

Resuscitation of Preterm Infants with Reduced Oxygen Results in Less Oxidative Stress than Resuscitation with 100% Oxygen

Shoichi Ezaki^{1,*}, Keiji Suzuki¹, Clara Kurishima¹, Masumi Miura², Wan Weilin³, Reiichi Hoshi¹, Shizue Tanitsu¹, Yuzo Tomita⁴, Chikako Takayama¹, Masaki Wada⁵, Tsutomu Kondo⁵, and Masanori Tamura¹

¹Division of Neonatal Medicine, Center for Maternal, Fetal and Neonatal Medicine, Saitama Medical Center, Saitama Medical University, 1981 Kamoda-Tujido, Kawagoe, Saitama 350-8550, Japan

²Division of Pediatrics, Tottori University School of Medicine, 36-1 Nishi, Yonago, Tottori 683-8504, Japan

³Department of Pediatrics, Peking Union Medical College Hospital, 1 Shuaifuyuan, Dongcheng district Beijing 100730, China

⁴Division of Pediatrics, Koshigaya Hospital, Dokkyo Medical University, 2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan

⁵Division of Neonatal Medicine, Yachiyo Medical Center, Tokyo Women's Medical University, 477-96 Owada-Nitta, Yachiyo, Chiba 276-8524, Japan

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Summary The objective of this study was to determine the effects of the level of inhaled oxygen during resuscitation on the levels of free radicals and anti-oxidative capacity in the heparinized venous blood of preterm infants. Forty four preterm infants <35 weeks of gestation with mild to moderate neonatal asphyxia were randomized into two groups. The first group of infants were resuscitated with 100% oxygen (100% O₂ group), while in the other group (reduced O₂ group), the oxygen concentration was titrated according to pulse oximeter readings. We measured total hydroperoxide (TH) and redox potential (RP) in the plasma within 60 min of birth. The integrated excessive oxygen ($\sum(\text{FiO}_2-0.21) \times \text{Time}(\text{min})$) was higher in the 100% O₂ group than in the reduced O₂ group ($p < 0.0001$). TH was higher in the 100% O₂ group than in the reduced O₂ group ($p < 0.0001$). RP was not different between the 100% O₂ and reduced O₂ groups ($p = 0.399$). RP/TH ratio was lower in the 100% O₂ group than in the reduced O₂ group ($p < 0.01$). We conclude that in the resuscitation of preterm infants with mild to moderate asphyxia, oxidative stress can be reduced by lowering the inspired oxygen concentration using a pulse oximeter.

Key Words: resuscitation, preterm infants, oxygen, oxidative stress, pulse oximeter

Introduction

It is known that free radicals (FR) in plasma and cerebrospinal fluid increase in post-ischemic reperfusion [1–3].

Some animal studies have shown that use of 100% oxygen for resuscitation of asphyxiated newborns induces potential tissue damaging factors and may cause developmental disorders of the central nervous system. For example, levels of matrix metalloproteinase 2 (MMP2; a marker of nervous tissue injury and inflammation) and extracellular glycerol (a marker of cell damage) were elevated in cerebral tissue after exposure to 100% oxygen in experimental cerebral ischemia [4]. Nuclear factor-kappa B (NF- κ B; an indicator of inflam-

*To whom correspondence should be addressed.
Tel +81-49-228-3727 Fax +81-49-226-2325
E-mail: s_ezaki@saitama-med.ac.jp

matory response) activity was also higher after exposure to 100% oxygen than to 21% oxygen [5].

A study in full-term neonates reported that both an indicator of oxidative stress (oxidized glutathione) and indicators of redox potential (RP) activity (glutathione peroxidase, superoxide dismutase) were increased in the first few minutes after birth; these increases were more evident in infants resuscitated with 100% oxygen than in those resuscitated with air [6]. However, there have been no reports on FR or RP in preterm infants in relation to levels of inspired oxygen used during resuscitation. Compared to full-term infants, preterm infants frequently develop respiratory impairment requiring oxygen. They are also more susceptible to disturbances caused by exposure to high levels of oxygen, which are known to be associated with retinopathy of prematurity (ROP) and chronic lung disease (CLD) [7, 8].

