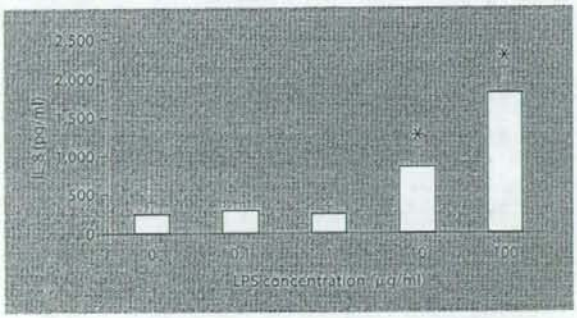

Fig. 2. The effect of LPS on release of IL-8 from alveolar epithelial cells (A549). Data are means ± SD (n = 3, * $p < 0.01$ in comparison with control).

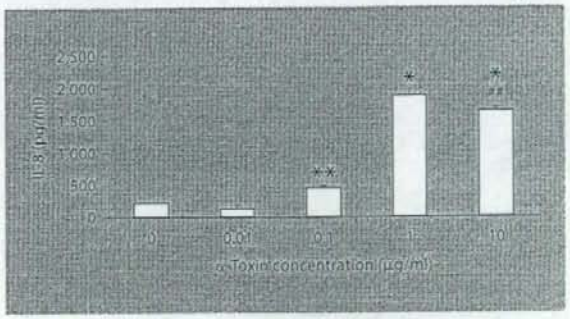

Fig. 3. Effects of α-toxin on release of IL-8 from alveolar epithelial cells (A549). Results are shown as means ± SD (n = 3, * $p <$ 0.01 in comparison with control; ** $p < 0.05$ in comparison with control; ## $p < 0.05$ in comparison with 1.0 μg/ml).

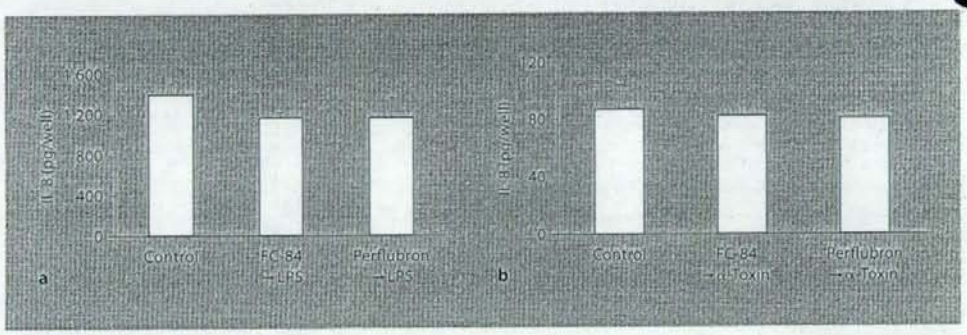

Fig. 4. Effects of pre-treatment of alveolar epithelial cells with PFC. Values are means ± SD. There were no significant differences between groups. **a** Control: incubation for 24 h, followed by addition of LPS (100 μg/ml) (n = 4). FC-84 → LPS: incubation with FC-84 in the upper chamber for 24 h, followed by aspiration of FC-84 and addition of LPS (100 μg/ml) (n = 3). Perflubron → LPS: incubation with perflubron in the upper chamber for 24 h, followed by aspiration of perflubron and addition of LPS (100 μg/ml) (n = 3). **b** Control: incubation for 24 h, followed by addition of α-toxin (1 μg/ml) (n = 3). FC-84 → α-toxin: incubation with FC-84 in the upper chamber for 24 h, followed by aspiration of FC-84 and addition of α-toxin (1 μg/ml). Perflubron → α-toxin: incubation with perflubron in the upper chamber for 24 h, followed by aspiration of perflubron and addition of α-toxin (1 μg/ml).

Effects of Pre-Treatment with PFC on Production of IL-8 by Alveolar Epithelial Cells

Pre-treatment of cells with PFC for 24 h did not affect production of IL-8 in our experiments (FC-84, 1,167 ± 55 pg/well, n = 3; perflubron, 1,163 ± 209 pg/well, n = 3) in comparison to the LPS control value (1,398 ± 110 pg/well, n = 4; $p = 0.19$, fig. 4a) (FC-84, 83 ± 7 pg/well; perflubron, 81 ± 10 pg/well), or to the α-toxin control value (87 ± 17 pg/well, $p = 1.5$, n = 3; fig. 4b).

After the experiments, the viability of cells in each of the groups was >90% as determined by trypan blue exclusion.

Effects of PFC

A significant decrease was observed in the production of IL-8 from the alveolar epithelial cells with PFC (1,398 ± 110 pg/well in LPS control vs. 686 ± 50 pg/well in FC-84 and 749 ± 137 pg/well in perflubron; $p < 0.05$, n = 4; fig. 5a, 260 ± 18 pg/well in α-toxin control vs. 127 ±

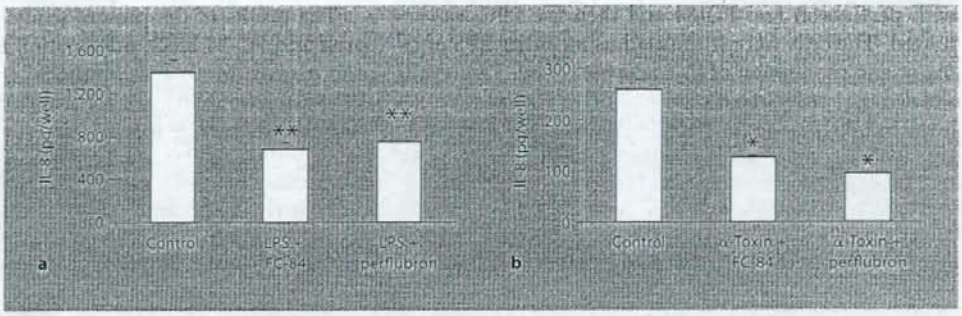

Fig. 5. PFC inhibits toxins-induced IL-8 release. Results are shown as means ± SD. **a** Control: no PFC. LPS + FC-84: LPS added to the upper chamber, followed immediately by FC-84. LPS + perflubron: LPS added to the upper chamber, followed immediately by perflubron (n = 4, ** $p < 0.05$ in comparison with control). **b** Control: no PFC. α-Toxin + FC-84: α-toxin added to the upper chamber, followed immediately by FC-84. α-Toxin + perflubron: α-toxin added to the upper chamber, followed immediately by perflubron (n = 3, * $p < 0.01$ in comparison with control).

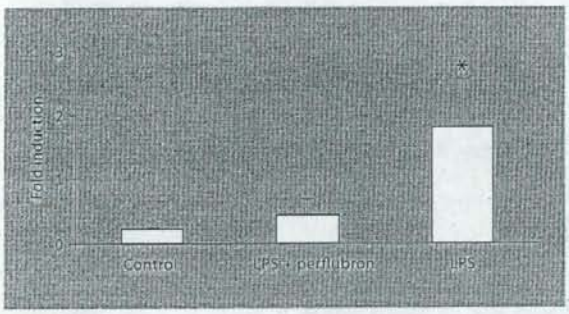

Fig. 6. Quantitative RT-PCR analysis of IL-8 mRNA expression in A549 cells. Data indicate the fold induction relative to expression in DMEM without co-culture with LPS. Results for each group were normalised relative to GAPDH expression measured in parallel in each sample. Control: no LPS. LPS + perflubron: LPS added to the upper chamber, followed immediately by perflubron. LPS: no perflubron (n = 3, * $p < 0.05$ in comparison with control and LPS + perflubron).

3 pg/well in FC-84 and 95 ± 0.3 pg/well in perflubron; $p < 0.01$, n = 3; fig. 5b).

After the experiments, the viability of cells in each of the groups was >90% as determined by trypan blue exclusion.

