Original Article

High frequency oscillation ventilation compared to conventional mechanical ventilation plus exogenous surfactant replacement in rabbits

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Abstract

Objectives: (a) to evaluate the effect on oxygenation and ventilation of rabbits with induced surfactant depletion when they are submitted to a conventional mechanical ventilation, plus a small dose of exogenous surfactant; (b) to compare this group with another group submitted to a High Frequency Oscillation (HFO) without exogenous surfactant administration.

Methods: twenty New Zealand White rabbits weighing (± 3 kg) were anaesthetized and artificially induced to an endogenous surfactant depletion by successively lung lavage with normal saline (aliquots of 25 ml/kg) until to reach a persistent PaO₂ less than 100 mmHg when submitted to a mechanical ventilation in a pressure control mode with a target tidal volume of 10ml/kg, PEEP of 5cm H₂O, FiO₂ 1.0, respiratory rate 30/min, and inspiratory time of 0.65 s. Then the rabbits were divided in (a) CMV+S group, submitted to a conventional mechanical ventilation plus exogenous surfactant replacement; (b) HFO group, submitted to a High Frequency Oscillation Ventilation. Arterial blood gases were measured at control period, post lung lavage, 15, 16 and 120 minutes after treatment started. The groups were compared using Student t test.

Results: the post lung lavage PaO₂ in both groups was lower than 50mmHg (p=0.154), increasing after 15 min of treatment to 254 mmHg (CMV+S) and 288 mmHg (HFO, p=0.626). The PaO₂ at 60 and 120 minutes were higher (p=0.001) in the HFO group (431 e 431 mmHg) when compared with the CMV+S group, which showed a progressive fall (148 e 126 mmHg). At 60 minutes of treatment, the PaCO₂ was lower (p=0.008) in the CMV+S group (29 versus 41 mmHg).

Conclusions: in ARDS animal model a protect mechanical ventilation strategy as HFO by itself promotes a fast and persistent increase in the oxygenation, with superior levels than those observed in animals treated with conventional mechanical ventilation plus exogenous surfactant replacement.

Introduction

In acute respiratory distress syndrome (ARDS) pulmonary involvement is not homogeneous; in some areas, compliance is reduced, whereas in others it is nearly normal.¹,² As a consequence, there is a progressive decrease in pulmonary volume, which causes lungs with ARDS to be frequently described as “small,” an expression that replaces the older expression “stiff lungs”.³ Although mechanical ventilation is necessary to maintain life in patients with ARDS, the specific method or the ideal ventilation pattern have yet to be defined. Depending on the adopted ventilation pattern, it may lead to progressive pulmonary lesions. The technique used to ventilate “low compliance” areas may not be adequate to ventilate “normal compliance” areas, possibly causing ventilator-induced lung injury (VILI).²⁻⁶

References

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Ventilator-induced lung injury in ARDS has been associated with: a) barotrauma, when the excessive pressure used during mechanical ventilation causes air leaks (pneumothorax, interstitial emphysema, pneumomediastium, ...); b) volutrauma, when the tidal volume administered preferably distends areas of normal or increased compliance, occasioning stretching and tissue rupture, followed by capillary overflowing, alveolar edema, abnormalities in the production and distribution of surfactant; c) atelectrauma, a lesion related to the opening and closing (collapse and distension) of the alveolar units. In this case, lungs are ventilated by using low tidal volumes, inferior to the inflexion point of the volume pressure curve, and/or the final pressure of expiration is not able to maintain terminal airways and alveoli open, leading to progressive pulmonary collapse; to reopen these units a higher pressure will be necessary; d) biotrauma, when mechanical ventilation causes collapse, stretching, or pulmonary tissue rupture leading to cellular injury with increase of local inflammatory mediators (cytokines, oxygen free radicals, etc.).