In recent years, monitoring of oxygen saturation by pulse oximetry (SpO₂) from birth in the delivery room has been recommended and is becoming more widely used [9]. However, there is no agreement on the optimal SpO₂ in preterm infants during resuscitation. We therefore compared plasma levels of FR and RP in two groups of preterm infants with asphyxia at the time of admission to the neonatal nursery: one group received reduced concentrations of oxygen according to SpO₂ readings; the other group was given 100% oxygen continuously during resuscitation before transfer to the neonatal nursery. The objective of this study was to determine the effects of the level of inhaled oxygen during resuscitation on the levels of free radicals and anti-oxidative capacity in the blood of preterm infants.

Methods

Patients

Premature infants delivered by cesarean section before 35 weeks gestation with attendance of neonatal physicians in Saitama Medical Center, Saitama Medical University, between June 2005 and May 2006 were involved in the study, provided parental consent had been obtained prior to delivery. Infants with congenital malformations were excluded from the study.

Resuscitation and oxygen administration

Immediately after delivery, an infant was transferred to a radiant warmer resuscitation table by a midwife. Then she took a blood sample from the umbilical artery blood gas analysis. Resuscitation procedures were performed by three physicians. The first physician determined the 1 and 5-min Apgar scores. The second physician played a central part in the resuscitation such as wiping the infant, suctioning the upper airway, giving respiratory stimuli, and checking pulse rate. The third physician placed a sensor of the pulse oximeter (N-550 Nellcor™, Boulder, CO) on the patient's

right upper extremity and controlled the FiO₂. Free-flow 100% oxygen was given to all infants for the first minute after birth. When the infant's 1 min Apgar score was below ≤6, the third physician started adjusting FiO₂ according to the infant's group of pre-allocation and recorded the SpO₂ and FiO₂ every minute. Information on the infant's group and FiO₂ were blinded to both the first and second physicians. The first group of infants was given 100% oxygen continuously during resuscitation (100% O₂ group). The other group was resuscitated with a titrated concentration of oxygen such that SpO₂ measured on the right upper extremity was maintained within the 90–95% range (reduced O₂ group). The concentration of oxygen was regulated by an oxygen blender (OA 2000, Metran™, Japan). The physician who placed the pulse oximeter sensor, reduced the oxygen concentration according to SpO₂ readings and recorded changes of oxygen concentration. When SpO₂ exceeded 95% in the reduced O₂ group, oxygen was reduced by 10% increments every minute for an FiO₂ >0.5 or by 5% increments every minute for an FiO₂ ≤0.5. The 'integrated excessive oxygen' was calculated by summing the products of (FiO₂—0.21) and the duration of for each FiO₂ (*i.e.* $\sum(\text{FiO}_2 - 0.21) \times \text{Time}(\text{min})$).

For mask-bag ventilation, inflation pressure was controlled using a manometer (Mercury Medical, North Clearwater, FL) with an initial pressure of 30 cmH₂O followed by a pressure of 15–20 cmH₂O thereafter. If 30 seconds of positive pressure ventilation by mask and bag produced no improvement, infants were intubated, followed by further resuscitation.

For infants whose spontaneous respiration was steady with a heart rate >100 beats/min and without a need for intubation, free-flow mixed gas with regulated oxygen concentration was given through a face mask and Jackson-Rees circuit during resuscitation at delivery room. After the resuscitation in the delivery room, during transfer from delivery room to neonatal nursery and in the neonatal nursery, oxygen concentration was adjusted in both groups of infants so that SpO₂ was maintained at 90–95% until blood collection for FR and RP measurement.

Respiratory distress syndrome (RDS) was defined as the presence of respiratory distress and a characteristic chest radiograph (reticular granular pattern with air bronchograms and low lung volumes). The data of microbubble test [10] of the gastric aspirate and arterial blood gas analysis were also used for diagnosis.

