

Oxygen Increases Lung Inflammatory Response in Spontaneous One-Lung Ventilation in Rabbits: A Prospective Randomized Experimental Study

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Abstract

Study objective: The purpose of this study was to investigate if oxygen supplementation would increase lung inflammatory response in a spontaneous one-lung ventilation animal model, when compared to room-air oxygen fraction.

Design: *In vivo* prospective randomized animal study

Setting: University research laboratory

Subjects: New Zealand rabbits

Interventions: Rabbits (n=20) were randomly assigned to 2 groups (n=10 each group). Groups (OS – Oxygen Supplemented, and NOS – Non-Oxygen Supplemented) were submitted to spontaneous One-Lung Ventilation (OLV) during 60 minutes; OS group had a 2-liter/minute oxygen supplement, and NOS group was kept on room-air. Ketamine/xylazine was administered for induction and maintenance of anesthesia. One-lung ventilation was achieved by administration of air into interpleural space, and left lung collapse was visually confirmed through the center of diaphragm. Clinical monitoring and arterial blood gas analyses were performed in all rabbits.

Measurements: Lung histology plates were observed under light microscopy for quantification of inflammatory response (light, moderate and severe).

Main results: All subjects had at least light inflammatory response. However, rabbits submitted to oxygen supplementation had a statistically significant value for the occurrence of moderate inflammation (p<0.001). The inflammatory cells found were mainly eosinophils and neutrophils in an average proportion of 80/20. Oxygen partial pressure increased in both groups with a higher proportion in OS group (p<0.001).

Conclusion: In this spontaneous OLV model, the use of oxygen supplementation was associated with a greater inflammatory response.

Keywords: Oxygen supplement; Lung inflammatory response; Spontaneous one-lung ventilation

Introduction

The complex relations and interactions of volutrauma (overdistension), barotrauma (increased pulmonary pressures), atelectotrauma (cyclic opening and closing of alveoli) and biotrauma (inflammatory mediators) play an important role on development of ventilator induced lung injury (VILI) [1,2]. Mechanical ventilation can influence the extent and course of perioperative well-being [3,4]. Even healthy individuals when submitted to mechanical ventilation are prone to develop lung injuries [5]. Recent studies suggest that even protective ventilatory strategies may induce subclinical ventilation lung injury in previous healthy lungs, by initiation of subclinical VILI, and possibly by sensitizing the lung to a second hit [4].

Using near physiological tidal volumes, approximately 6 ml/kg as standard mammalian spontaneous tidal volumes strategy [6,7], may imply some atelectasis formation. This condition will be present due to local alveolar collapse, with a subsequent formation of shunt and hypoxia. Moreover, an augmented inspired oxygen fraction will commonly be used to mitigate this condition, which will generate hyperoxia in aerated lung. Hypoxia can generate inflammation [8] and hyperoxia can worsen atelectasis and lead to excess of reactive oxygen species, all worsening acute lung injury (ALI) [6,9,10]

In the one-lung ventilation (OLV) situations, tidal volumes < 6 ml/kg, positive end-expiratory pressure (PEEP) between 5 and 10 cm H₂O, and the lowest possible oxygen inspired fraction (FiO₂) to maintain peripheral oxygen arterial saturation over 90 %, are the main features for a protective ventilatory strategy [11]. The lowest FiO₂ possible to provide adequate peripheral saturation has been accepted as the best option to reduce oxidative damage and to prevent absorption atelectasis [11-13]. Nevertheless, spontaneous OLV, on its own, has been observed as a condition in which inflammation is more present when compared to a spontaneous two-lung ventilation mode [14].

The objective of this study was to evaluate the contribution of oxygen

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supplementation to the occurrence of acute inflammatory response in both lungs, in an OLV model using a spontaneous breathing mode. In this regard, the study hypothesis is that oxygen will not influence the occurrence of inflammation in this model, since OLV is present, but no controlled ventilation is applied.

