RAPID COMMUNICATION

Assessment of Exhaled Gases in Ventilated Preterm Infants

P. HITKA1, M. ČERNÝ2, M. VÍZEK1, J. WILHELM2, P. ZOBAN3

1Department of Pathophysiology, 2Department of Medical Chemistry and Biochemistry, 3Department of Neonatology, Second Faculty of Medicine, Charles University, Prague, Czech Republic

Received January 20, 2004
Accepted March 3, 2004

Summary
Hydrogen peroxide (H2O2) production in exhaled air was measured in ventilated preterm newborns at 5, 24 and 48 hours after delivery, using originally designed method of exhaled breath condensate (EBC) collection. H2O2 production in expired gas was 812±34 pmol/20 min during the first measurement and then declined to 389±21 at 24 hours and 259±26 pmol/20 min at 48 hours.

Key words
Exhaled breath condensate • Hydrogen peroxide • Ventilated newborns

Although an enhancement of the production of reactive oxygen species (ROS) is believed to be a crucial component of the injurious process in the diseased lung (RDS, BPD), a reliable assessment of an oxidative load in the lungs of premature newborns is not available. Because of its position in the metabolic pathway of ROS, its relative stability and the fact that it can be detected in expired air, H2O2 could be a useful marker of immediate oxidative load in the lungs. Its concentration in exhaled air was already used as a noninvasive marker of ROS production in the respiratory tract in adults and children (Kharitonov and Barnes 2002). However, the attempt of Cheah et al. (2003) to measure exhaled H2O2 in neonates failed. In a similar attempt we adapted the technique of the collection of exhaled breath condensate (EBC) in small laboratory animals (Hitka et al. 2002) for ventilated preterm newborns.

Eleven preterm infants, including four infants (ELBW) with extremely low birth weight 803±69 g (mean ± S.E.M.) and five very low birth weight infants (VLBW, 1350±33 g), and three term newborns (3137±211 g) entered the study. However, the EBC was collected three times in five preterm babies only (1152±288 g, mean GA 29.2±2.4 wks), others were excluded for different reasons (in six infants was ventilation stopped before 20th hour after delivery, one was transferred to high frequency ventilation (HFV) 5 hours after the first collecting, two infants were excluded for cardio-pulmonary instability during first day). The Apgar scores assigned at the first minute were 3-8 points and reached 8-9 points at the fifth minute, while the babies were intubated and manually ventilated via an endotracheal tube. All babies were mechanically ventilated (Babylog 8000 plus) in the SIMV mode, flow, Pin, PEEP, MAP, f, and Fio2 were controlled. Routinely monitored parameters were VT, HR, ECG, RR, IBP, and
SaO₂ (see List of Abbreviations). The ventilation, blood gases and acid base balance were controlled before and after each sampling procedure. Four of five preterm infants studied, received artificial pulmonary surfactant (Survanta). First EBC samples were collected from infants after clinical stabilization, 3–9 hours after birth (mean 5 hours). The second and third collections of EBC were performed around 24 and 48 hours after birth. The ethical approval for this study was obtained from the institutional ethics committee.

The double valve outlet (AMBU Paedi-Ventil Mark) was connected to the endotracheal tube adapter (Fig. 1). This device directed expired gases into specially constructed glass chamber submerged in a salt/ice bath (13 g NaCl/100 g ice) with temperature around –15 °C. Pressure in the expiratory limb never exceeded 3 cm H₂O and was therefore below that set by PEEP. Volumes of condensate collected during 20 min were 40–370 µl, but in one newborn, the amount of EBC increased to 800 µl because of a leak in the valve system (and therefore H₂O₂ concentration in this EBC decreased about 5-fold).

Fig. 1. Scheme of breath condensate collection by a special cold trap and detail of double valve system.

The condensate was then kept in plastic tubes in –75 °C until the analysis (maximally for 1 week). H₂O₂ content in the inspired gas was measured by flowing the same volume of gas as that expired by the newborn through the condensation chamber. The H₂O₂ concentrations in EBC were measured by chemiluminescence (Wilhelm et al. 2003) with a detection limit of 2 nM. The mean amounts of H₂O₂ in an adequate volume of inspired gas condensate were 150±2 pmol for air and 140±2 pmol for oxygen. Production of H₂O₂ by individual newborn during 20 min was calculated as the difference between an amount of H₂O₂ in respective samples and its amount found in the same volume of condensate from given inspired gas.

ANOVA and nonlinear regression analysis were used for statistical evaluation of the data, p<0.05 was considered as significant.

The H₂O₂ production was measured altogether in 27 attempts in 14 babies. The values ranged from 88 pmol/20min to 4440 pmol/20min, the two highest values were found in an infant after surgery in the orofacial region. In 5 ventilated preterm newborns measured repeatedly during 48 hours after delivery the production of hydrogen peroxide decreased from 812.2±34.0 pmol/20 min to 388.6±20.8 and 258.6±25.9 pmol/20 min, respectively. In these infants, the production correlated with the time elapsed from the delivery (Fig. 2).