During the past three years, several studies showed the importance of using of mechanical ventilation protective techniques in patients with ARDS, reducing the incidence of VILI and influencing survival. 5, 9

In addition, in patients with ARDS surfactant dysfunction promotes instability of alveolar units, favoring repetitive collapse and reexpansion. 3, 5, 6, 10 Protective ventilation in this situation is based on increasing pulmonary expiratory volume (for example, increasing positive-end expiratory pressure, PEEP) in order to prevent alveolar collapse, as well as on using low tidal volumes to prevent alveolar hyperinflation (distension). Strategies involving recruiting maneuvers, maintaining pulmonary volume by using PEEP, surfactant-associated use, liquid ventilation, or ventilation by oscillation with an airway pressure superior to that used in conventional ventilation may reduce VILI, promote a more physiological alveolar insufflation, and reduce pulmonary inflammation. 2, 5, 7, 9, 11-15

High frequency oscillation (HFO) ventilation was developed about 50 years ago and is based on the use of tiny tidal volumes with constant mean airway pressure, and thus avoiding extreme pulmonary volumes (both low and elevated). 13, 16-18 Several investigators were able to demonstrate in different animal models that HFO may protect lungs from induced lesion when compared to conventional ventilation. 11, 17, 19, 20 Although clinical studies with humans are still controversial, HFO is being recognized as an efficient alternative for children and newborns with respiratory insufficiency. Clinical studies have suggested that HFO could be associated with a smaller incidence of conventional VILI. 5, 13, 16, 18, 21

In animals with ARDS, VILI was avoided when exogenous surfactant associated with a PEEP of 4 cmH2O was administered. 11, 14, 15, 22, 23 However, the use of exogenous surfactant in clinical series of patients with ARDS presented controversial results. 18, 24, 25 This may be attributed to the following factors: a) type and origin of the surfactant used; b) method of surfactant administration; c) dose and stage of disease in which the surfactant was administered; d) presence of inhibitor proteins on the terminal airway; and d) ventilator strategy used concomitantly with surfactant administration. 14, 15, 24, 26

Alveolar surfactant is found in two different structural forms: a) large aggregates (active); and b) small aggregates (inactive). 14, 15, 24, 27 Exogenous surfactant consists basically of large aggregates. Once deposited in the lung, the exogenous surfactant may be converted into its active form. Studies demonstrate that using small tidal volumes during mechanical ventilation is one of the best ways to preserve endogenous surfactant. 12, 14, 15, 17 On the other hand, the use of large tidal volumes was associated with a higher rate of conversion from the large aggregates form (active) into the small aggregates form (inactive). 14, 15, 24, 27

Our objective in this study was: a) to evaluate oxygenation and ventilation in rabbits in a situation of artificial surfactant depletion when submitted to conventional mechanical ventilation, using a tidal volume of 10 ml/kg and PEEP of 5 cmH2O associated with partial exogenous surfactant replacement; b) to compare the evolution of this group with that of another group submitted to HFO ventilation without surfactant replacement.

Methods

The present study followed the guidelines of the National Institute of Health for use of experimental animals (Canada). The study was approved by the Institutional Animal Care and Use Committee (Canada).

Twenty New Zealand white rabbits, weighing approximately 3 kg each were pre-medicated with acepromazine (0.5mg/kg, intramuscular) and anaesthetized with sodium pentobarbital (10-20mg/kg, intravenous). A peripheral venous access was created for fluid infusion and an arterial line was inserted in the auricular artery for continuous hemodynamic monitoring (Hewlett-Packard pressure transducer model 1280). It also allowed seriate blood collections for arterial gasometry (Radiometer ABL 3300). An endotracheal tube with a diameter of 3.5 mm or 4.0 mm was inserted through a tracheostomy. Anesthesia and muscular paralysis were achieved through continuous infusion with pentobarbital (6mg/kg/h) and pancuronium (0.2mg/kg/h). Hydric maintenance was 7 ml/kg/hour, with a saline solution (NaCl at 0.9%) to which glucose at 5% was added. Hemoglobin saturation was continuously monitored (Nellcor) and body temperature was monitored and kept constant (between 38 and 39 degrees Celsius) with the use of a heat irradiating source and thermal blankets. Tidal volume was controlled through a monitor (thermistor
pneumotachograph - BEAR NVM-1, BEAR medical Systems, Riverside, CA) with reduced dead space (1.3 ml), inserted between the tracheal tube and the respirator circuit.

