Short courses of mechanical ventilation with high-O_2 levels in elderly rat lungs[1]

Ventilação mecânica de curta duração com altos níveis de O_2 em pulmões de ratos idosos

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ABSTRACT

Purpose: To evaluate the effects of mechanical ventilation (MV) of high-oxygen concentration in pulmonary dysfunction in adult and elderly rats. **Methods**: Twenty-eight adult (A) and elderly (E), male rats were ventilated for 1 hour (G-AV1 and G-EV1) or for 3 hours (G-AV3 and G-EV3). A and E groups received a tidal volume of 7 mL/kg, a positive end-expiratory pressure of 5 cm H2O, respiratory rate of 70 cycles per minute, and an inspiratory fraction of oxygen of 1. We evaluated total protein content and malondialdehyde in bronchoalveolar lavages (BAL) and performed lung histomorphometrical analyses. **Results**: In G-EV1 animals, total protein in BAL was higher (33.0±1.9 µg/mL) compared with G-AV1 (23.0±2.0 µg/mL). Upon 180 minutes of MV, malondialdehyde levels increased in elderly (G-EV3) compared with adult (G-AV3) groups. Malondialdehyde and total proteins in BAL after 3 hours of MV were higher in elderly group than in adults. In G-EV3 group we observed alveolar septa dilatation and significative increase in neutrofiles number in relation to adult group at 60 and 180 minutes on MV. **Conclusion**: A higher fraction of inspired oxygen in short courses of mechanical ventilation ameliorates the parameters studied in elderly lungs.

Key words: Aging. Lung injury. Ventilator-Induced Lung Injury. Neutrophil Infiltration. Oxidative Stress. Rats.

RESUMO

Objetivo: Avaliar os efeitos da ventilação mecânica com alta concentração de oxigênio em animais adultos e idosos. **Métodos**: Vinte e oito ratos machos (adultos e idosos; n=7 por grupo) foram divididos em ventilados por 1 hora (G-AV1 e G-EV1) e ventilados por 3 horas (G-AV3 e G-EV3). Os grupos receberam volume corrente de 7 mL/kg, pressão positive ao final da expiração de 5 cmH_2O, frequência respiratória de 70 ciclos por minuto e fração inspirada de oxigênio de 100%. Ao final do experimento avaliamos no lavado brônquio-alveolar as proteínas totais, malondialdeído, celularidade e a histomorfometria do parênquima pulmonar. **Resultados**: Em animais idosos ventilados por 1 hora (G-EV1) as proteínas totais no lavado brônquio-alveolar aumentaram (33.0±1.9 µg/mL) quando comparados com os adultos (G-AV1; 23.0±2.0 µg/mL). Após 180 minutos de ventilação mecânica os níveis de malondialdeído e as proteínas totais estavam elevadas nos animais idosos (G-EV3) quando comparadas com os adultos (G-AV3; $p<0.001$). No grupo de animais idosos (G-EV3) observamos ainda dilatação dos septos alveolares e aumento significativo no número de neutrófilos em relação aos adultos ventilados, tanto aos 60 quanto aos 180 minutos de ventilação mecânica ($p<0.001$). **Conclusão**: A ventilação mecânica com alta fração inspirada de oxigênio por um curto período de tempo favoreceu os parâmetros pulmonares estudados nos animais idosos.

Descritores: Envelhecimento. Lesão Pulmonar. Lesão Pulmonar Induzida por Ventilação Mecânica. Infiltração de Neutrófilos. Estresse Oxidativo. Ratos.

Introduction

Elderly patients have become an increasingly prevalent proportion of the intensive care unit (ICU) population. Outcomes of patients with acute respiratory distress syndrome (ARDS) have been significantly improved in recent years[1].

In the very elderly population (85 years and older), the acute severity of illness is the most significant predictor of mortality after an ICU admission associated with a significant functional deterioration[2,3]. The senescence process itself decrease of the muscular strength and aerobics capacity, vasomotor instability, reduction of the bone mass, reduction of the lung ventilation etc.[4]. Regarding the mortality rate post-ICU, a study by Gordo *et al*.[5] showed that among patients over 75 years the age can be associated with mortality as an independent factor, whereas Iapichino *et al.*[6] demonstrated that after discharging ICU the mortality rate was increased in patients over 78 years.

Artificial ventilation is one of the most important techniques in the ICU, besides being an important tool in the approach of the acute lung injury/acute respiratory distress syndrome (ALI/ARDS) of any nature or in other cases[7,8]. In many cases of respiratory diseases, the use of ventilatory support is required. Besides that, a ventilatory support is needed in other situations, including sepsis and sepsis shock; neuromuscular disease; during postoperative state; and in cases of altered mental status with loss of consciousness[9,10].