Methods

Animals

The experimental protocol used in this study was approved by appropriate local ethic committee ("Comité de Ética do ICBAS/UP"), and was done according to the European Union Directive nº 63/2010/EU. Twenty adult New Zealand rabbits were purchased from a Portuguese breeder (NORLAP - Rui M.S. Gonçalo, 4825-466 Água-Longa, Portugal) and kept under standard housing conditions with unrestricted access to food and water, with attendance by veterinary doctors and daily inspection. Study inclusion criteria for rabbits were based on general well being status, namely normal food and water ingestion, with absence of self-mutilation and weigh loss.

Study groups

This study was conducted as a prospective randomized animal experiment; two groups were studied, with 10 rabbits each, on spontaneous one-lung ventilation. Group 1 had oxygen supplementation (OS) with a fresh gas flow mixture on air of 2 liters/minute with a Jackson-Rees system; Group 2 had no oxygen supplementation (NOS) to the breathing system. Both groups had a 60-minute length of procedure.

Instrumentation

The rabbits were anesthetized with intramuscular ketamine 50 mg/kg (Imalgene®, Merial Laboratorios, S.A, Spain) and xylazine 4 mg/kg (Rompum®, Bayer, Leverkusen, Germany). Anesthesia maintenance was attained with half the initial dose of ketamine and xylazine every 20 minutes. The anterior region of the neck and the abdomen were shaved, and a 2 cm vertical midline incision being made through the anterior cervical region; after exposing the trachea, an incision was made to allow the introduction of a 2.5 tracheal tube to be connected to a spontaneous breathing system (Jackson-Rees type). In all rabbits, a 4 cm vertical abdominal incision was performed in order to allow aortic blood samples collection and visualization of the left inferior face of the diaphragm; once the diaphragm was visualized 10 milliliters of air was administered thru its left half. Immediately after this, left lung recoil was observed thru the medial tendinous center of the diaphragm. This fact was consistently confirmed in all rabbits, by direct visualization, which also allowed a visualization of the spontaneous and normal ventilation of the right lung [14]. At the end of procedure all rabbits were euthanized with ketamine (100 mg/kg) and xylazine (8 mg/kg), followed by section of abdominal aorta for exsanguination.

Monitoring

During the procedure non-invasive monitoring was used to monitor heart rate (HR) and oxygen peripheral arterial saturation (SpO₂) (Nellcor Oximax N600X®, Tyco Healthcare group LP, Nellcor Puritan Bennett Division, Pleasanton, CA 94588, USA); respiratory rate (RR) was monitored by direct observation. Measurements were obtained immediately after setup and every 15 minutes.

Blood samples were obtained directly from aortic puncture, five minutes after set up and immediately before the end of procedure, and analyzed for arterial oxygen (PaO₂) and carbon dioxide (PaCO₂) partial pressures, pH, Bicarbonate (HCO₃⁻) and peripheral arterial saturation

with oxygen (SatO₂) (Radiometer ABL 90flex®, Radiometer medical APS, akandevvej 21, 2700 Bronshoj, Denmark).

Tissue samples

The rabbits' lungs were harvested and fixed in 10% formaldehyde and embedded in paraffin for a Light Microscopy (LM) study. Three micra sections from the left and right lungs (superior, dorsal medial, ventral medial and lower aspects respectively) were obtained from all rabbits. Lung sections were stained in haematoxylin and eosin. A total of 8 plates were collected for each rabbit.

Histopathology

An arbitrarily defined four level inflammatory score was assigned to the lungs depending on the intensity of inflammatory infiltrates in each lung plate: no inflammation, light, moderate and severe inflammation.

Infrequent inflammatory cells and/or inflammation confined to a few areas (corresponding to less than 15 cells per high magnification field of 40×) [15] were defined as a light inflammatory response. Multiple areas in the tissue or a large area of inflammatory cells (corresponding to an average of 16 to 25 cells per high magnification field 40×) [15] were defined as a moderate inflammatory response. Large multifocal areas of tissue with inflammatory cells or almost all areas of tissue affected (corresponding to more than 25 cells per high magnification field 40×) [15] were defined as severe inflammation. Thus, plates were classified as light, moderate or severe inflammatory responders according to the intensity of inflammation assessed.

