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Hydrogen peroxide in expired breath condensate of patients with acute respiratory failure and with ARDS

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Abstract. *Objective:* Measurement of hydrogen peroxide concentrations in breath condensate of mechanically ventilated patients with ARDS and with risk factors for developing ARDS.
Design: Open study in intensive care patients.
Setting: Intensive care units of the Clinics of the University of Goettingen, a primary care center.
Patients: 10 post-operatively ventilated patients as a control group and 26 patients with acute respiratory failure, 7 of them with ARDS, 12 with polytrauma, 4 with pneumonia, 3 with cardiogenic or nephrogenic pulmonary edema.
Interventions: None.
Measurements: Breath condensate was collected by a special cold trap and was analysed for H_2O_2 by a chemiluminescence method. Daily measurements were performed for 4.2±2.6 days (mean±SD) as soon as possible after manifestation of respiratory failure.
Results: Patients with acute respiratory failure exhibited higher H_2O_2 concentrations than control patients (median 95 nmol/l, range 76–144 nmol/l), with the highest median value found in the ARDS group (552 nmol/l, range 154–893). After clinical improvement, H_2O_2 concentrations decreased to the range of the control group.
Conclusion: Since high concentrations of H_2O_2 in breath condensate were only found in patients with ARDS or with risk factors for ARDS, the results add to the existing evidence that reactive oxygen species are associated with some acute lung diseases.

Key words: Hydrogen peroxide – Oxidant activity – Breath condensate – Respiratory failure – ARDS

In several types of acute lung disease, especially ARDS, polymorphonuclear leucocytes in the alveolar infiltrates release toxic oxygen metabolites [1–5]. These may reach the airways and can be detected in the expired breath, since they are not degraded there by catalase.

Baldwin et al. [6] and Sznajder et al. [7] first measured increased hydrogen peroxide concentrations in the breath condensate of mechanically ventilated intensive care patients, especially with ARDS.

This study was designed to determine hydrogen peroxide concentrations in the expired breath of patients with ARDS or with risk factors for developing ARDS. Furthermore, the prognostic significance of the course of H_2O_2 concentration and its suitability as a diagnostic mean was elucidated.

Materials and methods

Patients

The study was conducted according to the principles established in Helsinki. Hydrogen peroxide concentrations in the expired breath condensate were measured in 36 mechanically ventilated patients at the different intensive care units of the Clinics of the University of Goettingen (see Table 1). Of these patients 10 were ventilated post-operatively, had no signs of pulmonary disease and served as a control group. The other patients were included in the study if they met the following criteria for "ARDS" or for "risk factors": "ARDS" was supposed to exist if 5 criteria were fulfilled: acute development of respiratory failure; bilateral, diffuse alveolar infiltrates on chest X-ray; pulmonary edema not caused by heart failure; increased airway pressure on mechanical ventilation (plateau pressure minus PEEP was at least 25 mmHg), indicating decreased lung compliance; and a ratio of arterial to alveolar oxygen tension less than 0.2. The cause of ARDS in these 7 patients were: Pneumonia (3), eclampsia with DIC (1), DIC after shock due to severe intraoperative haemolysis (1), pulmonary edema caused by acute renal failure (2).

The patients with "risk factors" for developing ARDS were divided into the following groups: Polytrauma without chest trauma, including 2 patients with skull and brain trauma; polytrauma with chest trauma (lung contusion), including 5 patients with skull and brain trauma; pneumonia. Additionally, a group of patients with cardiogenic (2) or nephrogenic (1) pulmonary edema were studied.

The indices of mechanical ventilation were documented in all patients, as were body temperature, heart rate, arterial pressure and routine laboratory reports.

The breath condensate was collected once in the patients of the control group. In all other patients the first sample was taken as soon as possible (2.0±1.6 days) after the clinical manifestation of the respiratory failure. H_2O_2 was measured once per day for several successive days (4.2±2.6 days) up to extubation if possible.