Real-time RT-PCR revealed increased expression of IL-8 mRNA in the LPS group (0.23 ± 0.01-fold in control and 0.44 ± 0.25-fold in LPS + perflubron vs. LPS group 1.81 ± 0.75-fold; $p < 0.05$, n = 3; fig. 6).

Discussion

The major findings of the present study were that perflubron and FC-84 suppressed IL-8 production in A549 cells stimulated with LPS or α-toxin. Similar effects were observed on the expression of IL-8 mRNA. The major actions of IL-8 on neutrophils include chemotaxis, transendothelial migration with concomitant shedding of leucocyte adhesion molecules, induction of lysosomal enzyme release, respiratory burst, shape change and generation of superoxide anions [27]. There have been reports suggesting that IL-8 facilitates pulmonary inflammatory reactions in vivo [7–9]. Therefore, we investigated the blocking effects of perflubron and FC-84 using a system in which alveolar epithelial cells were stimulated to produce IL-8.

The levels of IL-8 production released from A549 cells stimulated by α-toxin are decreased slightly when the cells are stimulated at higher concentrations [26]. The same phenomenon was observed in the present study. Therefore, we stimulated A549 cells with α-toxin at a concentration of 1 μg/ml.

The present study was conducted using a double-chamber system, where the upper and lower chambers were considered to represent the conditions inside and outside the alveoli, respectively. This model was used to determine whether administration of PFC from the trachea at the onset of ventilator-associated bacterial pneumonia can suppress IL-8 production in type II alveolar epithelial cells. In the present study, A549 cells were pre-incubated

with perflubron and FC-84 and then the PFC was removed. The cells were stimulated using either LPS or α-toxin, and production of IL-8 was measured. The results showed that pre-incubation did not alter IL-8 production levels in LPS- or α-toxin-stimulated A549 cells suggesting that PFCs do not directly affect IL-8 production.

PFCs are insoluble in water, and have high specific gravity. We used perflubron (specific gravity, 1,930 kg/m^3; surface tension, 18 mN/m; oxygen solubility, 53 m^3/100 m^3; carbon dioxide solubility, 210 m^3/100 m^3), which is used clinically, as well as FC-84 (specific gravity, 1,730 kg/m^3; surface tension, 13 mN/m; oxygen solubility, 59 m^3/100 m^3; carbon dioxide solubility, 224 m^3/100 m^3), which is used in animal studies in Japan. When PFCs were added to the medium, they spread on top of the A549 cell layer, separating the cells and the medium. In a previous study using the same system, A549 cells were stimulated from the lower side using TNF-α, but when perflubron was added to the upper surface, IL-8 release was not suppressed. These observations also suggest that PFCs do not directly affect IL-8 production in A549 cells, and it appears that perflubron physically blocks information transmission on the cell surface [20]. We did not examine the cells by electron microscopy and A549 cells were not stimulated from the lower side using LPS or α-toxin in the present study. However, the results of this study suggest that perflubron and FC-84 suppress IL-8 production before IL-8 mRNA is expressed. *E. coli* LPS has been reported to evoke TNF-α release from A549 cells [28]. Therefore, it is possible that perflubron and FC-84 also block TNF-α release from A549 cells stimulated by *P. aeruginosa* LPS or α-toxin. In the present study, perflubron and FC-84 were shown to block IL-8 release from A549 cells stimulated by *P. aeruginosa* LPS or α-toxin.

The results of the present study suggest that perflubron and FC-84 block information transmission of LPS and α-toxin in alveolar epithelial cells. Perflubron and FC-84 may be able to protect alveolar epithelial cells from compounds harmful to the lung – not only LPS and α-toxin, but also cytokines. Perflubron and FC-84 may also be able to block information transmission from alveoli to other interstitial cells, such as fibroblasts. It has been reported that phagocytosis of perflubron by macrophages leads to anti-inflammatory effects [18]. It has also been suggested that physical blockage by perflubron protects lung epithelial cells from neutrophil-mediated injury [19]. It will be necessary to investigate whether another PFC physically block transmission of various types of information on the surfaces of macrophages, neutrophils and other inflammatory cells. If it is possible to physically block such transmission on the surface of alveolar epithelial cells, it may be possible to clinically utilise perfluorocarbons in the treatment of several diseases involving pulmonary inflammation in the alveolar space.

Conclusions

The results of the present study show that perflubron and FC-84 block stimulation of pulmonary epithelial cells by LPS or α-toxin.

Acknowledgements

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Non-Pathogenic Bacterial Flora May Inhibit Colonization by Methicillin-Resistant *Staphylococcus aureus* in Extremely Low Birth Weight Infants

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Key Words

Non-pathogenic bacterial flora · Methicillin-resistant *Staphylococcus aureus* · Extremely low birth weight infants

Abstract

Objectives: To evaluate our hypothesis that non-pathogenic bacterial flora inhibit later colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) in extremely low birth weight (ELBW) infants, we performed a retrospective investigation of the association between non-pathogenic bacterial flora and later inhibition of colonization by MRSA in ELBW infants. ***Methods:*** A total of 110 preterm infants with birth weight <1,000 g admitted to the Neonatal Intensive Care Unit of Nagano Children's Hospital from January 1997 to December 2003 were analyzed retrospectively with regard to colonization by MRSA during hospitalization. We investigated the incidence of MRSA colonization in 56 infants with non-pathogenic bacterial flora in the oral cavity during the first week after birth and compared them with 54 infants lacking non-pathogenic bacteria. ***Results:*** Incidence rate of colonization by MRSA at postnatal week 6 was significantly lower in infants with non-pathogenic bacterial flora in the oral cavity (32.1%) than in infants without such bacteria during the first week of life (77.8%; $p < 0.001$). ***Conclusions:*** The present results suggest an important role for non-pathogenic bacterial flora in the oral cavity during early life in prevention of later MRSA colonization in ELBW infants.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is usually considered a nosocomial pathogen. However, MRSA infection can become a major problem in neonates. Reducing colonization and infection by MRSA in the neonatal intensive care unit (NICU) is thus important. Although various control measures have been introduced, including hand-washing, reduction of overcrowding, increased number of nurses and treatment of staff with mupirocin, these measures have not prevented the spread of MRSA [1–3]. The exponential increase in the isolation rate of MRSA represents one of the most serious problems in NICUs in Japan [4].

Non-pathogenic bacterial flora has been shown to inhibit colonization with pathogenic bacteria in older people and animals [5–8]. The birth canal of the mother may play an important role in colonization by non-pathogenic bacteria in neonates. *Corynebacteria* and coagulase-negative staphylococci (CNS) are usually isolated from the skin of newborn infants within a few hours after birth. However, neonates have no detectable bacterial flora in the nasal or oral cavities during the first several days after birth [9, 10]. *Corynebacterium* species have been shown to eliminate MRSA colonization in the adult nasal cavity [11]. However, no previous studies have examined whether non-pathogenic flora can inhibit MRSA colonization in newborn infants.

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Table 1. Clinical characteristics of the infants

	Group 1 n = 56	Group 2 n = 54	p value
Gestation, weeks	26.5 ± 2.0	26.5 ± 1.9	0.97
Birth weight, g	791.4 ± 142.7	812.0 ± 138.2	0.45
Apgar score at 1 min	3.8 ± 2.3	4.4 ± 2.2	0.15
Cesarean section	46/56 (82%)	44/54 (81%)	0.92
Antenatal steroids exposure	30/56 (54%)	34/54 (63%)	0.31
PROM and antibiotics use to mother	30/56 (54%)	30/54 (56%)	0.83
Antibiotics used before 7 days old	15/56 (27%)	18/54 (33%)	0.45
BPD at 28 days of age	30/56 (54%)	30/54 (56%)	0.83
Duration of intubation, days	49.5 ± 35.0	54.0 ± 36.6	0.51
PDA needed surgery	4/56 (7%)	5/54 (9%)	0.7
Sepsis	1/56 (2%)	6/54 (10%)	0.045
Duration of hospitalization, days	114.5 ± 58.9	130.0 ± 62.5	0.19
Death	0/56 (0%)	1/54 (2%)	0.31

We hypothesized that non-pathogenic bacterial flora in the oral cavity may inhibit later colonization by MRSA in extremely low birth weight (ELBW) infants. The present study retrospectively investigated the possible roles of non-pathogenic bacterial flora in inhibiting later colonization by MRSA in ELBW infants.