Staff investigators who collected data during the procedure were aware of the group assignments. An independent and experienced pathologist (unaware of which subject was being evaluated) classified all plates accordingly to the criteria set.

The plates were classified as light, moderate or severe inflammatory responders, according to the intensity of inflammation encountered

Statistical analysis

The Resource Equation method [16] was used to determine if the sample size was appropriate for the experiment, considering a laboratory animal study with a controlled set-up. A sample size of 10 rabbits per group with a total of 2 groups (n=20) were considered adequate for the questions being asked, resulting in an adequate error component (df) between 10 and 20. Kruskal-Wallis non-parametric test was used to compare variables between groups. Friedman test (Nonparametric two-way ANOVA) was used to compare variations within groups. Pearson's chi squared test was used to test for independence in inflammation. The statistical hypothesis is that the occurrence of moderate inflammation is independent of the technique (OS or NOS); the results presented by Machado et al. [14] were considered for the effect of OLV, to estimate the expected proportion of plates with moderate inflammation. A p-value<0.05 was considered statically significant. The IBM SPSS ® 21 and Microsoft Excel 2013 were used for all calculations. Data is presented with median and range variables.

Results

The results founded were accepted as valid, and only light or moderate inflammatory patterns were found. No severe inflammatory response was seen in any of the groups.

The median rabbit weight was 2209 g (1540-2670; Standard Deviation [SD] 241,4), the rabbits were randomly assigned to each group.

The median values and range of non-invasive monitored parameters are shown in Table 1. The first recorded values of HR, RR and SpO₂ were statistically different between Group 1 and Group 2 (p<0.05). The initial rectal temperature is not statistically different between groups. During the procedure SpO₂ significantly increased in both groups (p<0.05) by 12.4%. Temperature also statistically decreased in both groups (p<0.05) by 2.6%. HR only increased in Group 1 by 4.9% (p<0.05).

Arterial blood gas values are shown in Figure 1. The pO₂ and SatO₂ were different between groups (p<0.001) on first sample. At the end of the procedure the median pO₂ increased by 116% in Group 1, and by 65% in Group 2, this change was significantly different (p<0.001). Sat O₂ did not change (between first and final samples) for Group 1, but it increased significantly by 15.7% in Group 2. No significant differences were observed either within the groups or between groups regarding initial and final measurements of pH, and Bicarbonate.

All plates had some degree of inflammation (Table 2). The oxygen supplemented (OS) Group showed moderate inflammation in 12 out of 80 plates, whereas the non-oxygen supplemented (NOS) Group showed 6 plates; in this regard, the study hypothesis, that postulated that the occurrence of moderate inflammation was independent of

	Moderate inflammatory response	Light inflammatory response	Total
OS Group	12	68	80
NOS Group	6	74	80
Total	18	142	160

Table 2: Occurrence of moderate and mild inflammatory response in the oxygen supplemented Group (OS) (n=80: 8 plates per rabbit) and non-oxygen supplemented Group (NOS) (n=80: 8 plates per rabbit) groups (Chi-Square p<0.001).

oxygen supplementation, was rejected (Chi-square p<0.001). No differences were observed between inflammatory response in left and right lungs (respectively, collapsed and ventilated) within groups. The inflammatory cells found in these histological plates were neutrophils and eosinophils; with a proportion of about 75% eosinophils and 25% neutrophils.

Discussion

Both groups exhibit some degree of inflammation, evidenced by the appearance of at least mild inflammation in all subjects. This fact confirms the tendency of OLV is prone to the occurrence of acute lung injury in spontaneous breathing subjects [14], reinforced by evidence that near physiologic tidal volumes may also be somehow harmful [17].

The occurrence of more pronounced inflammatory response on those animals supplemented with oxygen when compared to those on room air ventilation (p<0.001), confirms that a certain degree of oxygen content might induce an enhanced population of acute inflammatory cells. Hyperoxia itself might induce atelectasis formation and diminish the aerated areas of the lung [4], inducing the occurrence of non ventilated areas that are prone to generate local hypoxia and acidic environments.