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Table 1. Patient characteristics

	Control group ($n = 10$)	Polytrauma without chest trauma ($n = 4$)	Polytrauma with chest trauma ($n = 8$)	Pneumonia ($n = 4$)	Cardiogenic or nephrogenic pulmonary edema ($n = 3$)	ARDS ($n = 7$)
Sex (m/f)	5/5	1/3	6/2	4/0	3/0	3/4
Age (years)						
Mean ± SD	42 ± 15	25 ± 7	31 ± 12	58 ± 16	60 ± 8	50 ± 16
Range	22 – 64	15 – 31	18 – 48	40 – 76	51 – 66	28 – 76
Lethality (n)	0	0	1	0	0	5

Breath collection

Hydrogen peroxide in the expired breath was collected together with the water fraction by condensation in a special cold trap. In the bronchi and alveoli the air is saturated with water vapour by 100% (44 mg/l) and has a temperature of 37 °C [8, 9], independent of the humidity and temperature of the inspired gas mixture. At the end of the endotracheal tube the expired breath has already partly condensed and lost approximately 40% of its humidity and heat, the water content being only 27 mg/l and the temperature 29 °C [9]. Since H_2O_2 and H_2O have different vapour pressures, boiling and melting points, they do not condense at the same rate. Therefore it is indispensable to standardize the condensation conditions. Figure 1 outlines the sampling method used. A bypass tube with a diameter of 7 mm was put into the expiration tube and connected with a pump equipped with a gas flowmeter. This pump sucked a portion of the expired breath with a continuous flow of approximately 1.5 l/min constantly during in- and expiration phase through a special cold trap which was precooled to approximately −80 °C in a mixture of acetone and dry ice, according to data by Sakugawa and Kaplan [10] on condensation of H_2O_2 from atmospheric air. The expiration tube and the bypass tube up to the glass cold trap were heated to 40 °C with a heating tape for preventing the expired breath from condensing before reaching the cold trap. By connecting a second glass cold trap in series we assured the completeness of condensation, as the second cold trap always remained completely dry. Air contains varying amounts of H_2O_2 in the vapour phase [10]. To assure that all humidity and all H_2O_2 in the expired breath originated from the patients' lungs, ventilation during the 10 min breath collection period was performed with completely dry gas mixtures of oxygen and compressed air at the individually required FIO_2. The moisturized silicone ventilation tubes were replaced by new disposable PVC tubes during the sampling period.

Analytical procedure

Hydrogen peroxide was assayed by a chemiluminescence method previously described by Yoshizumi et al. [11]. Briefly, the sample solution (minimum volume 50 µl) and an equal volume of reagent solution consisting of 1 ml of luminol-NaOH (1.7 mg luminol in 1 l 0.069 M NaOH) and 99 ml of haemin-NaOH (4.5 mg haemin in 99 ml 0.069 M NaOH) are mixed in a reaction cell to undergo a chemiluminescence reaction. Luminol (5-amino-1,2,3,4-tetrahydrophthalazine-1,4-dione) is oxidized by H_2O_2 in the presence of haemin as a catalyst. The phthalic acid derivative formed emits light with a maximum at 462 nm. The intensity of light emission is directly proportional to the H_2O_2 concentration in the sample and was detected with a 6-Channel-Multi-Biolumat LB9505C from Berthold (Wildbad, Germany). The calibration curve was linear for concentrations of 200 nmol/l up to 1500 nmol/l ($r = 0.992$). The lower detection limit of the method was 70 nmol/l. The intraday variation was 4.5% ($n = 10$), interday variation between different calibration curves was 12% ($n = 10$). Standard addition tests yielded 10% – 15% lower values than calculated ($n = 5$). Samples were assayed immediately after collection, or the whole cold trap was stored frozen at −80 °C for a few days. The specificity of the assay was confirmed by pretreating representative samples with catalase, which resulted in a complete loss of detectable H_2O_2.