Materials and Methods

Subjects comprised 110 infants admitted soon after birth and hospitalized for more than 42 days from among 164 ELBW infants admitted to the NICU at Nagano Children's Hospital from January 1997 to December 2003. We excluded infants with major congenital malformations, hydrops fetalis, inherited metabolic disease and infants back-transferred before postnatal day (PD) 42. Parental consent was obtained for all subjects prior to enrolment in the study, and all protocols were approved by the hospital ethics committee. Oral bacterial samples were collected from infants until PD 42. We compared incidence rates of MRSA colonization between infants with non-pathogenic bacterial colonization during the first week (group 1, n = 56) and infants with no significant growth of bacteria except MRSA (group 2, n = 54). Our analysis of the timing of colonization by non-pathological bacteria showed that the most significant difference existed between day 7 and days 14 and 21. The reason for comparing rates of MRSA colonization until PD 42 was that most infants were back-transferred to the second level of the NICU in another hospital after PD 42, and the numbers of infants in groups 1 and 2 therefore decreased. No prophylactic oral hygiene or antibiotics treatments were used during the study period.

Surveillance Methods

In our NICU, routine surveillance culture for all neonates was first performed on admission and then weekly thereafter. According to clinical requirements, cultures were occasionally performed between surveillance cultures, and were also used for epidemiological analysis. At the time of admission, specimens of feces and umbilical exudate and oral cavity swabs were obtained for culture for every newborn infant, and specimens from oral cavities were cultured every week as a part of routine surveillance with sterile rayon-tipped swabs (Seed swab No. 2; Eiken Kizai, Tokyo, Japan). All swabs were inoculated onto plates with 5% sheep blood agar, chocolate agar, modified Drigalski agar, and OPA *Staphylococcus* agar (all plates were purchased from Becton Dickinson, Franklin Lakes, N.J., USA). Plates were incubated for 24 h at 37°C in 5% CO_2 in air. Bacterial identification and antibiotic susceptibility testing was performed. MRSA was defined as *S. aureus* using oxacillin plates for which the minimum inhibitory concentration of oxacillin was >4 μg/ml.

Statistical Analysis

Data are presented as mean ± SD or as the number of patients/total number of patients (percentage). Outcomes were compared using Welch's t-test or Fisher's exact probability test as appropriate. For all tests, two-tailed values of $p < 0.05$ were accepted as significant.

Results

On admission, most cultures (99%) of oral cavity specimens showed no significant growth of bacteria. Bacteria detected during the first week in group 1 were *S. epidermidis* (80.3%), *Corynebacterium* (7.1%), *Lactobacillus* (7.1%) and *α-Streptococcus* (5.4%). 39% of patients in group 2 were already colonized with MRSA during the first week, 11% of patients showed other pathogenic bacteria *(S. aureus, Enterobacteriaceae, Escherichia coli)*, and the remaining 40% showed no significant bacterial colonization on in vitro culture. The clinical characteristics

of the two groups are listed in table 1. All infants were feeding on breast milk from their own mother. No significant differences in premature rupture of membranes (PROM), maternal antibiotic use, mean duration of maternal antibiotics (group 1: 2.5 days; group 2: 2.3 days), type of antibiotics (cefazolin or piperacillin) administered to mother, type of delivery or use of neonatal antibiotics (ampicillin or gentamicin) were seen between groups. The rate of sepsis was significantly greater in group 2 than in group 1 ($p < 0.05$). MRSA was the major cause of sepsis (1/1 in group 1; 5/6 in group 2), and 1 patient in group 2 died due to MRSA sepsis. Figure 1 shows cumulative rate of infants with MRSA colonization every week up to 6 weeks of hospitalization. Prevalence of MRSA colonization at 6 weeks was significantly lower in group 1 (18/56, 32.1%) than in group 2 (42/54, 77.8%; $p < 0.001$).

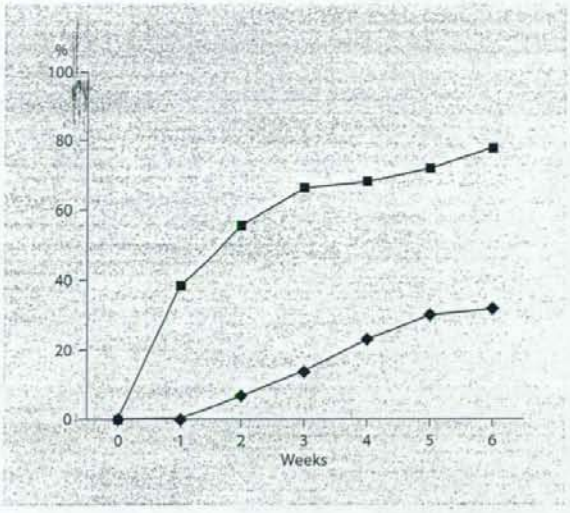

Fig. 1. Cumulative rate of infants with MRSA in groups 1 and 2 every week until 6 weeks of age. ◆ = Group 1, infants with non-pathogenic bacterial flora during the first week (n = 62); ■ = group 2, infants with no significant bacterial growth except MRSA during the first week (n = 48).

Discussion

The uncontrollable spread of MRSA in newborn infants in NICUs has been largely attributed to environmental risk factors. In addition, immune systems compromised by premature birth, illness and invasive procedures seem to play important roles in neonatal MRSA colonization. Most nosocomial infections in NICU patients result from person-to-person transmission via the hands of medical staff [12]. Colonization with MRSA is achieved through a number of continuous processes: arrival of bacteria from other sources to sites in the newborn infant, specific attachment of bacteria to molecules on epithelial cells, and proliferation of bacteria in the infant. Interruption of the continuous flow of colonization processes at any point is likely to inhibit colonization with MRSA. Current methods of preventing colonization focus on preventing either patient-pathogen contact or growth of colonizing microorganisms. However, none of the individual conventional control measures involved in these processes, such as an emphasis on hand-washing, gown- and glove-wearing, isolation of colonized and infected infants, or even administration of antibiotics, have succeeded in controlling the spread of MRSA in NICUs [1]. Only when the usual control measures are performed in combination with a reduction in overcrowding and an increase in staffing has control of the spread of MRSA been achieved [2].

Many mechanisms by which non-pathogenic bacterial flora inhibit the growth of pathogenic microorganisms have been reported. Production of bacteriocins is an example of a direct inhibitory mechanism. Production of these high molecular weight antibiotics by resident viridans streptococci is considered to form an important barrier to colonization by pathogenic bacteria in the oropharynx [5]. Other indirect inhibitory mechanisms, such as enhancement of host immune or clearance systems, have also been reported [5]. In vitro studies have established that various strains of viridans streptococci inhabiting the oropharynx suppress the growth of respiratory pathogens [13]. Normal bacterial flora can inhibit colonization by pathogenic bacteria in older people [11]. However, few investigations have examined whether normal bacterial flora can inhibit MRSA colonization in neonates. We recently reported that *Streptococcus viridans* during the first week after birth, as components of the normal bacterial flora, eliminated subsequent MRSA colonization in the oral cavities of newborn infants in our NICU [14]. The present study therefore investigated the effects of non-pathological bacterial colonization during the first week after birth on later MRSA colonization of ELBW infants.