**Intervention:** Immediately after tracheostomy the animals were ventilated in the controlled pressure mode so as to achieve a tidal volume of 10ml/kg, with PEEP of 0cm H₂O, 100% of FiO₂, respiratory frequency of 30 ventilations a minute, and inspiratory time of 0.65 seconds. (Humming V, Senko Medical Instruments, Tokyo, Japan). The animals were kept in this regimen for a period of 30 minutes (control). Later, endogenous surfactant depletion was artificially induced by successive lung lavages (aliquots of 25 ml/kg) with heated saline solution, administered through the tracheal tube. Concomitantly, the animals’ thorax was gently massaged for a better distribution of the fluid inside the lungs. As soon as a marked fall was observed in arterial pressure, cardiac frequency, or hemoglobin saturation, the liquid infused in the lungs was aspirated. The maneuver was repeated (usually from 4 to 6 times) until reaching a hemoglobin saturation inferior to 90% and a PaO₂ smaller than 100 mmHg with a FiO₂ of 100%. PEEP of 5 cmH₂O; respiratory frequency of 30 mpm; inspiratory time of 0.65 seconds and peak of inspiratory pressure (PIP) required to reach a tidal volume of 10 ml/kg.

Depending on the ventilation strategy to be adopted, the animals were divided in two main groups: A) Conventional ventilation associated with partial exogenous surfactant replacement (CMV+S); B) HFO ventilation. In each group, some animals received slightly different treatments, related to other associated experiments. Consequently, there were four different subgroups:

A) Conventional ventilation associated with partial exogenous surfactant replacement (CMV+S): eight rabbits were submitted to conventional mechanical ventilation with the following parameters: FiO₂ of 1.0; PEEP of 5 cmH₂O; respiratory frequency of 30 mpm; inspiratory time of 0.65 seconds and PIP needed to obtain a tidal volume of 10 ml/kg. These parameters were set before the administration of exogenous surfactant and remained unchanged during the entire period of study. According to the surfactant regimen used, the rabbits were placed in one of the two groups:

A1) CMV+Sa: conventional ventilation associated with partial exogenous surfactant replacement. Four rabbits (mean weight: 2.99±0.10 kg) received bovine surfactant extract (27 mg/ml) in a dose of 1 ml/kg immediately after ventilation parameters were reached and set.

A2) CMV+Sd: conventional ventilation associated with partial exogenous surfactant replacement and with Dextran. Four rabbits (mean weight: 3.00±0.14 kg) received bovine surfactant extract (27 mg/ml) in a dose of 1 ml/kg associated with 2 ml of Dextran (molecule weight: 70,000) at a concentration of 50mg/ml. Dextran was associated in order to investigate the possibility of increase in surfactant activity, as demonstrated in some *in vitro* studies. Bovine surfactant extract and dextran were administered only after conventional mechanical ventilation parameters were reached and set.

B) HFO ventilation without exogenous surfactant administration. Depending on the respiratory frequency used, the 12 rabbits in this group were divided into two subgroups:

B1) HFO₁₅ - oscillation ventilation with 15 Hz frequency, used in six rabbits (mean weight: 3.03±015 Kg), with mean airways pressure (MAP) of 15 cmH₂O, inspiratory time of 33%, 100% FiO₂. Amplitude and power were adjusted so that pCO₂ was kept around 40 mmHg.

B2) HFO₅ - oscillation ventilation with 5 Hz of frequency, used in six rabbits (mean weight of 2.93 ±0.22 kg), with mean airway pressure (MAP) of 15 cmH₂O, inspiratory time of 33%, 100% FiO₂. Amplitude and power were adjusted so that pCO₂ was kept around 40 mmHg.

**Evaluations:** arterial gasometries were collected at five distinct moments: prior to lung lavage (control), after lung lavage, 15, 60, and 120 minutes after the treatment was initiated. The two main groups (CMV+S versus HFO) and the four subgroups (CMV+Sa, CMV+Sd, HFO₁₅, HFO₅) were evaluated and compared, based on their differences concerning PaO₂, pH, PCO₂, oxygenation rate ([FiO₂ x MAP/PaO₂] x 100), and mean arterial pressure in these five observation moments.