Several studies consider the age as a factor influencing the clinical prognosis of a patient needing mechanical ventilation[11,12]. These are in disagreement with others that consider, in addition to the age, other factors like functional status and the seriousness of the acute process[11-14], and others yet which consider age as an associated factor[15-18]. Although no consistent data exist in this scenario, it is presumable that aged individuals have greater susceptibility to the harmful effects of mechanical ventilation than adults.

Animal studies the effects of hyperoxia have employed very high levels of inspired oxygen fraction (FiO_2 > 0.95) for extended periods of time. These experimental models produce severe lung injury and death in 48–72 h[19]. Patients without lung dysfunction requiring such high concentrations of supplemental oxygen, however, often receive concurrent ventilatory support. The deleterious effects of hypoxia are well known and physicians may be mostly concerned about avoiding hypoxia and giving additional oxygen 'to remain on the safe side[20,21].

Significant resistance to hyperoxia-induced oxidative stress has been demonstrated in pulmonary endothelial cells overexpressing manganous superoxide dismutase (Mn-SOD)[22]. Limited literature, however, exists on the role of reactive oxygen species (ROS) in modulating the inflammatory response during early or late phase of acute inflammation[23-25].

The present study tested in an animal model the effect of two different periods of mechanical ventilation and we hypothesize that elderly subjects are more susceptible than adults to the deleterious effects of a large period of mechanical ventilation using a higher fraction of inspired oxygen and to the accompanying oxidative stress.

Methods

Surgical procedure

All procedures were approved by the Institutional Research Committee at Federal University of Sao Paulo, in accordance with the National Institutes of Health (NIH) Guidelines Regarding Animal Experimentation and the principles of the Brazilian College on Animal Experimentation.

Twenty eight male Wistar rats (*Rattus norvegicus albinus*) from which 14 were adult (4 month-old, body weight [bw] - 320–360 g) and 14 were elderly (24 month-old, bw 430–465 g), were divided in four groups (n = 7 animals each) as follows: adult (G-AV1) and elderly (G-EV1) rats ventilated for 1 hour; and adult (G-AV3) and elderly (G-EV3) rats ventilated for 3 hours. The animals were individually housed, fed rat chow and water *ad libitum*, and were maintained on a 12-h light/dark cycle for 3 weeks prior to initiation of the experiments.

Mechanical ventilation (MV)

Rats were anesthetized with an intraperitoneal (ip) bolus injection of sodium thiopental (50 mg/kg; Thionembutal®, Abbott, SP, Brazil). After reaching a surgical plane of anesthesia and after tracheostomy, a 14G cannula was inserted into the trachea. Upon tracheostomy, a sterile PE50 catheter was inserted into the right carotid artery for blood gases sampling and another one into the left carotid artery for mean arterial pressure (MAP) control (Pressure transducer, Gould-Statham, CA, USA). Muscle relaxation was performed with ip succinylcholine chloride (0.8 mg/kg; Uniao Quimica Farmaceutica Nacional, SP, Brazil).

The animals were immediately connected to a rodent ventilator (Harvard Apparatus, MA, USA) and ventilated during 1 or 3 h using a tidal volume (V_T) of 7 mL/kg, a positive end-expiratory pressure (PEEP) of 5 cmH_2O, a fraction of inspired oxygen (FiO_2) of 1 and a respiratory rate (RR) of 70 cycles per minutes (cpm). Body temperature was maintained at nearest 37 °C by the use of a heating blanket and controlled by a rectal thermometer (YSI Inc., OH, EUA).

Arterial blood gases measurements

Arterial blood gases were collected in sterile vented plastic syringes (PICO 70, Radiometer, Copenhagen, Denmark) and measured at different times: in the groups ventilated for 1 hour at the times 0 min and 60 min; the ventilated groups for 3 h were evaluated at the times 0 min, 60 min and 180 min, using an automatic analyzer (AVL-Compact3, Roche Diagnostic, Germany).

Bronchoalveolar lavage (BAL)

At the end of experiment, the animals were euthanized (ip doses of 1 mL/100 g body weight; T-61 Euthanasia Solution, Schering-Plough, SP, Brazil). A sternotomy was performed and the lungs and structures were removed and weighed. The left main stem bronchus lung was tied and the right lung was washed three

times with 10 mL/kg of sterile saline solution. Lavage fluids were kept separate and centrifuged (400 x ***g*** for 10 min, 4 ºC) and the supernatants were immediately frozen on dry ice and stored at –80ºC.