Cultures from the nose, nasopharynx, throat, umbilicus and rectum are usually negative on admission in neonates. Infants are colonized by flora from their mothers

and from other human contacts [9]. The birth canal may play an important role in neonatal colonization by normal bacterial flora. *Corynebacteria* and CNS are usually isolated from the skin of neonates within a few hours after birth, and simultaneously from the vagina of the mother. *Bacteroides fragilis*, a bacterial component of the fecal flora, can be isolated from vaginally delivered neonates within 48 h after birth, but few such bacteria are isolated from neonates born by cesarean section [9]. The full-term neonate can obtain normal bacterial flora from the mother's breast and milk during holding and feeding. However, many ELBW infants are born by cesarean section, separated from the mother immediately after birth, and given tube feeding by nurses. They can thus experience difficulty in obtaining normal bacterial flora.

The present investigation has a few limitations, particularly that it was retrospective. Many practices, such as hand-washing and other nursing procedures, changed during the study period. Numerous other factors could represent potential confounders, increasing the risk of MRSA colonization. We hope to analyze the effects of these other practices on MRSA colonization in a prospective study. Although no significant clinical differences except the rate of sepsis were identified between groups, the present results suggest that non-pathogenic bacterial flora are correlated with resistance against colonization by MRSA in ELBW infants. We cannot answer why colonization with non-pathogenic bacteria during the first week might be important in inhibiting later MRSA colonization. Further prospective studies are needed to clarify the potential roles of bacterial flora in preventing the spread of MRSA in the NICU.

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Pregnancy complicated by diffuse chorioamniotic hemosiderosis: Obstetric features and influence on respiratory diseases of the infant

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Abstract

Aim: To clarify the clinical features of pregnancy and neonatal respiratory problems associated with diffuse chorioamniotic hemosiderosis (DCH).

Methods: Sixteen singleton cases of DCH without chorioamnionitis (CAM) were retrospectively analyzed and compared with gestation- and birthweight-matched controls (32 cases of CAM and 32 cases of non-DCH-non-CAM). Maternal symptoms and respiratory problems of the infants were investigated.

Results: All 16 cases with DCH resulted in preterm delivery from 23 to 35 weeks' gestation. The presence of subchorionic hematoma in the first trimester ($P < 0.001$), recurrent vaginal bleeding ($P < 0.001$), brownish amniotic fluid ($P < 0.001$) and amniotic necrosis or degeneration ($P < 0.001$) were significantly more frequent in the DCH group compared to the CAM and non-DCH-non-CAM groups. The incidence of dry lung syndrome and persistent pulmonary hypertension of the newborn (PPHN) was significantly higher in the DCH group than in the CAM ($P < 0.001$) and non-DCH-non-CAM ($P < 0.001$) groups.

Conclusion: Long-term exposure to degenerating red blood cells is supposed to damage amnion, fetal alveolar epithelial cells and fetal pulmonary arteries, and may lead to dry lung syndrome and PPHN in the infant complicated by DCH.

Key words: diffuse chorioamniotic hemosiderosis, dry lung syndrome, persistent pulmonary hypertension of the newborn, preterm delivery.

Introduction

Placental hemorrhage often causes premature rupture of the membranes (PROM), preterm delivery and fetal hypoxia, and these obstetric events affect the function of each organ of the infant. Abruptio placentae is supposed to result from acute placental hemorrhage, leading to acute breakdown of placental functions such as the oxygenation of fetal blood. This life-threatening dysfunction of the placenta is clinically detected by fetal heart rate monitoring. In contrast, chronic hemorrhage of the placenta is not always lethal to the fetus, and it may be possible to prolong the pregnancy period with appropriate medication.

Some placental findings such as peripheral placental separation and circumvallate placenta reflect venous and recurrent hemorrhage of the placenta.[1–3] These findings are macroscopic and subjective to some extent. To evaluate such chronic placental hemorrhage more objectively, the concept of diffuse chorioamniotic hemosiderosis (DCH) was suggested by Redline *et al.*[1] Diagnosis of DCH is made based on microscopic

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findings of the chorioamnion, and the essence is the deposition of hemosiderin within chorionic plate or membrane, which is formed from phagocytosis of hemoglobin or hemoglobin breakdown products by macrophages. This cellular reaction triggered by macrophages may cause dysfunction of chorioamnion and neonatal organs.

In this decade, the impact of DCH on obstetric and neonatal aspects has been reported.[1,4] According to the report by Ohyama *et al.*,[4] DCH is closely associated with preterm delivery, persistent pulmonary hypertension of the newborn (PPHN), and dry lung syndrome, and is a significant risk factor for chronic lung disease (CLD). However, since many of the cases in their study were simultaneously complicated by chorioamnionitis (CAM), their findings might be affected by this condition. We investigated the obstetric characteristics and respiratory problems of the infants associated with DCH without CAM.

Materials and Methods

This retrospective study investigated cases cared for through their pregnancy and delivery in our center during the period from September 2000 to October 2005. Cases with stillbirth, multiple pregnancy, fetal anomaly and maternal uterine deformity such as bicornuate uterus were excluded from our survey. In the case of threatened preterm delivery before 34 weeks' gestation, we routinely injected the mother with 12 mg betamethasone i.m. twice per 24 h interval. Placentas were pathologically examined in all cases, and diagnosis of DCH was histopathologically made with scattered brown pigment granules by hematoxylin–eosin staining (Fig. 1). The stages of CAM were determined according to the criteria by Blanc.[5] Maternal neutrophils are observed as follows: stage 1, in the subchorionic membranes at the junction between the decidua and chorioamnion; stage 2, in the connective tissues of the chorionic plate and membranous chorioamnion; stage 3, in the amniotic basement membrane and the layer of amniotic epithelial cells.

There were 22 cases with DCH in this study period. However, two cases were multiple pregnancy, and another four were complicated by CAM; therefore we analyzed the remaining 16 cases with DCH that were singletons and without CAM. As gestation- and birthweight-matched controls, 32 cases of CAM and 32 cases of non-DCH-non-CAM were selected. For the CAM group, we selected stage 2 or stage 3 patients. Patients with pre-eclampsia or maternal complications

Figure 1 Histopathological findings of diffuse chorioamniotic hemosiderosis. Deposition of hemosiderin within chorionic membrane is shown as scattered brown pigment granules in macrophages (arrows). Hematoxylin–eosin staining, ×400.

were not included in the study and control groups, because such maternal factors may cause therapeutic preterm deliveries and affect histopathological findings of the placenta by themselves. In each of these three groups, clinical records were reviewed and the following factors of the mothers and the infants were examined.

Maternal factors: (i) maternal age; (ii) subchorionic hematoma (SCH) diagnosed with ultrasound in the first trimester; (iii) recurrent vaginal bleeding (for more than 7 days); (iv) PROM; (v) placenta previa; (vi) brownish amniotic fluid; and (vii) amniotic necrosis or amniotic degeneration confirmed by histopathological examinations (Fig. 2).

Neonatal factors: (i) Apgar score at 5 min; (ii) small for gestational age (SGA); (iii) respiratory distress syndrome (RDS); (iv) dry lung syndrome; (v) PPHN; and (vi) CLD.

The criteria of the neonatal factors are as follows. RDS: result of microbubble test and response to the surfactant, in addition to chest radiographic findings. Dry lung syndrome: very high requirement for ventilation with dramatic improvement during the first 24–36 h, RDS and infection excluded.[4] PPHN: evidence of right-to-left shunting of blood across the foramen ovale and ductus arteriosus by cardiac sonography. CLD: oxygen requirement greater than that obtainable in room air at 28 days after birth, with symptoms of persistent respiratory distress and a hazy or emphysematous and fibrous appearance on chest radiograph.[6]

As each of CAM group and non-DCH-non-CAM group included one infant who died during treatment,

Figure 2 Histopathological findings of amniotic degeneration. Amnion is equally stained with eosin, and nuclei of the amniotic epithelial cells are almost lost (double arrows). Deposition of hemosiderin is also shown (arrows). Hematoxylin–eosin staining, ×100.

we performed statistical analysis of the remaining 31 infants in each of these two groups (except Apgar scores and SGA). These 62 infants in the control groups and all 16 infants in DCH group were discharged alive.