Assay of FR and RP

Fifty microliters of heparinized venous blood was collected within 60 min of birth to measure total hydroperoxide (TH) levels and RP by using a FRAS4 system (Diacron srl, Grosseto, Italy). TH represents the total of radical oxygen metabolites produced as a result of peroxida-

tion chain reaction of proteins, lipids, and amino acids, which are deprived of hydrogen by reactive oxygen species (ROS) and FR. TH levels were measured using the d-ROM (Reactive Oxygen Metabolites) kit that applies Fenton's and Haber-Weiss reactions [11–13], where one U.CARR (unit of TH value) is equivalent to 0.08 mg/dl of H₂O₂.

“Endogenous” anti-oxidants (*i.e.* albumin, transferrin, ceruloplasmin, bilirubin, uric acid, reduced glutathione, etc.) or “exogenous” anti-oxidants (*i.e.* tocopherols, carotenes, ubiquinol, ascorbate, methionine, flavonoids, polyphenols, etc.) are able to give electrons and block potential damage by ROS and FR. RP indicates a total of both endogenous and exogenous anti-oxidative ability to reduce oxides by inactivating, decomposing and eliminating FR and ROS. RP levels were determined using the biological antioxidant potential (BAP) test, which is based on the ability of a colored solution, containing a source of ferric (Fe³⁺) ions adequately bound to a special chromogenic substrate, to decolor when Fe³⁺ ions are reduced to ferrous ions (Fe²⁺), as it occurs by adding a reducing/antioxidant system [14]. The BAP test, by exploiting the same chemical principle of the well-known ferric reducing ability of plasma (FRAP) test—*i. e.* the reduction of ferric to ferrous ions—provides a reliable measure of biological antioxidant potential of blood plasma [15].

Statistical analysis

An unpaired *t* test was used for comparison of numerical data and the Yates 2 × 2 chi-square test or Fisher exact probability test was used for comparison of categorical data between the two groups. All *p* values were based on two-sided tests. Statistics were compiled using SPSS10.0J (SPSS Inc, Chicago, IL) and Graph Pad Prism 5 (Graph Pad Software, Inc., San Diego, CA).

Results

A schema showing the study design and allocation of subjects to study groups is shown in Fig. 1. Of 147 infants born before 35 weeks gestation with the attendance of a neonatal physician during the study period, parental consent was obtained in 144 infants. Of these 144 infants, 52 had asphyxia (1-min Apgar score ≤6). We excluded from the study 2 infants, one with trisomy 18, one with congenital chylothorax. The remaining 50 infants were randomized for the study; 6 of these infants were excluded because of failure of blood sample collection within 60 min of birth. Consequently, data from 44 infants were subjected to statistical analysis.

Table 1 shows background characteristics of patients on admission to the NICU. There were no differences in gestational age, birth weight, and 1-min Apgar score, umbilical arterial blood pH between the two groups. The interval from