Continuous data were expressed as means and standard deviation (SD). The means of the two main groups (HFO vs. CMV+S) were compared by using Student’s t test, while the one way ANOVA was used to compare the means of each variable in the four subgroups (CMV+Sa, CMV+Sd, HFO₁₅, and HFO₅). A P value of less than 0.05 was considered as significant.

**Results**

During the control period (pre-lavage), the four subgroups did not present any difference concerning mean weight of the rabbits or number of lung lavages. Mean PaO₂, PaCO₂, and pH in the four subgroups were also similar (one way ANOVA) before the lung lavage. Similar results were also observed during the other four evaluations (post-lung lavage, 15, 60, and 120 minutes post-treatment) when we compared the four subgroups (CMV+Sa, CMV+Sd, HFO₁₅, and HFO₅) using one way ANOVA, and when the two main groups were compared (HFO and CMV+S, Student’s t test). Thus, for practical reasons, the results of this study will be presented (Table 1) for the two main groups: HFO (HFO₁₅ plus HFO₅) and CMV+S (CMV+Sa plus CMV+Sd).
After lung lavage, mean PaO₂, in the group submitted to conventional ventilation associated with surfactant replacement (CMV+S), was 43.6±9.9 mmHg. This was not statistically different (P=0.154) from the result obtained in rabbits submitted HFO ventilation (50.7±10.9 mmHg). After 15 minutes of treatment, we observed an important increase in mean PaO₂ in both groups. Mean PaO₂ at 15 minutes in rabbits in the CMV+S group increased to 254.2±107.7 mmHg, while in the HFO group, it reached 288.5±173.6 mmHg, without statistical difference (P=0.626). However, after 1 and 2 hours of treatment (Figure 1, Table 1), the HFO group presented rather elevated PaO₂ values (431.8±65.4 mmHg and 431.4±72.4 mmHg, respectively) when compared to the CMV+S group (148.8±101.6 mmHg and 126.1±88.1 mmHg, respectively) (P<0.001).

We did not observe any difference in oxygenation rates between the two groups after lung lavage (P=0.166) and after 15 minutes (P=0.187). However, after 60 and 120 minutes (Figure 2, Table 1), the oxygenation rate in the group submitted to HFO ventilation was smaller (3.6±0.6 and 3.4±0.8, respectively) when compared (P<0.001) to the rabbits receiving CMV+S (10.3±6.1; 12.3±3.4; respectively).

After lung lavage, mean airway pressure (MAP) was similar in both groups (P=0.980). However, at 15, 60, and 120 minutes after being allocated for HFO or CMV+S, we observed that the CMV+S group presented a significantly smaller MAP (P<0.001) than the HFO group (Table 1 and Figure 3).

In the post-lung lavage period, we did not observe any differences between the two groups in PaCO₂ levels (P=0.508). However, after 1 hour (Table 1, Figure 4) the groups submitted to CMS+S presented a significantly smaller (P=0.008) mean PaCO₂ (29.0±5.4 mmHg) than the rabbits submitted to HFO (41.5±12.6 mmHg).

### Table 1 - Evolution of PaO₂, PaCO₂, oxygenation rate, mean airway pressure, mean arterial pressure, and pH during at four moments in two groups of rabbits: CMV+S (conventional mechanical ventilation associated with partial bovine surfactant replacement) and HFO (5 and 15 Hz high frequency oscillation ventilation)

