RAPID COMMUNICATION

Assessment of Exhaled Gases in Ventilated Preterm Infants

P. HITKA[1], M. ČERNÝ[3], M. VÍZEK[1], J. WILHELM[2], P. ZOBAN[3]

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

Summary

Hydrogen peroxide (H_2O_2) 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. H_2O_2 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.

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, H_2O_2 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 H_2O_2 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 F_IO_2 were controlled. Routinely monitored parameters were V_T, HR, ECG, RR, IBP, and

SaO$_2$ (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 H$_2$O 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 H$_2$O$_2$ concentration in this EBC decreased about 5-fold).
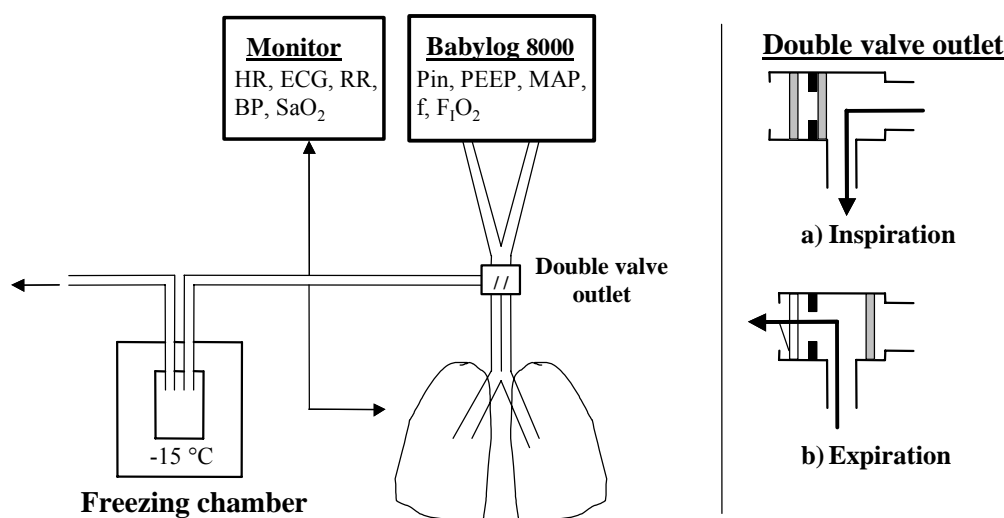

Fig. 1. Scheme of breath condensate collection by a special cold trap and detail of double valve system.

The condensate was then kept in plastic tubes in −75 °C until the analysis (maximally for 1 week). H$_2$O$_2$ 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 H$_2$O$_2$ concentrations in EBC were measured by chemiluminiscence (Wilhelm *et al.* 2003) with a detection limit of 2 nM. The mean amounts of H$_2$O$_2$ in an adequate volume of inspired gas condensate were 150±2 pmol for air and 140±2 pmol for oxygen. Production of H$_2$O$_2$ by individual newborn during 20 min was calculated as the difference between an amount of H$_2$O$_2$ 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 H$_2$O$_2$ 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 H$_2$O$_2$ 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 H$_2$O$_2$ measurement was more sensitive, and e) we measured H$_2$O$_2$ production three times during the first two days, while they cumulated measurements during this period into a single value.
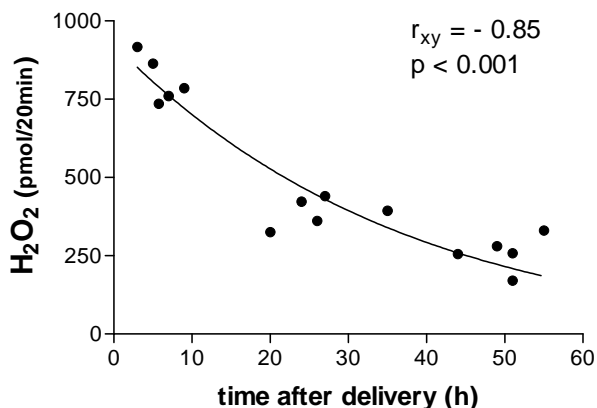
Fig. 2. Correlation between the time elapsed from delivery and H_2O_2 production in five ventilated premature babies.

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 H_2O_2 degradation.

We preferred the assessment of the H_2O_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 H_2O_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 H_2O_2, the differences due to flow were eliminated.

Our system affected the breathing of tested babies very slightly and transiently. We had to increase F_IO_2 in our newborns for up to 5 min after the beginning of collection by maximally 0.05 to avoid a change of 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 H_2O_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 H_2O_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 H_2O_2 production shortly after birth is not clear. A persisting effect of previous hypoxia or the increase in the lung tissue PO_2 during delivery could be responsible, since there are experiments showing that hypoxia as well as the transition from hypoxia to normoxia increased H_2O_2 production in rats (Hitka *et al.* 2003). However, other causes like activation of neutrophils during the delivery cannot be excluded. The fact that H_2O_2 concentration in exhaled breath of premature babies ventilated with slightly increased FiO_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 H_2O_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 F_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 $F_IO_2 \leq 0.4$, H_2O_2 production significantly decreased during 48 h after delivery.

Acknowledgements

List of Abbreviations

SIMV mode - synchronized intermittent mandatory ventilation
Pin - inspirative pressure
PEEP - positive end-expiratory pressure
MAP - mean airway pressure
f - frequency
F_IO_2 - inspired oxygen fraction
V_T - tidal volume
HR - heart rate
ECG - lectrocardiography
RR - respiratory rate
IBP - invasive blood pressure
SaO_2 - arterial oxygen saturation

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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