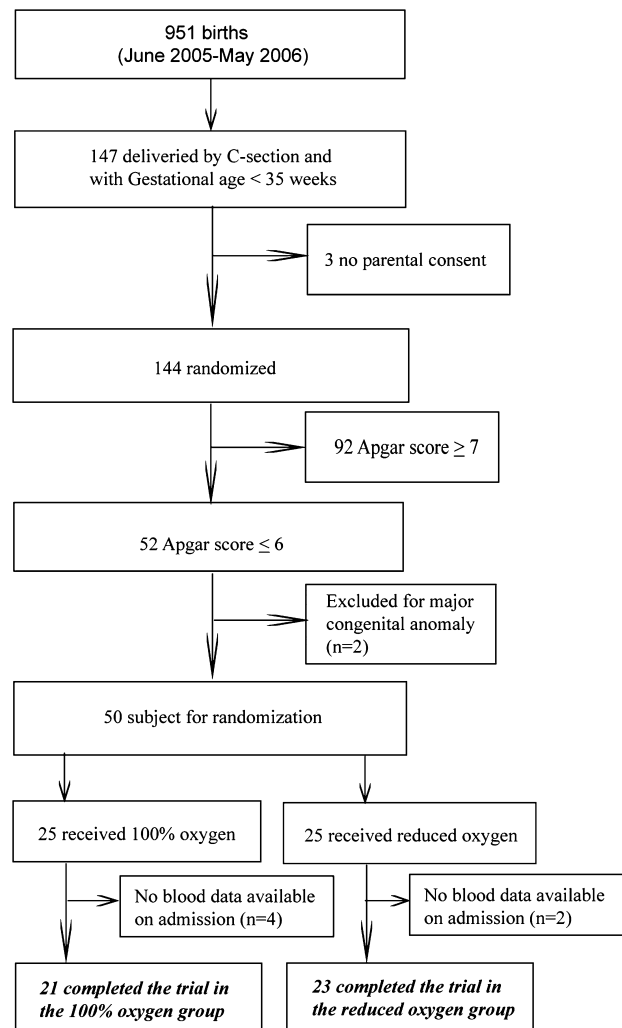


Fig. 1. Flow diagram showing the distribution of infants born during the study period. A total of 44 infants completed the study; 21 infants received 100% oxygen and 23 infants received reduced oxygen.

birth to neonatal nursery admission was not different ($p = 0.576$). However, the integrated excessive oxygen was higher in the 100% O₂ group than in the reduced O₂ group ($p < 0.0001$). The rate of infants requiring intubation was not different between groups ($p = 0.29$). Similarly, the incidence of RDS, which required higher concentration of inspired oxygen, was not different ($p = 0.105$). However, the 5-min Apgar score was lower in the 100% O₂ group than in the reduced O₂ group ($p = 0.022$).

Fig. 2. shows TH values in both groups obtained by the dROM test. Values in the 100% O₂ group (234.1 ± 13.2 U.CARR) were significantly higher than in the reduced O₂ group (131.5 ± 13.7 U.CARR; $p < 0.0001$).

Fig. 3. shows RP in both groups obtained by the BAP test. Values were not different between groups (2725 ± 113 μM in the 100% O₂ group vs 2540 ± 192 μM in the reduced O₂

Table 1. Characteristics and outcome measures of the patients

	100% O ₂ (n = 21)	Reduced O ₂ (n = 23)
Gestational age, weeks	29.0 ± 0.8	29.2 ± 0.7
Birth weight, g	1177 ± 124.3	1089 ± 104.4
Apgar score		
1 min	3.5 ± 0.4	4.3 ± 0.3
5 min	6.4 ± 0.5	7.6* ± 0.2
Umbilical artery pH	7.34 ± 0.09	7.34 ± 0.10
Time from birth to admission, min	27.7 ± 7.6	26.0 ± 9.4
The integrated excessive oxygen ($\sum(\text{FiO}_2 - 0.21) \times \text{Time (minutes)}$)	19.2 ± 1.7	8.0** ± 1.1
Number of infants requiring intubation, n (%)	18 (86)	16 (70)
Incidence of RDS, n (%)	16 (76)	11 (48)

Values are expressed as mean ± SD.

Significance is expressed as follows: * $p < 0.05$, ** $p < 0.0001$.

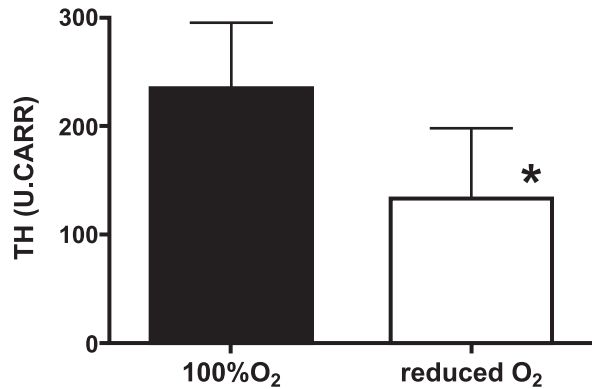


Fig. 2. Plasma levels of total hydroperoxide (TH, by dROM test) in the 100% O₂ and reduced O₂ groups (mean ± SD). The dROM value in the 100% O₂ group was significantly higher than in the reduced O₂ group (* $p < 0.0001$).

