

Microvascular Lung Tissue Oxygenation-A Methodological Study in the Pig

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Abstract

The objective of the present study was to investigate the possibility of measuring lung tissue oxygen pressure (P_{tO_2}) distributions at the microvascular level, and also if a change in the lung tissue oxygenation could be detected during hypoventilation (50% reduction in ventilatory settings). Experiments were carried out on eight mechanically ventilated ketamine-anaesthetised pigs. A thoracotomy was performed through the third right intercostal space. P_{tO_2} measurements were made using a Clark-type multiwire microelectrode placed onto the pleural surface of the middle lobe. P_{tO_2} was measured during normoventilation, hypoventilation (3 minutes) (reduction of respiratory volume/minute and frequency by 50%), and a second period of normoventilation. Baseline P_{tO_2} was 5.8 (range 4.4 to 10.3) kPa and decreased to 2.9 (range 1.6 to 4.2) kPa during hypoventilation, associated with some P_{tO_2} values close to zero. The P_{tO_2} increased to 5.4 (range 3.4 to 8.4) kPa during the second normoventilatory period, some values still close to zero. This study demonstrates that lung tissue PO_2 registrations can be made in a suitable animal (pig) model, and that hypoventilation induced an almost reversible decrease in lung tissue oxygen pressure distributions. In addition, no microscopically visible tissue damage was inflicted by the electrode on the underlying lung surface.

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Introduction

Although the lung is mostly referred to as a respiratory organ it also exhibits a variety of metabolic functions, e.g. uptake, storage and/or synthesis and metabolism of compounds as different as biogenic amines,^{1,2} prostaglandins³ and angiotensin I.⁴ In spite of this vast metabolic capacity and the respiratory role of the lung, the oxygenation of lung tissue itself has been less studied, *vide infra*.

The tissue oxygen tension in a given organ varies with location and time. The use of a Clark-type surface multiwire microelectrode⁵ takes this heterogeneity into consideration, and makes it possible to investigate local tissue oxygen pressure at the microvascular level. This type of oxygen electrode has been used to investigate the tissue oxygenation in several organs such as the liver, skeletal muscle, gut, brain,⁶⁻¹⁰ as well as in a constantly moving organ, i.e. the heart.¹¹ Few studies on lung tissue oxygenation have hitherto been published.^{12,13}

The present study was undertaken to explore if a

porcine model could be used to investigate lung tissue oxygen pressure (P_{tO_2}) at the microcirculatory level; the pig being more related to the human, from an anatomical and physiological point of view, than the dog or rabbit.^{12,13} It was also of interest to investigate if any change in P_{tO_2} distributions could be seen during reduction in arterial O_2 saturation by hypoventilatory provocation, and if the use of the Clark-type electrode induced any microscopically visible lung surface damage.

Materials and Methods

Animal Preparation

Technically successful observations were carried out on eight Swedish land-race pigs, weighing 21 ± 1 kg. The experimental protocol was approved by the appropriate Animal Ethical Committee (Umeå, Sweden, no. A137/94). The conduct of the investigation conformed with the "Guide for the Care and Use of Laboratory Animals (NIH Publication # 85 - 23, Bethesda, MD, USA, revised 1985). Animals were housed, one or two per pen, in an accredited animal facility that provided environmental

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temperature of $20 \pm 2^\circ\text{C}$ and artificial illumination with a photoperiod of 12 hours light/12 hours darkness. Animals were allowed to accommodate to the facilities for at least three days prior to experiments. They were fed a standard diet and had free access to tap water. The animals were premedicated with intramuscular ketamine, 500 mg (Ketalar®, Parke Davis & Co, Pontypool, Gwent, Great Britain), atropine, 0.5 mg (Kabi Vitrum, Stockholm, Sweden) and diazepam (Stesolid Novum® 5 mg, Dumex, Denmark). Anaesthesia was induced with pentobarbital sodium 15 mg/kg (Apoteksbolaget, Umeå, Sweden) in one marginal ear vein and thereafter maintained with a constant infusion of ketamine (Ketalar®) 60 mg/kg/h using an infusion pump. The animals were tracheotomised or intubated orally and ventilated on room air with a Servo ventilator 900 B (Siemens-Elema, Sweden). Through a cervical cut down, a catheter was introduced into the right common carotid artery for blood sampling. Blood gases, haemoglobin and haematocrit were measured (six animals) (BGE analyser and CO-Oximeter 482, Instrumentation Laboratory Srl, Milano, Italy). The left jugular vein was catheterised for continuous infusion of anaesthetics and fluid administration (ringer acetate, 10 ml/kg/h, Kabi Pharmacia, Sweden). A pulse oximeter (OxiCap 4700, BOC Ohmeda, Västra Frölunda, Sweden) was used to record the pulse frequency. The probe was placed either on one of the ears or the tongue. With the animal on its left side, a thoracotomy was performed through the third right intercostal space using standard surgical techniques. An electrode holder was carefully sutured onto the pleural surface of the middle lobe, using 6/0 monofilament (Ethilon®, Ethicon, Norderstedt, Germany). After completion of the experiments, animals were killed with an overdose of intravenous pentobarbital.