A cross-over test was made with the staff of a laboratory of the Institute of Bioclimatology of the University of Goettingen, where H_2O_2 was measured with a fluorescence method (p-hydroxyphenyl-acetic-acid) in the presence of horseradish peroxidase: The difference between measured concentrations of samples assayed with both methods was 2.2% – 11.6%, mean 5.7%, $n = 4$.

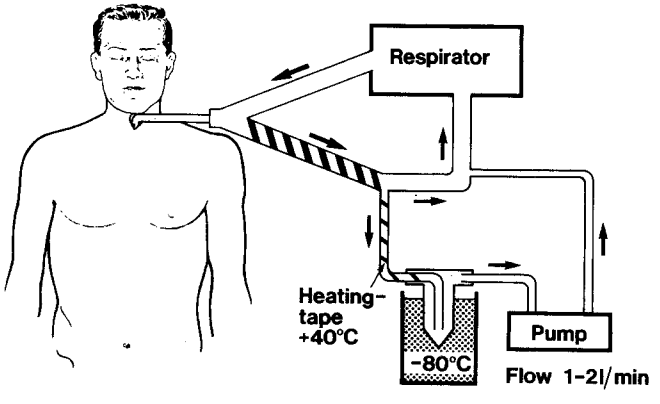

Fig. 1. Schematic diagram of breath condensate collection by a special glass cold trap

Data analysis

The Mann-Whitney U test was used to compare data of all patients between groups. $p < 0.05$ was considered significant.

Results

All patients were included in the study within 2.0 ± 1.6 days (range 0 – 7 days) after the beginning of mechanical ventilation. The 7 patients with ARDS were included within 1.4 ± 1.2 days (range 0 – 4 days) after the onset of ARDS. Table 1 shows the characteristics of the investigated patients. By chance, the sex ratio was different between groups. Mean age was different between groups, too, but typical for the underlying disease states.

In Table 2 the median values of maximum and minimum H_2O_2 concentrations in the groups are listed. In patients without acute lung disease (we investigated the 10 patients of the control group and 7 other intensive care patients not included in the study) we never measured H_2O_2 concentrations higher than 200 nmol/l breath condensate.

Patients with acute lung infiltrates, whether focal or diffuse, exhibited markedly higher values than control patients, with the highest median value (by 5 times higher than in the control group) found in the ARDS group ($p < 0.01$ for H_2O_2 of control group versus maximum H_2O_2 of all other patients; $n = 10$ vs 26). However, the differences between the groups with polytrauma, pneumonia, pulmonary edema and ARDS were not statistically significant.

Table 2. Patient data and results of H_2O_2 measurements and clinical parameters. H_2O_2 max = Maximum H_2O_2-concentrations in breath condensate; PaO_2: FIO_2 = PaO_2 divided by the inspired FIO_2 at the time of maximum H_2O_2; $V_t/(P_p - PEEP)$ = tidal volume (V_t) divided by the difference of plateau pressure (P_p) and PEEP as a parameter of the respiratory system compliance at the time of max. H_2O_2; no. of quadr. = number of quadrants with lung infiltrates or edema on the X-ray at the time of max. H_2O_2; H_2O_2 min = minimum concentrations of H_2O_2 at clinical recovery to a required $FIO_2 < 0.45$. Data are given as median values (range)

Group	*n*	H_2O_2 max [nmol/l]	PaO_2: FIO_2 [mmHg]	$V_t/(P_p - PEEP)$ [ml/cmH_2O]	No. of quadr. with infiltr.	*n*	H_2O_2 min [nmol/l]
Control group	10	95 (76–144)	438 (388–550)	66 (44–71)	0 –	0	–
Polytrauma, no chest trauma	4	233 (156–427)	273 (260–506)	51 (36–57)	0 (0–1)	4	110 (86–204)
Polytrauma, chest trauma	8	334 (158–1008)	255 (152–420)	45 (33–63)	1 (1–3)	7	128 (92–227)
Pneumonia	4	343 (241–862)	171 (150–303)	37 (30–50)	2.5 (1–4)	4	124 (95–250)
Pulmonary edema	3	368 (222–671)	146 (139–200)	43 (30–62)	4 (2–4)	3	170 (68–172)
ARDS	7	552 (154–893)	78 (38–129)	29 (22–40)	4 (2–4)	2	137 (104–170)