The hydrogen peroxide concentration in EBC of our ventilated babies was in the range reported by other authors for children and adults (for review see Kharitonov and Barnes 2002). Our method of EBC collection differed from that used by Cheah et al. (2003) in several aspects: a) we collected the EBC only from the air expired by the baby, b) we measured the H₂O₂ concentration in inhaled air in each trial, c) we used a lower temperature to condensate water vapor from the expired air, d) our method for H₂O₂ measurement was more sensitive, and e) we measured H₂O₂ production three times during the first two days, while they cumulated measurements during this period into a single value.
We believe that the first and last mentioned differences are the most important. The average minute ventilation of our babies was around 300 ml, which was less than 4% of the flow through the ventilator. This proportion illustrates why the collection of only the expired air of the infant is necessary. Lower temperature of our cooling bath could also be of some importance since the water vapor is in fact frozen in this case, which should prevent \( \text{H}_2\text{O}_2 \) degradation.

We preferred the assessment of the \( \text{H}_2\text{O}_2 \) production to the more usual measurement of its concentration in the condensate since the different concentrations could result from different levels of dilution of the \( \text{H}_2\text{O}_2 \) formed in the lung tissue due to changes in minute ventilation. Indeed, Schleiss et al. (2000) showed that the increase in expiratory flow caused a significant decrease in the concentration of hydrogen peroxide in exhaled air. However, when their data were recalculated as the production of \( \text{H}_2\text{O}_2 \), the differences due to flow were eliminated.

Our system affected the breathing of tested babies very slightly and transiently. We had to increase \( \text{F}_\text{IO}_2 \) in our newborns for up to 5 min after the beginning of collection by maximally 0.05 to avoid a change of \( \text{SaO}_2 \). Similar valve system had been used by Nycyk et al. (1998) who collected expired breath to measure pentane production by the lung of preterm infants.

Although we can present only preliminary results, the values of \( \text{H}_2\text{O}_2 \) production in each group were consistent and we believe that our study, like that of Cheah et al. (2003) may increase the effort to use analysis of EBC also in newborns. The non-invasive assessment of \( \text{H}_2\text{O}_2 \) production may provide useful information about ROS formation in the lungs and therefore help to induce and to control the appropriate antioxidative therapy.

A cause of high \( \text{H}_2\text{O}_2 \) production shortly after birth is not clear. A persisting effect of previous hypoxia or the increase in the lung tissue \( \text{PO}_2 \) during delivery could be responsible, since there are experiments showing that hypoxia as well as the transition from hypoxia to normoxia increased \( \text{H}_2\text{O}_2 \) production in rats (Hitka et al. 2003). However, other causes like activation of neutrophils during the delivery cannot be excluded. The fact that \( \text{H}_2\text{O}_2 \) concentration in exhaled breath of premature babies ventilated with slightly increased \( \text{F}_\text{IO}_2 \) (maximally up to 0.4) substantially decreased during 48 hours after delivery may also be of clinical significance. This decrease resulted either from a decrease in \( \text{H}_2\text{O}_2 \) formation or an increase of antioxidant capacity. The reduction of formation of hydrogen peroxide seems to be more likely because Vento et al. (2000) showed that antioxidant capacity did not increase with time in newborns ventilated with low \( \text{F}_\text{IO}_2 \). In fact, mean values of total antioxidant capacity decreased from the first day to the sixth day after birth.

It can be concluded that the system for the collection of EBC in ventilated premature infants has been developed. The pilot clinical study showed that in preterm babies, ventilated with \( \text{F}_\text{IO}_2 \leq 0.4 \), \( \text{H}_2\text{O}_2 \) production significantly decreased during 48 h after delivery.

Acknowledgements
This work was supported by grants IGA MZ CR NE6450-3, GACR 305/97/S070 and research project MŠMT 111300002.

List of Abbreviations
SIMV mode - synchronized intermittent mandatory ventilation 
\( \text{Pin} \) - inspirative pressure 
PEEP - positive end-expiratory pressure 
MAP - mean airway pressure 
\( f \) - frequency 
\( \text{F}_\text{IO}_2 \) - inspired oxygen fraction 
\( V_T \) - tidal volume 
HR - heart rate 
ECG - electrocardiography 
RR - respiratory rate 
IBP - invasive blood pressure 
\( \text{SaO}_2 \) - arterial oxygen saturation
References


Reprint requests
Patrik Hitka, M.D., Department of Pathophysiology, Charles University, Second Faculty of Medicine, Praha 5, Plzeňská 221, Czech Republic, e-mail: patrikhitka@hotmail.com