Lung Tissue Oxygen Pressure Recordings

For lung tissue oxygen pressure (P_{tO_2}) measurement (eight animals), the eight-channel Clark-type surface oxygen microelectrode (Mehrdracht Oberfläche, Eschweiler, Kiel, Germany) was used.^{5,7-9,14} This electrode consists of eight platinum wires (0.15 µm each) embedded in a glass cylinder. The electrode was covered with an inner cellophane membrane (12 µm) soaked in 0.20 M KCl and with an outer teflon membrane (12 µm). The multiwire electrode was calibrated at 0% O_2 (zero solution, Radiometer, Copenhagen, Denmark) and at 21% O_2 (air saturated distilled water, at 37°C). The electrode values were corrected for drift, temperature and atmospheric pressure. The electrode was placed in the holder sutured to the lung surface. The connecting wire was long enough to allow the holder and the electrode to follow the respiratory movements without dislocation of the electrode or compression of the lung surface. The electrode was rotated slightly 15 times in 10 seconds intervals. In this way 120 oxygen

pressure values were collected for each measuring situation. Total sampling time for each P_{tO_2} measurement was approximately 2.5 min. A highly shielded high-impedance measuring system for rapid measurement of low currents was used. The analog-to-digital converter has a 14-bit resolution, and the eight channels could be sampled at maximum 24 Hz frequency. It was interfaced to an IBM-AT compatible PC computer (OMtech 386) running at 8 MHz with a mathematical coprocessor. A computer program for on-line measurements was used. Computation was performed after correction for drift, atmospheric pressure and local tissue temperature.¹⁵

Experimental Design

First a 10 min period of normoventilation (normoventilation 1) was carried out at the end of which a P_{tO_2} measurement was performed. Ventilation was set at 5.5 to 6.0 liters/min with a frequency of 28/min. Thereafter hypoventilation was induced by reducing the ventilator settings by 50%. After 3 min of hypoventilation a new P_{tO_2} measurement was made. A second period of normoventilation (normoventilation 2) followed (reversing the ventilator settings) in which the P_{tO_2} recording was performed after 5 min. Arterial samples for blood gas analyses were drawn immediately after the P_{tO_2} measurements.

Morphologic Study

At the end of the experiments, a lung biopsy was taken from the area underlying the electrode and from a randomly chosen pulmonary region for comparison. The specimens were immersion-fixed in 4% formalin, dehydrated in a graded series of ethanols and embedded in Histowax® (Histolab Products, Göteborg, Sweden). Sagittal sections (6 µm) were cut on a microtome (HM 350, Microm, Heidelberg, Germany), mounted on object slides, counterstained with haematoxylin-eosin and cover-slipped with Entellan® Neu (E. Merck, Darmstadt, Germany). All slides were coded and examined in a light-field microscope (Microphot - FXA®, Nikon Corp, Tokyo, Japan).

Statistics

For statistical evaluation the nonparametric Friedman test and Wilcoxon signed rank test were used.¹⁶ Statistical significance was considered at a $P < 0.05$ level.

Results

It was possible to suture the electrode holder onto the lung surface without inflicting any visible gross tissue damage. Once the electrode had been placed in the holder, respiratory movements of the thorax and lungs did not interfere with the actual P_{tO_2} recordings. The slight rotation of the electrode could also be carried out without difficulties.

