentane (13). Second, tissue oxygen tension may influence the formation of the alkanes (27, 28). Third, the alkanes may be metabolized differently (29). Finally, with the use of the present method the quantitation of pentane is more accurate than that of ethane (14). These factors may account for the differences found here between ethane and pentane. However, concerning the rapid increase in lipid peroxidation occurring on the 5th postnatal d, and probably reflecting free radical–mediated tissue damage, the patterns of the two alkanes show striking similarity.

After its maximum level toward the end of the 1st wk, lipid peroxidation gradually returns to a level close to that measured during the 1st postnatal d. The latter phenomenon is in accordance with previous data by Pitkänen *et al.* (10) and seems to be a constant pattern of free radical–mediated effects in premature infants. It is not caused by artefacts, such as changes in the patient population (for instance, the death of moribund patients). One reason might be lack of substrate for lipid peroxidation, *i.e.* polyunsaturated fatty acids. However, this reason is improbable with regard to the lungs because the content of polyunsaturated fatty acids of tracheal aspirates of preterm infants remains unchanged during the 1st wk of life despite changes in lipid peroxidation (30) (unpublished results). Another explanation is that the lipid peroxidation process is self-limiting. The reason for this limitation might be either decreased generation of free radicals, perhaps because of a lower degree of inflammation or hyperoxia, or adaptation of the antioxidant defense.

In the present patients, however, only high lipid peroxidation (alkane excretion exceeding 40 pmol $\times$ kg^{-1} $\times$ min^{-1}) correlated with complications. The level of lipid peroxidation after the 1st wk, potentially indicative of the capacity of the patient to limit the effects of free radicals, did not correlate with the clinical course.

In conclusion, immaturity in premature infants was the single most important denominator linked with free radical–mediated lipid peroxidation. In these patients a rapid increase in lipid peroxidation is seen on the 5th d of life, and this high level correlates with a high risk of dying or with having BPD develop. Therapies for reduction of the effects of free radicals should be started before birth or immediately postnatally in premature infants to be effective.

Acknowledgments. The authors thank Maija Pohjavuori, M.D., Kari Raivio, M.D., and the personnel of the Neonatal Intensive Care Unit, Children's Hospital, for their kind cooperation. We are also grateful to Sinikka Tsupari for skillful technical assistance.

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