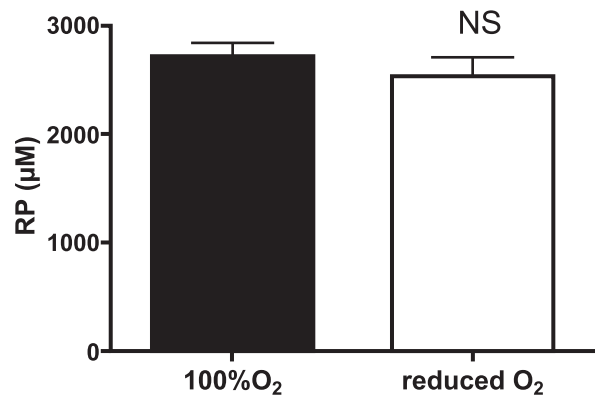


Fig. 3. Plasma levels of redox potential (RP) in the 100% O₂ and reduced O₂ groups (mean ± SD). There was no significant (NS) difference between the two groups.

group; $p = 0.399$).

The RP/TH ratio was significantly lower in the 100% O₂ group than in the reduced O₂ group (12.3 ± 0.8 vs 29.9 ± 6.6 ; $p < 0.01$) (Fig. 4).

Discussion

Our major finding was that TH levels were lower in the preterm infants given reduced concentration of oxygen during resuscitation compared to those given continuously 100% oxygen. Increased plasma FR in early neonatal period may have a significant negative impact on the short and long term outcome of these preterm infants. Vento *et al.* have shown that term infants resuscitated with 100% oxygen had higher FR levels compared to those resuscitated with air and this change was associated with increased plasma cardiac

troponin T (an index of myocardial damage) and urinary *N*-acetyl-glucosaminidase (an index of renal tubular damage) [16]. It is known that preterm infants have lower anti-oxidative potential than term infants [17]. Therefore, it is likely that preterm infants are more susceptible to oxidative stress causing cell damage compared with term infants.

Study protocol

Several studies have compared room air resuscitation with oxygen resuscitation in full term infants with neonatal asphyxia. However, similar studies have never been performed for preterm infants. The main reason for this may be that preterm infants often need oxygen in the delivery room because of insufficient oxygenation due to lung immaturity and not because of asphyxia. That's the main reason why we resuscitated infants with a certain amount of supplemental oxygen and not with air in this study.

In previous studies on the resuscitation of term infants

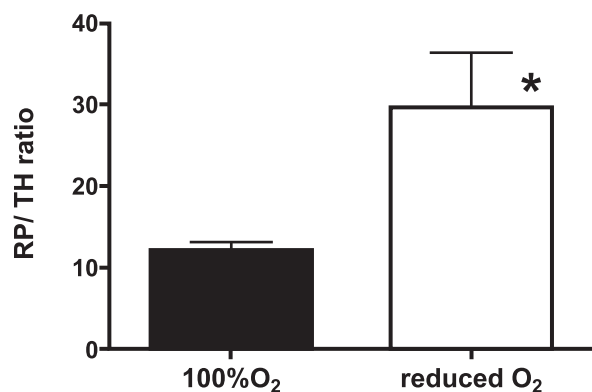


Fig. 4. The ratio of redox potential (RP) to total hydroperoxide (TH) in the 100% O₂ and reduced O₂ groups (mean ± SD). The RP/TH ratio in the 100% O₂ group was significantly lower than in the reduced O₂ group (* $p < 0.01$).

with air, asphyxia has been defined as acidemia of the cord blood, pale skin, bradycardia, nonresponsiveness to stimuli, or low 5-min Apgar score [6, 16, 18–20]. Except for acidemia of the cord blood, the factors used to diagnose asphyxia in these previous reports (pale skin, bradycardia and nonresponsiveness to stimuli) are all elements of Apgar score, by which we determined presence or absence of asphyxia at 1 min after birth. Use of the Apgar score was also advantageous for us because we routinely check 1 and 5 min Apgar scores during resuscitation. We were not able to include umbilical arterial blood pH in our study because its measurement results were not available by one minute after birth when we have to start controlling FiO₂. We defined asphyxia as a condition with a 1-min Apgar score of ≤ 6 , which is considered mild to moderate neonatal asphyxia [21].