TABLE I: ARTERIAL BLOOD GAS PARAMETERS, HAEMOGLOBIN CONTENT, OXYGEN SATURATION AND HEART RATE DURING THE FIRST PERIOD OF NORMOVENTILATION 1, HYPOVENTILATION AND THE SECOND PERIOD OF NORMOVENTILATION 2

	Normoventilation 1	Hypoventilation	Normoventilation 2
PO ₂ (kPa)	7.9 ± 0.6	4.4 ± 0.6*	7.7 ± 1.1*
PCO ₂ (kPa)	5.3 ± 0.7	7.5 ± 0.7"	5.4 ± 0.6*
pH	7.48 ± 0.03	7.36 ± 0.03*	7.47 ± 0.03"
Haematocrit (%)	32 ± 3	33 ± 3	31 ± 3
Haemoglobin (g/l)	99 ± 7	101 ± 8	99 ± 8
O ₂ saturation (%)	87.3	41.7*	84.6*
Heart rate	114 ± 14	118 ± 14	114 ± 12

Values are given as mean ± SD (n = 6)

*P < 0.05 compared to the preceding period

Blood Gas Analysis

Arterial blood gas values are presented in Table I (n = 6). During hypoventilation a reduction in arterial PO₂, oxygen saturation and pH was seen (P < 0.05) while PCO₂ increased (P < 0.05), compared to baseline. These parameters were reversed during the second period of normoventilation.

P_tO₂

The P_tO₂ histograms from all animals are shown in Figure 1 and numerical data are given in Table II. The P_tO₂ was 5.8 (4.4 to 10.3) kPa [median (25% to 75% percentiles)] (n = 960) during normoventilation 1, decreased to 2.9 (1.6 to 4.2) kPa (n = 928) (P < 0.001) during hypoventilation associated with some values close to zero, and increased again to 5.4 (3.6 to 8.4) kPa (n = 960) (P < 0.001; P < 0.001 compared to normo-ventilation 1) during normoventilation 2 with some prevalence of values close to zero.

Morphology

In all specimens a 4 to 8 µm thick layer of pulmonary tissue was observed between the surface of the lung and the most superficial alveolae. It consisted of collagenous tissue containing fibroblasts, scarce macrophages and

several layers of elastic fibers and was covered with a layer of mesothelial cells. No indications of tissue damage such as accumulation of leukocytes or disruption of the vascular or the alveolar walls were seen, neither in the specimens taken from the area underlying the electrode, nor in the control specimens. The findings were consistent in all animals.

Discussion

The lung is a metabolically active organ involved in e.g. uptake and degradation of biogenic amines such as serotonin, metabolism of angiotensin and bradykinin and metabolism and/or synthesis of prostanoids.¹⁻⁴ The role of the lungs in body oxygenation is self-evident, but in spite of its variety of metabolic functions, little is known about the oxygenation of the lung tissue itself.

This study demonstrates that lung tissue oxygen pressure measurements at the microvascular level, using the Clark-type multiwire microelectrode, can be carried out in a suitable animal model. Provocation by hypoventilation induced a left shift of the P_tO₂ histograms caused by the reduction in lung tissue oxygenation. No histologically demonstrable tissue damage was inflicted by the electrode.

P_tO₂ fields were measured with the Clark-type oxygen microelectrode. Each platinum wire measures the oxygen tension in a hemispherical tissue volume, with an approximate radius of 20 µm,¹¹ located directly below each wire. It was demonstrated microscopically, that the subpleural lung tissue in the pig has a thickness of 4 to 8 µm. This catchment area corresponds to the diameter of one to a few capillaries, and may also include organ cells and parts of arterioles and venules, thus describing a composite picture of PO₂ of the pleural surface layer, interstitial lung tissue and perhaps also to some extent alveolae. However, the fact that very few P_tO₂ values were above 16 kPa (Fig. 1) suggests that the major part of the P_tO₂ recordings was from the lung tissue, and not from room or alveolar air. The left shift of the histogram during hypoventilation also suggests recording of tissue

TABLE II: LUNG TISSUE OXYGEN PRESSURE (P_tO₂) (kPa) DURING THE FIRST PERIOD OF NORMOVENTILATION 1 (n = 960, 8 ANIMALS), HYPOVENTILATION (n = 928, 8 ANIMALS) AND THE SECOND PERIOD OF NORMOVENTILATION 2 (n = 960, 8 ANIMALS)

	Normoventilation 1	Hypoventilation	Normoventilation 2
Median	5.8	2.9***	5.4***†
Mean	7.2	3.0	6.3
25%	4.4	1.6	3.6
75%	10.3	4.2	8.4
Standard deviation	3.9	3.0	3.8