Figure 2 shows the individual values of maximum H_2O_2 concentration. The variation within groups is marked. The H_2O_2 concentrations in single patients did not correlate with clinical parameters such as FIO_2, PaO_2, severeness of infiltrates on the chest X-ray, airway pressure or laboratory parameters, e.g. number of leucocytes in blood (maximum H_2O_2 vs PaO_2: $r = -0.22$, $n = 33$; H_2O_2 vs FIO_2: $r = 0.013$, $n = 103$). In some cases ($n = 11$) with serial determination of H_2O_2 concentrations over several days a short lasting (24–48 h) peak developed, followed by a decrease over several days down to values near the lower detection limit. In Fig. 3 the course of H_2O_2 concentrations is plotted for all patients of the ARDS group. A decrease of H_2O_2 developed always if patients recovered but also in two cases in which the pulmonary gas exchange became desolate with increases in $PaCO_2$ followed by the death of the patient. Two other patients of those 5 who died had H_2O_2 concentrations higher than 500 nmol/l until a few hours before death, but in one case a patient died who never had concentrations above 400 nmol/l. In spite of exhibiting pulmonary infiltrates, some patients never had H_2O_2 values higher than 200 nmol/l (2 patients with chest trauma, 1 with ARDS). H_2O_2 median values were highest in the ARDS group, the group with lowest PaO_2, lowest lung compliance, maximum X-ray results and maximum lethality.

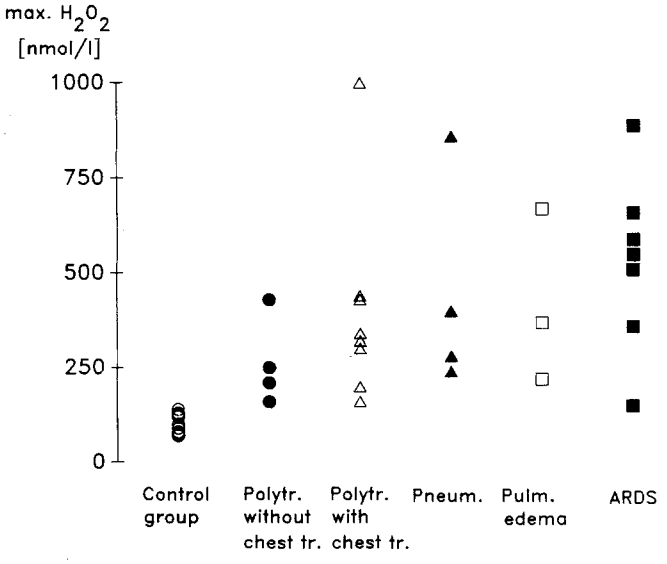

Fig. 2. Maximum H_2O_2 concentrations in the expired breath of mechanically ventilated patients. Individual values of all patients. *Polytr.*, Polytrauma; *tr.*, trauma; *Pneum.*, Pneumonia; *Pulm.*, Pulmonary

Discussion

The role of oxidants in acute lung injury has been examined in a variety of animal models (reviewed in [2]). In humans, indirect evidence such as the presence of α_1-proteinase inhibitor in the bronchoalveolar fluid [12] and of lipid hydroperoxides in pulmonary edema fluid [13] as well as direct detection of H_2O_2 in breath condensate [6, 7] and in this paper point to an elevated production of oxidants in the lung of ARDS patients. This is commonly ascribed to the margination of large quantities of neutrophils into the pulmonary interstitium and the alveolar space [14, 15].