Total cell countings and total protein determinations in BAL

The total number of cells in BAL was counted in a hemocytometer (Neubauer chambers) under 100x magnification. Total protein content was measured (in duplicate) in 1-mL aliquot of BAL by using the method of Lowry and colleagues [26].

Thiobarbituric acid-reactive substances (TBARS)

Products of lipid and protein oxidation were determined in the homogenate of the left lung by using the method of Das[27]. The concentrations of lipid peroxidation (LPO) products (thiobarbituric acid-reactive substances, TBARS) were determined through the absorbance at 532 nm and expressed as mmol/mg protein.

Morphology and morphometry

Lungs were removed *en bloc* and inflated at a pressure of 20 cmH_2O with 4% paraformaldehyde in phosphate solution (100 mM phosphate-buffered saline [PBS] pH 7.4) at room temperature for fixation during 12 h. Fragments of the left lung were washed in 50mM phosphate buffer, pH 7.4, dehydrated in graded concentrations of ethanol, and then embedded in paraffin. From every lung, 4-µm sections were obtained and stained with hematoxylin and eosin (HE) to evaluate lung morphology and morphometry. Neutrophils counts were performed in the alveolar septae by image capture with a light microscope using 400X magnification (Axiolab Standart 2.0, Carl Zeiss) coupled to a video camera (AxionCam, Carl Zeiss).

Statistical analyses

Data are presented as mean ± standard deviation (SD). Statistical intergroup comparisons of total cell, total protein, TBARS, and lung neutrophil were performed using one-way ANOVA. Statistical comparisons of mean arterial blood pressure, and blood gas analyses among the groups were performed using two-way ANOVA. Differences among groups were tested by the Tukey's multiple comparisons test. A *P* value less than 0.05 was considered significant. Statistical analyses were performed using a standard computer software (GraphPad Prism, GraphPad Software, CA, USA).

Results

Blood gas exchange

Table 1 shows that, upon mechanical ventilation (MV) during 60 min, the partial pressure of arterial oxygen (Pa_{O_2}) of the G-EV1 (355±3.9 mmHg) and G-EV3 (365±4.2 mmHg) groups were higher than that observed in the G-AV1 (342±4.5 mmHg) and G-AV3 (347±3.2 mmHg) groups. The Pa_{O_2} decrease in the 3-h ventilated elderly group (G-EV3 = 338±5.9 mmHg) as compared with the adult ventilated group (G-AV3 = 356±5.8 mmHg). The partial pressure of arterial carbon dioxide (Pa_{CO_2}), differed between elderly ventilated 1-h (G-EV1: 42±2.5 mmHg) and the adult ventilated group (G-AV1: 37±2.2 mmHg). Pa_{CO_2} was higher in the elderly group after 3-h MV (G-EV3: 58±4.1 mmHg) with regard to the adult ventilated animals (G-AV3: 50±2.4 mmHg) (Table 1). There was a pH (Table 2) reduction in elderly animals after 3-h MV (G-EV3: 7.22±0.03) compared with their adult counterparts of the same time (G-AV3: 7.31±0.02).

TABLE 1 - Effects of mechanical ventilation on the arterial blood Pa_{O_2} and Pa_{CO_2} at the time points 0 (zero), 60 and 180 minutes in the groups G-AV1, G-EV1, G-AV3 and G-EV3 (n=7 per group). Data are mean ± SD.

	Pa_{O_2} (mmHg)			Pa_{CO_2} (mmHg)		
Groups	**0 min**	**60 min**	**180 min**	**0 min**	**60 min**	**180 min**
G-AV1	90.0±3.2	342±4.5	-	37±1.9	37±2.2	-
G-EV1	91±5.8	355±3.9[a]	-	39±2.0	42±2.5[d]	-
G-AV3	91±5.9	347±3.2	356±5.8	39±3.5	47±1.6	50±2.4
G-EV3	88±2.7	365±4.2[b]	339±5.9[c]	42±2.5	51±2.6[e]	58±4.1[f]

G-AV1= adults ventilated for 1 h; **G-AV3**= adults ventilated for 3 h; **G-EV1**= elderly ventilated for 1 h; **G-EV3**= elderly ventilated for 3 h. [a]*P*=0.001 compared with G-AV1 at the time 60 min – Pa_{O_2}; [b]*P*=0.001 compared with G-AV3 at the time 60 min – Pa_{O_2}; [c] *P*=0.001 compared with G-AV3 at the time 180 min – Pa_{O_2}; [d] *P*=0.01 compared with G-AV1 at the time 60 min – Pa_{CO_2}; [e] *P*=0.01 compared with G-AV3 at the time 60 min – Pa_{CO_2}; [f] *P*=0.01 compared with G-AV3 at the time 180 min – Pa_{CO_2}.