*** P < 0.001 compared to preceding state;

† P < 0.001 compared to Normoventilation 1

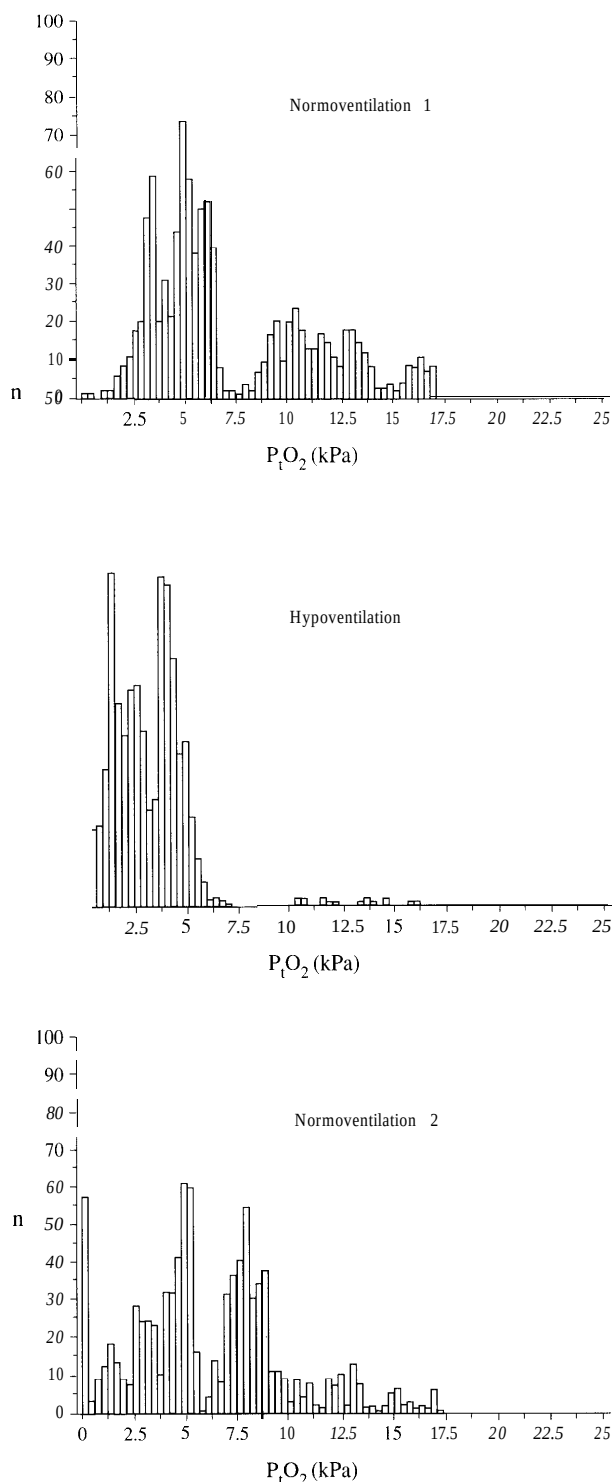


Fig. 1. Summarised lung tissue oxygen pressure (P_{tO_2}) distributions (P_{tO_2} , intervals of 0.3 kPa) of 8 animals during the first period of normoventilation (upper diagram, $n = 960$), hypoventilation (middle histogram, $n = 928$) and the second period of normoventilation (lower histogram, $n = 960$).

oxygenation, since it can be presumed that values recorded from air would still be in the range of 16 kPa, even if the ventilatory settings were reduced by 50%.

The distribution shape of the P_{tO_2} histogram during

baseline (normoventilation) was found to have a double-peak form. This pattern of P_{tO_2} distribution may reflect two different levels of oxygen needs and/or the difference between alveolar space and interstitial tissue. On the other hand, it may represent a two compartment phenomenon of microvascular blood flow in the lung tissue (arterioles versus capillaries and venules). During hypoventilation a left-shift of the histogram was found, associated with some values close to zero. With normoventilation again, the P_{tO_2} histogram was almost reversed, although still with some values close to zero. Even though the arterial PO_2 and oxygen saturation were reversed, a complete reversal of the P_{tO_2} was not found. A longer observation period of normoventilation 2 may well have resulted in a complete reversal.

Optimal arterial PO_2 (> 12 kPa) and arterial O_2 saturation (>93%) were not found in either normoventilatory period. An open thoratotomy with the animals lying on one side, may reduce these parameters. Furthermore, we believe that breathing room air is a more realistic model simulating the situation prior to resuscitation in a hospital setting. Therefore, additional oxygen breathing was not used in this study.

In summary, it was demonstrated that the Clark-type electrode can be used for measurement of lung tissue oxygenation in the pig. This animal is, for anatomical and physiological reasons, more suitable in studies simulating the human situation than other animals (e.g. rabbits or dogs) previously used for similar investigations. Respiratory movements of the lungs and thorax did not interfere with the recordings. No histologically demonstrable lung tissue damage was caused by the electrode.

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