Our findings as well as those of Baldwin et al. [6] and Sznajder et al. [7] do not point to the source of the H_2O_2 recovered in the breath condensate. Baldwin et al. [6] were the first to measure increased hydrogen peroxide lev-

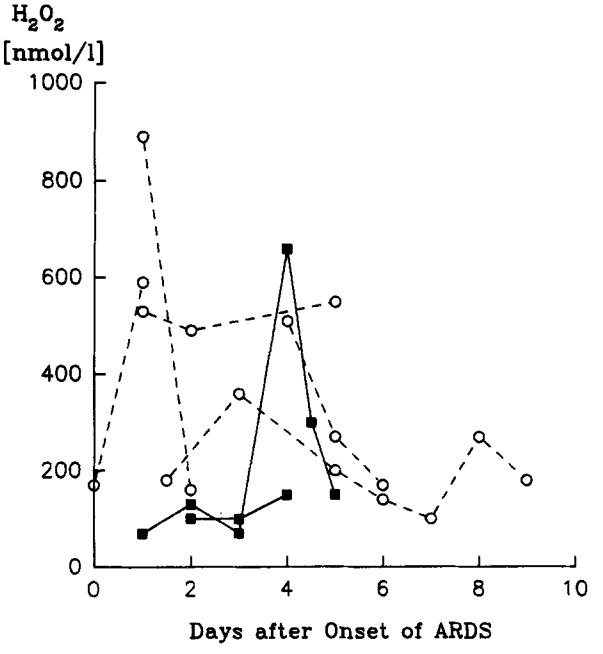

Fig. 3. Course of the H_2O_2 concentrations in all patients with ARDS. ○ – ○ 5 patients who died from ARDS; ■ – ■ 2 patients survived and could be extubated later

els in breath condensate of patients with ARDS, values being 5 times higher than in a heterogeneous group of patients with risk factors for developing ARDS, including some patients with focal pulmonary infiltrates due to pneumonia or lung contusion. The authors did not differentiate the group of non-ARDS patients into subgroups. It is therefore not known whether there was a difference in H_2O_2 concentrations between patients with focal pulmonary infiltrates and without any infiltrates. As the surviving patients showed a decrease of H_2O_2 with clinical improvement, Baldwin et al. suggested that measurement of breath condensate peroxide might serve as a diagnostic parameter in patients with acute lung disease. Unlike the results of Baldwin et al. [6], the recent findings of Sznajder et al. [7] showed high H_2O_2 concentrations in breath condensate of patients with ARDS as well as in patients with focal pulmonary infiltrates and in patients with severe brain injury without pulmonary infiltrates. Patients with different types of lung disease such as lung contusion, pneumonia and pulmonary edema showed similar values of H_2O_2 in our study while patients with ARDS exhibited higher values. The difference, however, was not statistically significant. Larger numbers of patients may provide a means of differentiation between groups. This is suggested by the observation that a correlation is found between the rank order of peak H_2O_2 concentration, degree of infiltration and arterial oxygen pressure between groups (Table 2). However, no correlation does exist between H_2O_2 concentration and clinical parameters for individual patients.

The variation of H_2O_2 breath concentrations within groups was similar as in the studies of Baldwin et al. [6] and Sznajder et al. [7] and may be caused by the pathophysiology of H_2O_2 generation and detoxification itself: the antioxidant activity in the extracellular lining fluid may differ as well as the level of enzyme activity (e. g. catalase, glutathione peroxidase) in the alveolar infiltrates. In infiltrated lung sections, inequalities of ventilation and perfusion may decrease the efficiency of H_2O_2 exchange. Under such conditions H_2O_2, though possibly released by neutrophils, may not reach the airways and the breath condensate. As the ventilation-perfusion ratio may vary between and within patients, a large variation of H_2O_2 concentrations may be inevitable. In patients with alveolar high-protein edema in both lungs H_2O_2 concentrations may decrease concomitantly with the decrease of PaO_2 and the increase of $PaCO_2$ since exchange of gases and possibly H_2O_2 is extremely impaired (2 ARDS patients of this study). Additionally, in cases with focal infiltrates H_2O_2 may be diluted by the water vapour of the unaffected lung parts. From these reasons H_2O_2 in breath condensate cannot be considered as a quantitative parameter of lung injury.