TABLE 2 - Effects of mechanical ventilation on the arterial blood pH at the time points 0 (zero), 60 and 180 minutes in groups G-AV1, G-EV1, G-AV3 and G-EV3 (n=7 per group). Data are mean ± SD.

	pH		
Groups	**0 min**	**60 min**	**180 min**
G-AV1	7.43 ± 0.03	7.36 ± 0.02	-
G-EV1	7.42 ± 0.02	7.33 ± 0.02	-
G-AV3	7.43 ± 0.02	7.36 ± 0.01	7.31 ±0.02
G-EV3	7.41 ± 0.01	7.33 ± 0.02[a]	7.22 ± 0.03[b]

G-AV1= adults ventilated for 1 h; **G-AV3**= adults ventilated for 3 h; **G-EV1**= elderly ventilated for 1 h; **G-EV3**= elderly ventilated for 3 h. [a] *P*<0.05 G-EV3 (at the time 60 min) compared with G-EV3 (at the time 180 min). [b] *P*<0.05 compared with G-AV3 at the time 180 min.

Mean arterial pressure (MAP)

In elderly animals the MAP decrease in 180 min MV (G-EV3: 97±2.9 mmHg) compared with elderly rats ventilated for 60 min (G-EV1 = 103±3.7 mmHg and G-EV3 = 104±3.2 mmHg). On the other hand, MAP was lower in the elderly animal ventilated during 180 min compared with the adults (P<0.05) (Table 3).

TABLE 3 - Effects of mechanical ventilation on the mean arterial pressure (MAP) at the time points 0 (zero), 60 and 180 minutes in groups G-AV1, G-EV1, G-AV3 and G-EV3 (n=7 per group). Data are mean ± SD.

	MAP(mmHg)		
Groups	**0 min**	**60 min**	**180 min**
G-AV1	115.0 ± 3.7	104.0 ± 2.9	-
G-EV1	115.1 ± 3.6	103.0 ± 3.7	-
G-AV3	114.0 ± 3.6	103.0 ± 3.8	104.0 ± 2.8
G-EV3	116.0 ± 3.8	104.0 ± 3.2[a]	97.0 ± 2.9[b]

G-AV1= adults ventilated for 1 h; **G-AV3**= adults ventilated for 3 h; **G-EV1**= elderly ventilated for 1 h; **G-EV3**= elderly ventilated for 3 h. [a] P<0.05 compared with G-EV3 at the time 180 min. [b] P<0.05 compared with G-AV3 at the time 180 min.

Total cell count and protein leakage in bal

The total cell counting were higher in the G-EV1 (5.0 ± 0.9 x10^6 mL^{-1}) compared with AV1 (3.0±0.9 x10^6 mL^{-1}) groups, and G-EV3 (7.0 ± 1.0 x10^6 mL^{-1}) compared with AV3 group (5.0 ± 1.1 x10^6 mL^{-1}) (Figure 1). The total protein (Figure 2) was lower in the G-AV1 group as compared with G-EV1 group (23.0 ± 2.0 µg/mL versus 27.0 ± 1.7 µg/mL, respectively). The total protein was higher in the G-EV3 (43.0 ± 2.3 µg/mL) when compared with G-AV3 (34.0 ± 1.9 µg/mL).

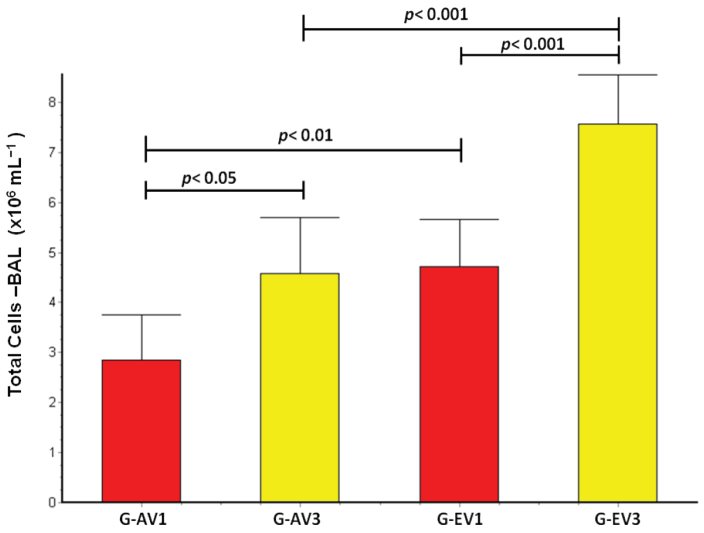

FIGURE 1 - Effects of mechanical ventilation (MV) on total cells in bronchoalveolar lavage at the end of 1-h and 3-h MV. Data are mean ± SD.