Statistical analysis was performed by Mann–Whitney *U*-test, and $P < 0.05$ was considered significant. Numerical variables were expressed as mean ± SD.

Results

Table 1 shows the mean gestational age and birthweight of the study population and their controls. All pregnancies ended in preterm deliveries from 23 to 35 weeks' gestation. Analysis of the obstetric features in the DCH group revealed that the presence of SCH in the first trimester ($P < 0.001$), recurrent vaginal bleeding ($P < 0.001$) and brownish amniotic fluid ($P < 0.001$) were significantly more frequent than in the control groups (Table 2). Histopathological findings of amniotic necrosis or degeneration were more commonly noted ($P < 0.001$). Apgar scores at 5 min in DCH group were significantly lower compared to the non-DCH-non-CAM group ($P = 0.04$) and the incidence of dry lung syndrome and PPHN was significantly increased in the DCH group compared to the control groups (in each disorder, DCH *vs* CAM: $P < 0.001$; DCH *vs* non-DCH-non-CAM: $P < 0.001$; Table 3).

Discussion

DCH is defined as the diffuse deposition of hemosiderin in chorionic plate or membrane. These hemosiderin crystals are formed in the process of phagocytosis of hemoglobin or degenerating hemoglobin products by macrophages. Metabolism of hemoglobin to hemosiderin in macrophages requires 3 to 8 days.[1] In a circumvallate placenta which macroscopically represents chronic placental bleeding, local hemosiderin deposition is the rule, and in severe cases DCH affects the entire placenta.[3] Taking these facts into consideration, DCH is a more objective and useful marker that reflects chronic bleeding of the placenta.

Degenerating red blood cells (RBC) undergo morphological, physicochemical, enzymatic and biochemical changes. These RBC gain cell toxicity, for instance producing superoxide radicals.[7] Macrophages recognize them and take them in. In macrophages, hemoglobin is cleaved to heme and globin protein, and heme is further catabolized to iron and porphyrin. Iron is stocked in macrophages as ferritin or hemosiderin.[8] Stocked hemosiderin is biochemically modified and adequately released out of macrophages for hemoglobin synthesis in erythroblasts.[9] In this way, macrophages act as a scavenger and also play an important role in iron recycling.

Activated macrophages release numerous kinds of products that can consequently injure cells, such as interleukins, complements, enzymes and activated oxygen. As chorioamnion complicated by DCH is abundant in macrophages, these products derived from macrophages may cause inflammatory reactions and affect the structure and function of the chorioamnion.

Although amniotic necrosis was reported to occur more frequently in cases with DCH compared to those without DCH,[4] comparison of the incidence of necrotic changes in the amnion between cases with DCH and those with CAM has not been made as far as we know. This study revealed that amniotic necrosis or degeneration was confirmed more frequently in the DCH group than in the CAM group. As for respiratory diseases of the infants, dry lung syndrome/pulmonary hypoplasia, PPHN and CLD were reported to develop more often in cases with DCH compared to those without DCH.[4] We also analyzed the incidence of RDS, dry lung syndrome, PPHN and CLD in the DCH and CAM groups, and found that dry lung syndrome and PPHN occurred significantly more often in the DCH group.

These differences between DCH and CAM may result from long-term exposure of the amnion and fetal

Table 1 Study population (DCH group) and control (CAM and non-DCH-non-CAM) groups

	DCH (n = 16)	CAM (n = 32)	non-DCH-non-CAM (n = 32)	Significance
Gestational week at delivery	30.9 ± 3.3	30.7 ± 3.2	31.2 ± 3.4	NS
Birthweight (g)	1584 ± 535	1562 ± 507	1611 ± 515	NS

CAM, Chorioamnionitis; DCH, diffuse chorioamniotic hemosiderosis; NS, not significant.

Table 2 Obstetric characteristics of DCH

	DCH ($n = 16$)	CAM ($n = 32$)	non-DCH-non-CAM ($n = 32$)	Significance
Maternal age	30.5 ± 3.8	30.9 ± 5.8	30.0 ± 4.2	DCH *vs* CAM: NS DCH *vs* non-DCH-non-CAM: NS CAM *vs* non-DCH-non-CAM: NS
Subchorionic hematoma	11 (69%)	0 (0%)	2 (6%)	DCH *vs* CAM: $P < 0.001$ DCH *vs* non-DCH-non-CAM: $P < 0.001$ CAM *vs* non-DCH-non-CAM: NS
Recurrent vaginal bleeding	16 (100%)	2 (6%)	1 (3%)	DCH *vs* CAM: $P < 0.001$ DCH *vs* non-DCH-non-CAM: $P < 0.001$ CAM *vs* non-DCH-non-CAM: NS
PROM	9 (56%)	23 (72%)	21 (66%)	DCH *vs* CAM: NS DCH *vs* non-DCH-non-CAM: NS CAM *vs* non-DCH-non-CAM: NS
Placenta previa	2 (13%)	0 (0%)	1 (3%)	DCH *vs* CAM: NS DCH *vs* non-DCH-non-CAM: NS CAM *vs* non-DCH-non-CAM: NS
Brownish amniotic fluid	13 (81%)	1 (3%)	0 (0%)	DCH *vs* CAM: $P < 0.001$ DCH *vs* non-DCH-non-CAM: $P < 0.001$ CAM *vs* non-DCH-non-CAM: NS
Amniotic necrosis/degeneration	10 (63%)	3 (9%)	1 (3%)	DCH *vs* CAM: $P < 0.001$ DCH *vs* non-DCH-non-CAM: $P < 0.001$ CAM *vs* non-DCH-non-CAM: NS

CAM, Chorioamnionitis; DCH, diffuse chorioamniotic hemosiderosis; NS, not significant; PROM, premature rupture of membranes.

Table 3 Apgar score, SGA and respiratory problems of infants born from mothers complicated by DCH

	DCH	CAM	non-DCH-non-CAM	Significance
Apgar score at 5 min	6.9 ± 1.9	7.9 ± 1.7	8.0 ± 1.3	DCH *vs* CAM: NS DCH *vs* non-DCH-non-CAM: $P = 0.04$ CAM *vs* non-DCH-non-CAM: NS
SGA	3/16 (19%)	0/32 (0%)	1/32 (3%)	DCH *vs* CAM: NS DCH *vs* non-DCH-non-CAM: NS CAM *vs* non-DCH-non-CAM: NS
RDS	1/16 (6%)	5/31 (16%)	9/31 (29%)	DCH *vs* CAM: NS DCH *vs* non-DCH-non-CAM: NS CAM *vs* non-DCH-non-CAM: NS
Dry lung syndrome	7/16 (44%)	1/31 (3%)	0/31 (0%)	DCH *vs* CAM: $P < 0.001$ DCH *vs* non-DCH-non-CAM: $P < 0.001$ CAM *vs* non-DCH-non-CAM: NS
PPHN	7/16 (44%)	1/31 (3%)	1/31 (3%)	DCH *vs* CAM: $P < 0.001$ DCH *vs* non-DCH-non-CAM: $P < 0.001$ CAM *vs* non-DCH-non-CAM: NS
CLD	3/16 (19%)	5/31 (16%)	4/31 (13%)	DCH *vs* CAM: NS DCH *vs* non-DCH-non-CAM: NS CAM *vs* non-DCH-non-CAM: NS

CAM, Chorioamnionitis; CLD, chronic lung disease; DCH, diffuse chorioamniotic hemosiderosis; NS, not significant; PPHN, persistent pulmonary hypertension of the newborn; RDS, respiratory distress syndrome; SGA, small for gestational age.

alveolar epithelial cells to degenerating RBC in DCH group rather than from activation of the immune system triggered by macrophages or neutrophils. It is obvious that SCH, recurrent vaginal bleeding, and brownish amniotic fluid are associated with DCH. These obstetric symptoms correlate with placental hemorrhage, which may lead to fetal alveolar dysfunction through aspiration of degenerating RBC. In an equine model, chronic aspiration of bloody substance in amniotic fluid may cause lung injury with chronic inflammation.[10] However, the same pathological process in a human lung has not been documented. Chronic peripheral separation is often accompanied by oligohydramnios, and this complication is called chronic abruption-oligohydramnios sequence (CAOS).[11] Alveolar injury caused by long-term exposure to bloody substance may reduce production of alveolar fluid, and lead to oligohydramnios. This qualitative and quantitative change could explain the mechanism of CAOS.