<table>
<thead>
<tr>
<th>Time</th>
<th>CMV+S (n=8)</th>
<th>HFO (n=12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂ (mmHg)</td>
<td></td>
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<td></td>
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<tr>
<td>Pós lavagem pulmonar</td>
<td>43.6±9.9</td>
<td>50.7±11.0</td>
<td>0.154</td>
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<tr>
<td>15 min</td>
<td>254.2±107.7</td>
<td>288.5±173.6</td>
<td>0.626</td>
</tr>
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<td>60 min</td>
<td>148.8±101.6</td>
<td>431.8±65.4</td>
<td>&lt;0.001</td>
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<tr>
<td>120 min</td>
<td>126.1±88.1</td>
<td>431.4±72.4</td>
<td>&lt;0.001</td>
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<td>PaCO₂ (mmHg)</td>
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<td></td>
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<tr>
<td>Post-lav</td>
<td>35.6±5.9</td>
<td>37.5±6.3</td>
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<tr>
<td>15 min</td>
<td>28.3±7.5</td>
<td>40.7±19.1</td>
<td>0.101</td>
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<td>60 min</td>
<td>29.0±5.4</td>
<td>41.5±12.6</td>
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<td>120 min</td>
<td>36.9±14.0</td>
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<td>Oxygenation rate</td>
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<tr>
<td>Post-lav</td>
<td>26.8±6.9</td>
<td>22.9±6.9</td>
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<td>15 min</td>
<td>5.0±2.7</td>
<td>8.3±6.4</td>
<td>0.187</td>
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<td>60 min</td>
<td>10.3±6.1</td>
<td>3.6±0.6</td>
<td>0.001</td>
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<tr>
<td>120 min</td>
<td>12.3±3.4</td>
<td>3.4±0.8</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean airways pressure (cmH₂O)</td>
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<tr>
<td>Post-lav</td>
<td>11.1±0.4</td>
<td>11.1±0.9</td>
<td>0.980</td>
</tr>
<tr>
<td>15 min</td>
<td>10.4±0.7</td>
<td>15.0±0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>60 min</td>
<td>10.3±0.9</td>
<td>15.4±1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>120 min</td>
<td>10.4±0.9</td>
<td>15.0±0.1</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean Arterial Pressure (mmHg)</td>
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<tr>
<td>Post-lav</td>
<td>70.4±18.5</td>
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<td>15 min</td>
<td>67.0±19.6</td>
<td>73.5±16.8</td>
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<td>60 min</td>
<td>55.6±11.1</td>
<td>71.8±18.5</td>
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<td>120 min</td>
<td>56.4±14.8</td>
<td>67.4±19.8</td>
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<td>pH</td>
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<td></td>
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<tr>
<td>Post-lav</td>
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<td>7.40±0.05</td>
<td>0.303</td>
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<td>15 min</td>
<td>7.46±0.04</td>
<td>7.43±0.16</td>
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<tr>
<td>60 min</td>
<td>7.41±0.03</td>
<td>7.38±0.09</td>
<td>0.325</td>
</tr>
<tr>
<td>120 min</td>
<td>7.36±0.09</td>
<td>7.39±0.06</td>
<td>0.420</td>
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</tbody>
</table>

Student’s t test.
After 1 hour of treatment, the rabbits in the CMV+S group presented mean blood pressures of 55.6±11.1mmHg, which were significantly smaller (P=0.026) than the blood pressure levels presented by the HFO group (71.8±18.5mmHg). During the other observation periods we did not find any statistical differences between the groups concerning this parameter (Table 1, Figure 5).

During the five observation moments, we did not observe any differences when the blood pH in the two groups were compared (Table 1).

Discussion

In this study, involving rabbits who suffered induced surfactant depletion and were later submitted to two different treatment strategies, it was possible to demonstrate: (a) that the use of a non-protective ventilation strategy associated with surfactant replacement promoted an immediate increase (15 min) in oxygenation. This effect, however, was quickly and progressively dissipated during the next 120 minutes; (b) that the use of a protective ventilation strategy (HFO), even if not associated with surfactant replacement, promoted...
a quick (15 min) and persistent (60 and 120 minutes) increase in oxygenation, at superior levels than those obtained in animals submitted to conventional mechanical ventilation (non-protective) associated with surfactant replacement during the two-hour observation.

Before discussing these results, some details of this experiment must be considered.

Definition of surfactant dose used: the estimated dose to replace the entire surfactant store in the lung is around 100 mg/kg. Similar studies with animals employed a dose ranging from 50 to 100 mg/kg. In good conditions the half-life of exogenous surfactant is estimated to be 5 hours. However, depending on the preparation used, administration method, disease course, presence of inhibitors, and on the ventilation strategy used, the half-life of exogenous surfactant may be significantly reduced. Thus, we opted to use what is considered to be the smallest effective dose of surfactant (27 mg/kg), obtained through previous studies in our laboratory. When analyzing our data, it is possible to observe that 15 minutes after surfactant administration there was a significant increase in PaO\textsubscript{2} (from 43.6 to 254.2 mmHg). There was also an increase in the oxygenation rate (from 26.8 to 4.96), demonstrating that the dose administered, although small, was effective. In addition, the choice of a minimal effective dose makes more evident the effect of conventional ventilation on the activity of the exogenous surfactant administered. If this form of ventilation is protective, or if it acts in synergy with the surfactant, its effect on oxygenation could be maintained for a long period. On the other hand, if the ventilation acts as inhibitor, the effect on oxygenation would be quickly lost.