There are several prospective clinical trials comparing outcome parameters in infants grouped according to higher or lower maintained oxygen saturation after birth. Those studies showed that the incidence of CLD and severe ROP was consistently lower in the lower SpO₂ groups (70–95%) than in the higher SpO₂ groups (88–100%) although the survival rate was not different [22]. All of these trials studied the SpO₂ levels in infants after the initial stabilization period in neonatal nurseries. However in the present study, we focused on the SpO₂ level during resuscitation. In premature infants born with asphyxia, sustained hypoxemia may interfere with adaptation of the respiration and pulmonary circulation. This is why we adopted a relatively high target SpO₂ range of 90–95% according to the report by Sun *et al.* [23]. They reported that the incidence of CLD or severe ROP requiring laser treatment was lower in infants with maintenance SpO₂ of $\leq 95\%$ than $\geq 95\%$. Though we could achieve considerable reduction of FiO₂ in the reduced O₂ group in comparison with the 100% O₂ group, the target

SpO₂ range of 90–95% which we adopted may still have been too high for these preterm infants, considering that SpO₂ in healthy term infants is below 90–95% range for the first ten min [24].

TH and RP

TH that we measured in this study is a sum of ROO[•] and RO[•]. ROO[•] is an oxidant that acts as oxidative stress on every molecule in the cell such as carbohydrates, lipids, amino acids, nucleotides, enzymes, structural and receptor proteins [13, 25]. ROO[•] mainly combines with lipids and continuously produces ROOH by lipid peroxidative reaction. We found lower TH values in the reduced O₂ group compared to the 100% O₂ group, which suggests that reducing inspired oxygen concentration can inhibit oxidative stress *in vivo*.

In contrast to previous reports on asphyxia in term infants, RP values were not elevated in the 100% O₂ group compared to reduced O₂ groups. There are some possible explanations for this. First, RP might already have reached the limit that preterm infants can achieve. We previously reported RP values in healthy preterm infants (<35 weeks) to be $2455 \pm 643 \mu\text{M}$ [26], which was not different from values in asphyxiated preterm infants in the present study ($2637 \pm 686 \mu\text{M}$, $p = 0.26$). Another possibility is that RP might have been higher in the 100% O₂ group than in the reduced O₂ group later in the neonatal period because endogenous antioxidants are produced more rapidly than exogenous antioxidants when FR and ROS levels increase [27]. Delayed production of exogenous antioxidants may explain why RP was not different within 60 min of birth.

RP/TH ratio was calculated in order to estimate the extent of response of RP relative to increased TH. The RP/TH ratio was lower in the 100% O₂ group than in the reduced O₂ group. This indicates that infants in the 100% O₂ group may have responded with insufficient production of RP and thereby have been at risk of cell damage due to oxidative stress compared with reduced O₂ infants.

Five-min Apgar score

It is interesting that, in spite of presumably better Apgar points for skin color in 100% O₂ infants, the Apgar score at 5 min in this group was significantly lower than in the reduced O₂ group. Saugstad *et al.* reported that the one minute Apgar score was higher and the time until first cry was shorter in an air resuscitation group compared to a 100% O₂ resuscitation group in term asphyxiated infants [18]. Similarly, Ramji *et al.* reported that air resuscitation and 100% O₂ resuscitation of full-term infants made no difference to the 1-min Apgar score, but the former resulted in a higher 5-min Apgar score [19] and an earlier first cry than the latter [20]. Blood levels of PO₂, PCO₂, and pH regulate breathing via various chemical receptors [28]. Elevation of

PaO₂ may have caused suppression of breathing and could have influenced the first cry in 100% O₂ infants. Another possible mechanism is that a surge of FR and ROS induced by high concentration of oxygen might have caused delayed initiation of breathing by damaging, either directly or via resultant inflammation, regions of the brain involved in the control of breathing [29].