Dry lung syndrome is related to oligohydramnios. Losa *et al.* hypothesized that external compression with continuous loss of lung fluid could squeeze out the fetal lung to such an extent that small bronchi and bronchioli would collapse completely.[12] In cases with DCH, oligohydramnios could be the result of PROM or the expression of CAOS with or without PROM. In our study, the incidence of dry lung syndrome is significantly higher in the DCH group, but that of PROM is not significantly different among the three groups. Moreover, all of seven cases complicated by dry lung syndrome in the DCH group developed PPHN. This suggests that the incidence of dry lung syndrome depends upon the quality of amniotic fluid, such as the presence of bloody substances, rather than the quantity of remaining fluid.

Our study revealed that the incidence of PPHN was significantly higher in the DCH group, but the duration of treatment with high frequency oscillatory ventilation (HFO) or oxygen, and the incidence of CLD, were not different among these three groups. And the number of cases which needed HFO treatment was not different, either (data not shown). These results suggest that although the infants born from mothers complicated by DCH develop PPHN more frequently than controls, PPHN itself does not seem to affect long-term respiratory conditions in premature infants. It should be investigated whether PPHN observed in the DCH group is more responsive to treatments than other respiratory problems, and whether antenatal corticosteroid treatment is effective in improving respiratory conditions of the infants complicated by DCH.

In conclusion, obstetric findings which suggest chronic placental hemorrhage, such as recurrent vaginal bleeding and brownish amniotic fluid, are considered to indicate clinical symptoms of DCH, and it seems that most pregnancies with DCH result in preterm deliveries. If the mother is complicated with DCH, neonatal care should be taken in preparation for dry lung syndrome and PPHN.

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Continuous negative extrathoracic pressure combined with high-frequency oscillation improves oxygenation with less impact on blood pressure than high-frequency oscillation alone in a rabbit model of surfactant depletion

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Abstract

Background: Negative air pressure ventilation has been used to maintain adequate functional residual capacity in patients with chronic muscular disease and to decrease transpulmonary pressure and improve cardiac output during right heart surgery. High-frequency oscillation (HFO) exerts beneficial effects on gas exchange in neonates with acute respiratory failure. We examined whether continuous negative extrathoracic pressure (CNEP) combined with HFO would be effective for treating acute respiratory failure in an animal model.

Methods: The effects of CNEP combined with HFO on pulmonary gas exchange and circulation were examined in a surfactant-depleted rabbit model. After induction of severe lung injury by repeated saline lung lavage, 18 adult white Japanese rabbits were randomly assigned to 3 groups: Group 1, CNEP (extra thoracic negative pressure, -10 cmH_2O) with HFO (mean airway pressure (MAP), 10 cmH_2O); Group 2, HFO (MAP, 10 cmH_2O); and Group 3, HFO (MAP, 15 cmH_2O). Physiological and blood gas data were compared among groups using analysis of variance.

Results: Group 1 showed significantly higher oxygenation than Group 2, and the same oxygenation with significantly higher mean blood pressure compared to Group 3.

Conclusion: Adequate CNEP combined with HFO improves oxygenation with less impact on blood pressure than high-frequency oscillation alone in an animal model of respiratory failure.

Background

Continuous negative extrathoracic pressure (CNEP) applied around the chest was been shown to be efficacious in the treatment of respiratory failure in infants [1-5]. CNEP can produce increased functional residual capacity and may lead to increased cardiac output by increasing cerebral venous return and decreasing pulmonary vascular resistance [6,7]. However, wide use of this technique has not been seen in the neonatal field, as creating negative pressure around the fragile chest wall is difficult in neonates.

High-frequency oscillation (HFO) has been shown to prevent both acute and chronic lung injury in neonatal man-

agement. Specifically, HFO has been shown to reduce the incidence of chronic lung disease in very low birth weight infants [8-10]. Studies of surfactant deficiency in animal models have demonstrated that volume recruitment is one of the important lung protective strategies during HFO [11,12].

In the present study, we hypothesized that CNEP combined with HFO would offer greater improvements in oxygenation than HFO alone in a rabbit model of surfactant depletion.

Materials and methods

Animal model

The study protocol was approved by the Institutional Animal Care and Committee of Nagano Children's Hospital, Nagano, Japan. Eighteen adult white Japanese rabbits weighing 2.0–2.5 kg were used for this study. All animals were premedicated by intramuscular administration of ketamine (10 ml/kg) and xyladine (5 mg/kg). The peripheral ear vein was cannulated using a 24-gauge angiocatheter for intravenous anesthesia and infusion of medication. Animals were placed in a supine position during the entire study period. A 3.5-Fr endotracheal tube without cuff (Mallinckrodt, St. Louis, Missouri, USA) was inserted into the trachea and tied to prevent gas leak. The carotid artery was cannulated using a 22-gauge angiocatheter and connected to a blood pressure monitor (Polygraph System; Nihon Koden, Tokyo, Japan) to monitor arterial blood pressure and heart rate, and to obtain arterial blood samples for blood gas analysis. Anesthesia was provided by continuous intravenous infusion of ketamine (5 mg/kg/h) and paralysis was maintained using pancuronium (0.1 mg/kg/h). Mechanical ventilation was performed using a time-cycled, pressure-limited ventilator (Humming II; Metran, Saitama, Japan). Animals were administered 10% glucose in 0.45% saline solution at 3 ml/kg/h throughout the study period without any colloid or catecholamine.

Measurements

Oxygen saturation, heart rate and blood pressure were monitored continuously using a pulse oximeter (Nihon Koden, Tokyo, Japan). Tidal volume (Vt) was measured intermittently using a low-dead space hot-wire pneumotachograph (Aivision Laminar Flow Meter LFM-317; Metabo, Lausanne, Switzerland). Arterial blood gas samples were analyzed intermittently (0, 30, 60, 90 and 120 min). Blood pressure, heart rate and ventilator settings were recorded before and after lung injury and at 30-min intervals during the 120-min study period.

Experimental protocol

After obtaining baseline measurements, acute respiratory failure was induced by repeated lung lavage with aliquots of 30 ml/kg of warmed normal saline. Lavage was considered adequate if PaO_2 was <80 mmHg by 15 min after last lavage with the following ventilator settings: FiO_2 1.0 at a respiratory rate of 30 breaths/min with positive end expiratory pressure (PEEP) of 5 cmH_2O; peak inspiratory pressure (PIP) to maintain Vt of 15 ml/kg; and inspiratory time of 1.0 s. To induce severe and stable lung injury, animals were ventilated mechanically for 60 min at the above settings.