Its prognostic potential is low since increasing H_2O_2 expiration does not necessarily indicate the onset of ARDS but may coincide with the fully developed disease and since decrease of H_2O_2 expiration does not necessarily indicate amelioration of lung injury (Fig. 3). Our results do not provide evidence whether the increased H_2O_2 formation is a primary event during the course of lung injury or an epiphenomenon accompanying manifest lung injury. Whether primary or secondary in nature, H_2O_2 production must be assumed to contribute to the lung damage. This may give rise to the development of antioxidant therapy strategies in the future.

In conclusion, our results demonstrate increased H_2O_2 expiration in various types of infiltrative lung disease, most markedly in ARDS. This adds to the evidence for a role of toxic oxygen species in human lung disease independent of the aetiology and of the individual prognosis.

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References

1. Simon RH, Ward PA (1988) Adult respiratory distress syndrome. In: Gallin JI, Goldstein IM, Snyderman R (eds) Inflammation: basic principles and clinical correlates. Raven Press, New York, pp 815–827
2. Junod AF (1989) Oxygen free radicals and lungs. Intensive Care Med 15:S21–S23
3. Demling RH (1990) Current concepts on the adult respiratory distress syndrome. Circ Shock 30:297–309
4. Wiener-Kronish JP, Gropper MA, Matthay MA (1990) The adult respiratory distress syndrome: definition and prognosis, pathogenesis and treatment. Br J Anaesth 65:107–129
5. Tate RM, Repine JE (1983) Neutrophils and the adult respiratory distress syndrome. Am Rev Respir Dis 128:552–559
6. Baldwin SR, Simon RH, Grumm CM, Ketai LH, Boxer LA, Devall LJ (1986) Oxidant activity in expired breath of patients with adult respiratory distress syndrome. Lancet I:11–14
7. Sznajder JI, Fraiman A, Hall JB, Sanders W, Schmidt G, Crawford G, Nahum A, Factor P, Wood LDH (1989) Increased hydrogen peroxidase in the expired breath of patients with acute hypoxemic respiratory failure. Chest 96:606–612
8. Ingelstedt S (1956) Studies of the conditioning of air in the respiratory tract. Acta Otolaryngol [Suppl]131:1–81
9. Dery R (1973) The evolution of heat and moisture in the respiratory tract during anaesthesia with a non-rebreathing system. Can Anaesth Soc J 20:296–306
10. Sakugawa H, Kaplan IR (1987) Atmospheric H_2O_2 measurement: comparison of cold trap method with impinger bubbling method. Atmospheric Environ 21:1791–1798
11. Yoshizumi K, Aoki K, Nouchi I, Okita T, Kobayashi T, Kamakura S, Tajima M (1984) Measurements of the concentration in rainwater and of Henry's law constant of hydrogen peroxide. Atmospheric Environ 18:395–401
12. Cochrane CG, Spragg RG, Revak SD, Cohen AB, McGuire WW (1983) The presence of oxidation activity in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. Am Rev Respir Dis 127:S25–S27
13. Cross CF, Forte T, Stocker R, Lowie S, Yamamoto Y, Ames BN, Frei B (1989) Oxidative stress and abnormal cholesterol metabolism in patients with adult respiratory distress syndrome. J Lab Clin Med 115:396–404
14. Weiland JE, Davis WB, Holter JF, Mohammed JR, Dorinsky PM, Gadek JE (1986) Lung neutrophils in the adult respiratory distress syndrome: clinical and pathophysiological significance. Am Rev Respir Dis 133:218–225
15. Thommasen H, Russel A, Boyho W (1984) Transient leukopenia associated with adult respiratory distress syndrome. Lancet I:809–812

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