Impact on long term outcome

Previous studies have shown that maximum expired ethane and pentane (markers of lipid peroxidation) levels were elevated in low birth weight infants with poor outcome (CLD, ROP, intraventricular hemorrhage or death) [30–32]. Similarly, high plasma lipid hydroperoxide levels in asphyxiated preterm infants were associated with neonatal adverse outcome [33]. Therefore, it is possible to improve the outcome of preterm infants by decreasing TH because lipid peroxidation in preterm infants is enhanced compared to term infants [17, 34]. Our study suggests that optimizing and reducing O₂ administration may improve long term outcome of preterm infants. Studies to examine the impact of reduced oxygen in the resuscitation of preterm infants on long term outcome measures such as incidence of CLD and ROP are required in the future to test this hypothesis.

Safety and feasibility

Only one infant in our reduced O₂ group who developed persistent pulmonary hypertension of the newborn (PPHN) was continuously given 100% oxygen until admission to the neonatal nursery. The TH value for this infant was the highest in the reduced O₂ group (285 U.CARR). There were no infants in the reduced O₂ group who required prolonged resuscitation or suffered from any adverse effects such as hypoxic-ischemic encephalopathy. It seems that most preterm infants with mild to moderate asphyxia do not require 100% oxygen continuously for resuscitation. Reducing oxygen using pulse oximetry, which was applied in this study, seems to be an easy, effective and safe method of resuscitating preterm infants.

Limitation of present study

This study has some limitations. It is small number of random examinations. A long term, excessive oxygen administration increases the incidence of rate of ROP and CLD [7, 8]. This research was not able to report the incidence rate of CLD and ROP because it did not depend on the strict oxygen management in long terms. The incidence of CLD and ROP might be prevented by managing strict oxygen over a long time in addition to the oxygen management after birth that we reported. A large-scale, random examination will be hoped for in the future. In this research, all preterm infants used 100% oxygen for one minute after birth. There might be a possibility that this influences TH

and RP levels. However, resuscitation of preterm infants with room air failed to achieve oxygen saturation $\geq 70\%$ by 3 min of life [35]. In addition, the 100% concentration of oxygen in the preterm's resuscitation is recommended at beginning by 2005 American Heart Association (AHA) guidelines for cardiopulmonary resuscitation (CPR) and emergency cardiovascular care (ECC) of pediatric and neonatal patients, and it is reported that it is necessary to withdraw an excessive oxygen administration afterward using a gas blender and a pulse oximeter [36]. The oxygen administration is begun for patients who have central cyanosis followed by 2005 AHA guidelines [36]. All infants in this study had asphyxia (1-min Apgar score ≤ 6). Consequently, oxygen administration was used for all infants who had admitted central cyanosis. This research supported the oxygen administration of the preterm infants of 2005 AHA guidelines for CPR and ECC of pediatric and neonatal patients [36] from the resuscitation of preterm infants with reduced oxygen results in less oxidative stress than resuscitation with 100% oxygen.

Conclusions

This is the first study that has demonstrated the beneficial effects of reducing oxygen concentrations during resuscitation on oxidative stress in preterm infants. We consider that a continuous use of 100% oxygen is unnecessary and may indeed be harmful, and we recommend a use of pulse oximetry to reduce inspired oxygen in the resuscitation of asphyxiated premature infants.

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Abbreviations

CLD, chronic lung disease; MMP2, matrix metalloproteinase 2, a marker of nervous tissue injury and inflammation; NF- κ B, nuclear factor-kappa B, an indicator of inflammatory response; PPHN, persistent pulmonary hypertension of the newborn; ROP, retinopathy of prematurity; RDS, respiratory distress syndrome of the newborn; ROS, reactive oxygen species; SpO₂, oxygen saturation by pulse oximetry.

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