To determine the adequate CNEP level combined with HFO (mean airway pressure (MAP), 10 cmH_2O) in our study, we conducted a preliminary examination of oxygenation at each CNEP level (extra thoracic negative pressures: -5 cmH_2O; -10 cmH_2O; and -15 cmH_2O) combined with HFO. CNEP (-5 cmH_2O) combined with HFO showed no change in oxygenation compared with HFO alone. CNEP combined with HFO (MAP, 10 cmH_2O) showed the same oxygenation level at -10 cmH_2O or -15 cmH_2O.

From these preliminary results, we decided to use CNEP (-10 cmH_2O) in our experimental protocol. Animals were randomly allocated to 3 therapy groups. Group 1 used CNEP (extra thoracic negative pressure -10 cmH_2O) with HFO (MAP 10 cmH_2O). CNEP (RTX; Medivent, London, UK) settings were as follows: CNEP at -10 cmH_2O and neonatal size selected for the cuirass. HFO settings were as follows: MAP at 10 cmH_2O and pressure amplitude adjusted to maintain $PaCO_2$ between 35 and 55 mmHg at a frequency of 15 Hz. Group 2 used HFO alone at MAP 10 cmH_2O, and Group 3 used HFO alone at MAP 15 cmH_2O.

Statistical Analysis

All results are expressed as mean ± standard deviation, and were compared using analysis of variance (ANOVA) for repeated measures with Scheffé's test. Values of $p < 0.05$ were considered statistically significant.

Results

Baseline and post-injury data were similar in all 3 groups. Changes in PaO_2 over time are shown in Figure 1. In Group 1 (-10 cmH_2O CNEP with HFO; MAP 10 cmH_2O), PaO_2 increased after starting CNEP and was significantly higher than in Group 2 (HFO; MAP, 15 cmH_2O) ($p < 0.05$). Group 3 (HFO; MAP 15 cmH_2O) displayed similar PaO_2 to Group 1. Changes in MAP during the observation period are shown in Figure 2. Mean arterial pressure was significantly lower in Group 3 than in Group 1 ($p < 0.05$) throughout the experimental period.

Discussion

PEEP is generally accepted to increase transpulmonary pressure, thus increasing lung volume and reopening some previously collapsed lung units. An alternative approach to increasing transpulmonary pressure, and

Figure 1

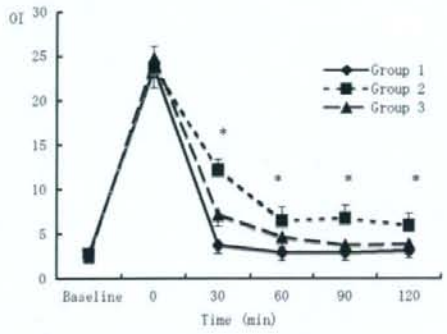

Figure 1
Changes in oxygen index (OI) in experiments. (Diamond) Group 1: CNEP (-10 cmH_2O) with low-MAP (10 cmH_2O) HFO. (Circle) Group 2: Low-MAP (10 cmH_2O) HFO. (Square) Group 3: High-MAP (15 cmH_2O) HFO. *p < 0.05 Groups 1, 3 vs. Group 2.

thus lung volume, is represented by application of negative pressure around the chest. Randomized trials to assess the benefits of CNEP and standard care in preterm infants have been described [3]. Telford et al. reported long-term

Figure 2

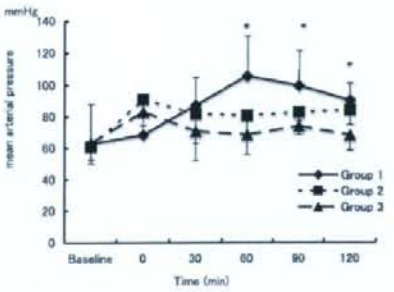

Figure 2
Changes in mean arterial pressure. (Diamond) Group 1: CNEP (-10 cmH_2O) with low-MAP (10 cmH_2O) HFO. (Circle) Group 2: Low-MAP (10 cmH_2O) HFO. (Square) Group 3: High-MAP (15 cmH_2O) HFO. *p < 0.05 Group 1 vs. Group 3.

outcomes after neonatal CNEP [5]. They showed that death or sever disability was equally distributed between CNEP group and standard treatment group. Full IQ did not differ significantly between groups, but mean performance IQ was higher in the CNEP group. CNEP was also useful in more mature infants with other types of respiratory failure [1,2]. In the treatment of acute lung injury, application of CNEP increased transpulmonary pressure, thus achieving improved lung function similar to that obtained with PEEP. As opposed to PEEP, which increases intrathoracic pressure, CNEP increases transpulmonary pressure by decreasing intrathoracic pressure, rather than by increasing airway pressure. CNEP has favorable effects on permeability and hydrostatic pulmonary edema [6,7]. In a sheep model inoculated with *Pseudomonas* bacteria, CNEP decreased hydrostatic filtration pressure and lung lymph flow [13]. In dogs with pulmonary edema induced by oleic acid, CNEP increased extravascular lung water volume, but did not change central blood volume [14]. Shekerdemian et al. reported that CNEP improved cardiac output in children after cardiac surgery [15-17]. However, CNEP has been not widely used in neonatal intensive care unit, as extrathoracic devices are not easy to fix to the chest wall of neonates, and maintaining constant extrathoracic negative pressure is difficult.

HFO is a gentler mechanical ventilation approach with very low tidal volume and fixed mean airway pressure, which decreases the pressure swing in the peripheral airways and alveoli, and may result in a reduction of lung injury. HFO started after birth can prevent the development of chronic lung disease in very low birth weight infants at high risk for respiratory distress syndrome [8-10]. Sustained increases in MAP could induce rapid, large increases in PaO_2 in the lungs, exhibiting some hysteresis in pressure/volume relationships [11,12]. However, higher MAP utilized during HFO could conceivably impede venous return and lead to hypotension. In neonates, this might result in intracranial hemorrhage [18].

Although the present study was limited by a lack of direct measurement of transpleural pressure and cardiac output, we showed that adequate CNEP combined with HFO results in the same level of oxygenation and significantly higher mean blood pressure compared with high MAP HFO-only groups. In neonate, high MAP HFO easily affect on circulation and need volume expander or catecholamine to keep adequate blood pressure. Although, further experiments are needed to develop a more comfortable and useful cuirass that can be adjusted to individual neonatal chest size for long-term use in human neonate, we can try this ventilator combination in neonate who has severe respiratory and circulatory failure. Based on these experimental data, we speculate that ade-

quate CNEP might play a role as a continuous volume recruitment maneuver during HFO or change in pulmonary blood flow or increases in cardiac output. Some articles have described comparative evaluations of hemodynamic effects for CNEP and positive end-expiratory pressure [19-22], no studies appear to have shown the combined effects of CNEP and HFO on oxygenation in an animal model of lung injury. We hope to look at adequate circulation in CNEP with HFO in further experiments.

We conclude that adequate CNEP combined with HFO improves oxygenation with less impact on blood pressure than HFO alone in an animal model of surfactant depletion.