In this study it is possible to observe a higher mean oxygen partial pressure (PaO₂) in the OS group, as expected. These higher quantities of oxygen might be responsible for a greater inflammatory response on this group, on top of the effect seen after atelectasis formed by the left lung collapse. Inflammatory mechanisms are enhanced by local tissue hypoxia [18], and the superimposed oxygen exposure itself, would trigger reactive oxygen intermediates (ROI) formation and the induction of further mechanisms of inflammatory cellular response [13].

Our study confirms that the use of physiological tidal volumes and room-air oxygen inspired fraction may reduce the possibility of the occurrence of significant acute inflammatory response; especially in the cases in which one-lung ventilation is used.

Our results, either in mild or moderate inflammatory plates, as expected, the acute inflammatory infiltrate is composed of polymorphonuclear leucocytes, however, more than 75% of the cells found are eosinophils. Existing evidence shows that there is an important link between hypoxia and eosinophil function [19]; hypoxic stress induces the transcription of several factors, like the hypoxia inducible factor (HIF-1), responsible for oxygen homeostasis [20,21], and changes in eosinophil cellular profile enhancing its ability to migrate thru blood vessels, decreasing cellular apoptosis, and prolonging its half-life [19,22,23].

First measurements of RR, SpO₂ and HR were significantly different between groups (p<0.05), but this is expected since the first measurement was done after the setup was ready, meaning Group 1 already had oxygen supplementation. This fact might explain the lower RR in the OS group, because of a lesser need to compensate hypoxia;

	OS Group (Oxygen supplemented)		NOS Group (Non-oxygen supplemented)	
	First Recording	Last Recording	First Recording	Last recording
RR, b/m	78 (58-96)	79 (62-96)	103 (71-118)	102 (74-142)
HR, bpm	211.5 (170-230)	187.5 (161-204)	183 (150-206)	192 (172-237)
SpO ₂ , %	92.5 (85-100)	99 (94-100)	85 (80-92)	95.5 (90-98)
Temperature, °c	38 (38-38)	36(35.5-37)	38(38-40)	37(36-38)

In the first recording, RR, HR, and SpO₂ were statistically different between groups (p<0.01). In the last recording RR, SpO₂ and Temperature were statically different between groups (p<0.05). Between the 1st and last recording, SpO₂ increased and the Temperature decreased significantly in both groups (p<0.05). HR increased only in Group 1 (p<0.05). Values are presented as mean (range). HR: heart rate; RR: respiratory rate; SpO₂: peripheral oxygen arterial saturation; bpm: beats per minute; b/m: breaths per minute.

Table 1: Physical parameters.

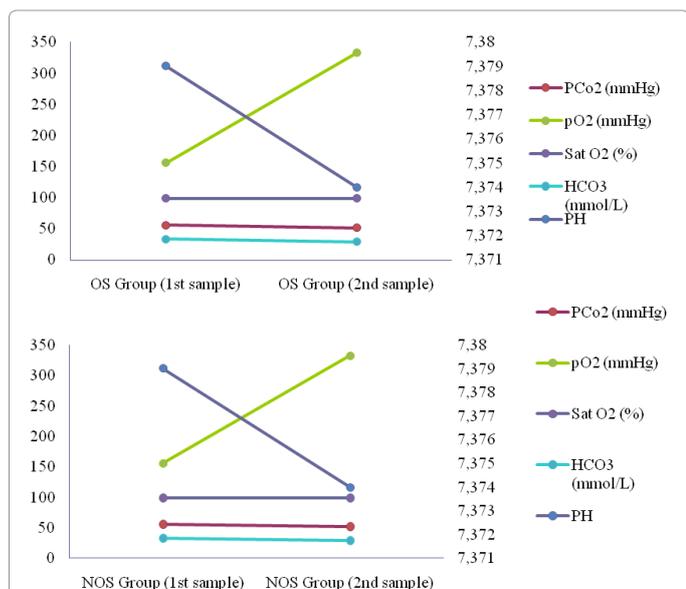


Figure 1: Arterial blood gas analysis

Arterial blood gas analysis at beginning of procedure (first sample) and at end of procedure (second sample) in OS Group (oxygen supplemented) and NOS Group (non-oxygen supplemented).