Why did a subgroup receive Dextram associated with surfactant? There are in vitro studies demonstrating that dextran, in addition to presenting a protective effect, could optimize surfactant action. Some of the animals in our experiment also belonged to this parallel study whose aim was to evaluate this possibility in vivo. However, since both subgroups (isolated surfactant and surfactant associated with dextran) in this study presented the same behavior, we decided to consider them as a single group.

Why was a more elevated PEEP not used? Previous results with the same animal model showed that applying a PEEP above 9 cmH\textsubscript{2}O resulted in a 100% mortality rate 1.5 h after the surfactant was administered. On the other hand, the use of a PEEP around 5 cmH\textsubscript{2}O proved effective, safe, and produced a longer effect on oxygenation.

At 15 minutes, oxygenation was similar in both the group of rabbits submitted to conventional ventilation (non-protective) associated with surfactant administration, and the rabbits submitted to HFO ventilation. Parallel to this effect, it is important to stress the marked fall in PaCO\textsubscript{2} (increase in the minute volume) in the group receiving surfactant. Since the respirator parameters were set (respiratory frequency and inspiratory pressure), we imagine that after surfactant administration, previously collapsed areas were ventilated again, contributing to the increase verified in minute volume. However, at 60 and 120 minutes, when respirator parameters had not yet been modified, there was an increase in PaCO\textsubscript{2} in the group receiving surfactant in association with a progressive diminution of PaO\textsubscript{2}. Following this reasoning, it is possible to speculate that at this point a progressive decrease in exchange surface area occurred, probably due to a progressive collapse of alveolar units.

In ARDS, the progressive pulmonary collapse during mechanical ventilation has been associated with a) the use of insufficient PEEP, allowing a reduction of alveolar volume at the end of expiration (atelectrauma); b) the use of high tidal volumes leading to alveolar hyperdistension (volutrauma), distension of the alveolar tissue with local inflammatory process (biotrauma), and surfactant progressive inactivation. Since we opted to use PEEP levels that are considered to be adequate and protective in this animal model, we believe that the progressive pulmonary collapse is mainly a consequence of volutrauma and biotrauma. In these two types of mechanical ventilation induced lesion, the elevated tidal volume (in this case 10 ml/kg), is the main causative agent. The iatrogenic power of elevated tidal volume as inductor of pulmonary lesions was so marked and fast in our study, that it neutralized the benefits obtained with surfactant replacement within less than 60 minutes.

On the other hand, the use of a non-conventional ventilation technique (HFO), based on extremely low tidal volumes administered at high respiratory frequencies (5 and 15 Hz), allowed immediate elevation in oxygenation, which was maintained throughout the 2-hour experiment. In this experiment with rabbits surfactant-depleted rabbit, it should be stressed that already in the 1\textsuperscript{st} hour, HFO ventilation proved to be more efficient to improve oxygenation than the use of surfactant associated with conventional ventilation. Although it is not part of our objectives, it is interesting to note that the improvement in oxygenation remained unaltered until the 6th hour of observation (Figure 1), when the evaluation was interrupted (data from another experiment not shown here).

HFO ventilation has yielded consistent results in laboratory animals with induced ARDS. HFO benefits in ARDS could be attributed to two factors: the maintenance of a constant airway pressure and the use of very small tidal volumes. The main advantage of these two factors is that they prevent progressive pulmonary collapse (since alveolar stability is maintained) and great oscillations in alveolar volume (collapse and reexpansion), saving surfactant and diminishing local inflammation.
From this perspective, HFO ventilation seems to adequately fulfill the required safety and effectiveness criteria, and to be an excellent treatment alternative.

References