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Consensus2005に基づく日本版新生児心肺蘇生法ガイドラインとその普及のための講習会推進事業

日本小児科学会蘇生法専門委員，日本未熟児新生児学会教育研修委員会委員長，日本周産期・新生児医学会教育研修委員会委員長，日本救急医療財団日本版救急蘇生ガイドライン策定小委員会委員，国際蘇生法連絡委員会新生児タスクフォース委員

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総　説

Consensus2005に基づく日本版新生児心肺蘇生法ガイドラインとその普及のための講習会推進事業

日本小児科学会蘇生法専門委員，日本未熟児新生児学会教育研修委員会委員長，日本周産期・新生児医学会教育研修委員会委員長，日本救急医療財団日本版救急蘇生ガイドライン策定小委員会委員，国際蘇生法連絡委員会新生児タスクフォース委員

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キーワード：国際蘇生法連絡委員会，Consensus2005，新生児心肺蘇生法ガイドライン，新生児心肺蘇生法講習会推進事業

Consensus2005に基づく日本版新生児心肺蘇生法ガイドライン作成の経緯

アメリカ心臓協会（以下AHA）の他にヨーロッパ蘇生会議，カナダ心臓・脳卒中財団，ラテン・アメリカ蘇生会議，オーストラリア・ニュージーランド蘇生会議，南アフリカ蘇生会議を構成メンバーとした国際蘇生法連絡委員会（以下ILCOR）は，2005年11月29日に，5年ぶりに新生児から成人までの心肺蘇生法の科学的な根拠に関する概要の大幅な改正を提言した（Consensus on Science With Treatment Recommendations以下Consensus2005）[1]．ILCORの構成メンバーは，このConsensus2005の基本的な考え方の枠内で，地域別の実情を踏まえた心肺蘇生法のガイドラインを作成し，各地域の国別に母国語で教材を作成し，その普及活動に務める事になっている．ILCORの主要メンバーであるAHAはConsensus2005の策定作業に深く関わっていたので，Consensus2005の発表と同時に，アメリカ小児科学会と協力して新しい新生児心肺蘇生法のガイドラインを公表した[2]．日本は2005年の時点ではILCORの正式メンバーでは無かったためConsensus2005の公開を待って，財団法人日本救急医療財団の日本版救急蘇生ガイドライン策定小委員会（委員長：丸川征四郎，小児科学会推薦委員：清水直樹，田村正徳）が，急遽「日本版救急蘇生ガイドライン」の作成作業を開始し，ホームページで公開[3]して意見聴取したのちに2007年春にやっと印刷物として出版するにいたった[4]．

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Consensus2005の主たる改正点

AHAの2000年版心肺蘇生国際ガイドライン[5]（以下AHA2000）と比較したときのConsensus2005の基本的な変更点を表1に示す．

北米における neonatal resuscitation program（NRP）

アメリカ小児科学会はAHAと協力して新生児心肺蘇生法の標準化とその関係者への普及事業に取り組んでいる．出生時に胎外生活に向けた呼吸循環動態の移行が順調に進行しない事例は，全出産の約10%にみられ，さらに全出生児の1%が救命のために本格的な蘇生手段（気管挿管，胸骨圧迫，薬物治療）を必要とし，適切な処置を受けなければ，死亡するか，重篤な障害を残すとされている[6]．そこで，AHA2000[5]では，「全ての分娩に新生児の蘇生を開始することのできる要員が少なくともひとり，専任で立ち会うべきである．」と推奨されている．この体制を確立するためにアメリカ小児科学会は，1987年より周産期医療従事者を対象とした新生児心肺蘇生法講習会を実施しており，8時間の講習会の後，筆記試験と実地試験をへた合格者（provider）のうち，更に半日間の指導法の講習会を修了したものにインストラクターの資格を与え，ともに2年ごとに資格更新をするシステムを全国的規模で構築している[6]．

表1　Consensus2005の基本的な変更点

1. 酸素投与は慎重に行う．
   ・出生直後のチェックポイントから「皮膚色のチェック」は除かれた．
   ・蘇生の初期処置を行っても中心性チアノーゼが続いたり，人工呼吸が必要な場合に酸素を投与する．
2. 胎便による羊水混濁時の分娩中の口腔咽頭吸引はルーチンには必要無い．
3. アドレナリンの投与は静注が原則で，ラインが無い場合に気管内投与する時は高用量を用いる．
4. ナロキサンは分娩室での使用は推奨され無い．
5. 極低出生体重児の保温法のオプションとしてプラスチックバッグやラップが推奨される．
6. 気管挿管チューブの位置の確認法として呼気中のCO2の検知が推奨される．

日本版新生児心肺蘇生法ガイドラインの普及のための講習会推進事業（NCPR）

厚生労働省こども家庭総合研究事業「アウトカムを指標としベンチマーク手法を用いた質の高いケアを提供する“周産期母子医療センターネットワーク”の構築に関する研究（H16―子ども―032）（主任研究者藤村正哲）の分担研究班「小児科医・産科医・助産師・看護師向けの新生児心肺蘇生法の研修プログラムの作成と研修システムの構築とその効果に関する研究班（分担研究者田村正徳）」では，「すべての分娩に，新生児の初期蘇生ができるスタッフが少なくとも一人，新生児の責任者として従事する．」という体制を我が国で整備するための基礎的および実証的な研究[7]を行い，新生児心肺蘇生法を関係者に修得させるための教材の開発や実技講習会のプログラム作りをしてきた．また，今回のConsensus2005に基づく日本版新生児心肺蘇生法ガイドライン作成にも深く関わってきた．日本周産期・新生児医学会ではこれらの研究成果を踏まえて日本版新生児心肺蘇生法ガイドラインを全国の周産期医療関係者に普及させるための実技講習会事業を開始することにした．

日本版新生児心肺蘇生法ガイドラインの対象患者[4]

新生児は医学的には，出生28日以内の児を指すが，Consensus2005では，AHA2000ガイドラインに比較すると，小児と新生児の心肺蘇生法の違いがより大きくなった．そのため，小児病棟や小児救急外来での生後1か月未満の乳児の心肺蘇生の実施に於いては混乱が生じることが予想される．ガイドライン作成小委員会では，新生児と小児の細かな分類にこだわって心肺蘇生が手控えられたり開始が遅れる事態を回避するために，乳児の心肺蘇生に関しては以下のような実施ポリシーを提言した．

1）分娩室や新生児室やNICU入院中の（修正月齢1か月未満）児の蘇生は，新生児蘇生法に則って行う．

2）分娩施設外での新生児仮死を新生児専門職でない術者（救急隊員など）が蘇生する場合は小児蘇生法で行っても良い．

3）一般小児科病棟や小児ICUや小児科外来で出生28日以内の児の蘇生を小児蘇生法で行うか新生児蘇生法で行うかは，それぞれの施設のポリシーに従ったので良い．

日本版新生児心肺蘇生法ガイドラインの概要[4]

日本版新生児心肺蘇生法ガイドラインのアルゴリズム図に沿ってガイドラインを解説したい．このアルゴリズム図の特色は，出生直後の新生児の評価で，“羊水の胎便混濁”の有無によって初期対応の基本的な流れが異なってくることを一枚の流れ図の中で分かりやすく明示していることである．Consensus2005では，この部分は二枚の図で構成されており，NRPでも，日本の様な統合されたアルゴリズム図とはなっていない．

1．出生直後の児の状態の評価（図-評価A1，表2）

出生時に蘇生処置が必要かどうかを迅速に判定するためには，表2の4項目を評価し，すべて問題なければルーチンケアを行い，もし異常があれば蘇生処置を開始する．

2．ルーチンケア（保温，気道開通，羊水の拭き取り）

出生時に特に問題の無い児では，低体温防止に努めながら，気道を開通（気道確保の体位をとらせ，ガーゼで鼻をぬぐい，喘鳴など上気道閉塞の兆候があれば口・鼻を優しく吸引する）し，皮膚の羊水を拭き取ってから皮膚色を評価する．

3．蘇生の初期対応（図-処置MCと処置C1）

本アルゴリズム図の特色は，出生直後の児の評価で，“羊水の胎便混濁”の有無によって初期対応の基本的な流れが異なってくることを明確に示していることである．

A：羊水の胎便混濁がある場合の処置（第一目標：気道からの胎便の除去，図-処置MC）

羊水が胎便で混濁している場合は，基本的には自発呼吸を誘発させる前に気道からの胎便の除去を優先する．低体温防止のために，気道からの胎便の除去は出来るだけラジアントウオーマ下で行うことが望ましい．どこまで，積極的に気道吸引を行うかは，児に“活気がある（表3）”か否かによって決まる．

a）羊水に胎便が混じていて出生時に児に“活気がない”（無呼吸・呼吸抑制，筋緊張が弱い，心拍数が100/