likewise the higher SpO₂ was expected in the OS group, nevertheless it raise from the first to last measurement in both groups. Temperature decrease in both groups mainly because passive cooling was present but with no clinical significance. The higher HR observed in the OS group (4.9%, p<0.05) might be linked to a higher local hypoxic representation, since oxygen might have enhanced this condition thru its ability to aggravate reabsorption atelectasis.

Median pO₂ value is higher in the OS group; this fact supports the higher median respiratory rate of NOS Group, as mentioned above. NOS group oxygenation was consistently sub-normal, since only room air was used for ventilation, and this is confirmed by the lower pO₂ values. Median pO₂ increased in both groups, but in a greater extent in OS group (p<0.001), which was expected since oxygen supplementation allowed a greater rise than a room-air oxygen fraction used in NOS group. NOS Group had a significantly increase of SatO₂, by 15.7%, this fact might be explained by the effort for normalization of the rather initial systemic hypoxic conditions; nevertheless, the observed lower inflammation response in this group confirms that oxygen supplementation might not be an adequate solution of this problem since it might improve oxygenation but aggravated local conditions, and induce more pronounced inflammatory response.

These data point out that a highly oxygenated group (when compared to a rather hypoxic one) exhibited a more pronounced acute inflammatory response in an OLV spontaneous ventilation model; in accordance to the available evidence. Moreover, since no controlled ventilation has been used, it is fare to assume that these results confirm that what is known about oxygen, regarding controlled ventilation, also applies to spontaneous ventilation.

At harvesting the lungs, the left lung was consistently collapsed and the right lung aerated, and both were untouched by any means, leaving them as their stood at the time of animal euthanasia. This methodology was used exactly to avoid any possible bias that might occur due to the use of a ventilator or organ manipulation. Mechanotransduction is the involved mechanism that transforms extreme physical lung forces into cellular signaling that induces polymorphonuclear leucocyte (PMN-L) recruitment, activation and apoptosis/necrosis balance [4]. These cells play a major role when activated by inflammatory mediators, which can be released without evidence of tissue damage; this phenomenon has been named as loss of compartmentalization [4], and its major consequence is the overall spillage of inflammatory mediators (biotrauma) not only locally but also systemically, aggravating eventual ongoing ALI but also having consequences in distal organs [4].

Since the inflammatory response impact is bilaterally present [24,25], a systemic explanation is may be involved in this process, which explains why moderate inflammation plates were found in both lungs.

The main findings in this study include:

- In a spontaneous OLV, oxygen supplementation further increases the occurrence of lung inflammation.
- In spontaneous OLV inflammation occurrence is of a bilateral nature, including the ventilated and the non-ventilated lung
- Inflammation cellular type includes polymorphonuclear leucocytes neutrophils and eosinophils, in an average of 20/80%, respectively.

The main conclusion of this study is that during procedures prone to the development of acute lung injury, namely thoracic operations with one-lung ventilation, or when safety margins are tight, common

biotrauma triggers, as inspired oxygen fraction, should be used with extreme caution, and thoroughly monitored; thus, oxygen utilization in the operative setting should be confined to the lowest inspired fraction possible, even in near physiologic tidal volumes in OLV situations.

Study limitations found during our work may be associated with the length of the procedure, since a longer exposure might have enhanced the findings. Likewise, this model is not similar to what is usually done in operating room for thoracic procedures; thus, this model's characteristics might be of significance for its ability to avoid interpretation bias (controlled ventilation, surgical manipulation), but, at same time might raise some difficulties to be compared to the classical clinical setting. Further studies to allow a closer resemblance to the common clinical approach may add value to the overall comprehension of this issue.

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