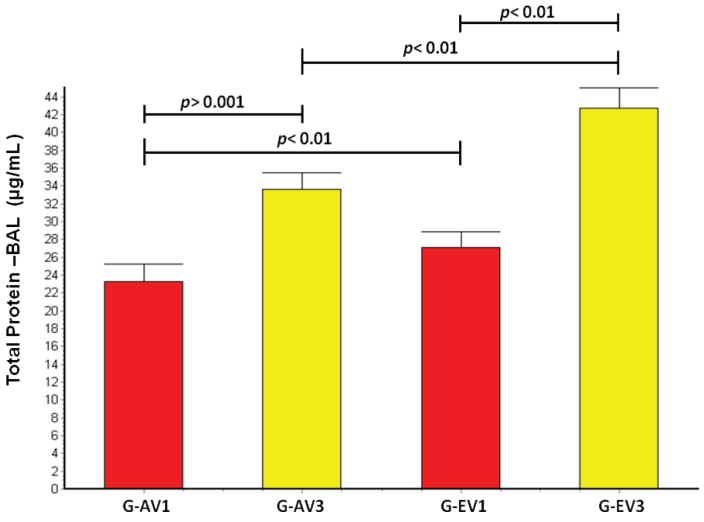

FIGURE 2 - Effects of mechanical ventilation on total protein in bronchoalveolar lavage at the end of 1-h and 3-h MV. Data are expressed as mean ± SD.

Thiobarbituric acid-reactive substances (TBARS) measurement in BAL

The TBARS concentrations (Figure 3) increased in the G-EV1 (0.22 ± 0.007 nmol/mg of protein) compared with G-AV1 (0.21 ± 0.008 nmol/mg of protein). In the elderly groups MV increased MDA in G-EV3 (0.51 ± 0.009 nmol/mg of protein) as compared with G-AV3 (0.24 ± 0.005 nmol/mg of protein).

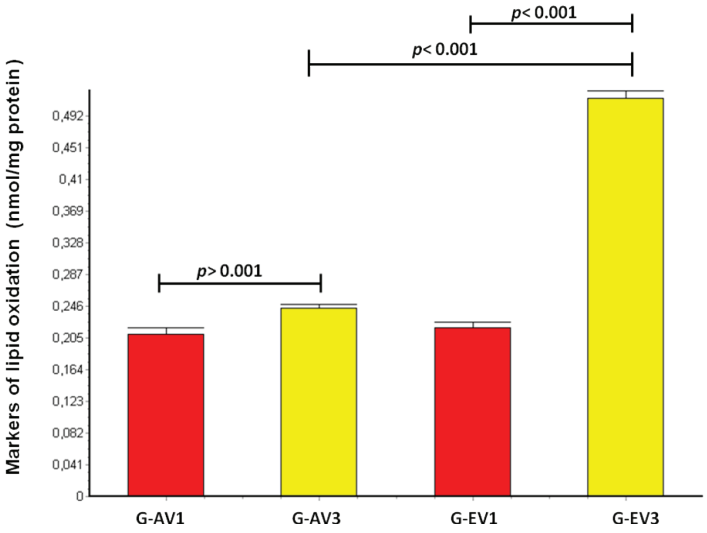

FIGURE 3 - Effects of mechanical ventilation on markers of lipid oxidation (TBARS; in nmol/mg protein) in the bronchoalveolar lavage at the end of 1-h and 3-h MV. Data are expressed as mean ± SD.

Histomorphometric analyses

Lungs from the elderly 3-h MV group showed typical cavities, septs with congested capillaries and few macrophages and neutrophils influx, and dilation of alveolar septae [G-EV3]; in adults ventilated for 3-h this effect was milder [G-AV3] (Figure 4). Lungs from adult [G-AV1] and elderly [G-EV1] ventilated for 1-h presented minimal changes at light microscopy. The neutrophil counting was higher in the elderly animals after 1-h of MV (19.0 ± 2.5/μm^2) compared with the adults (11.0 ± 4.5/μm^2; P<0.05). In the groups ventilated for 3 h the neutrophils counting were higher in G-EV3 (37.0 ± 2.6/μm^2) when compared with group G-AV3 (25.0 ± 3.0/μm^2) (Figure 5).

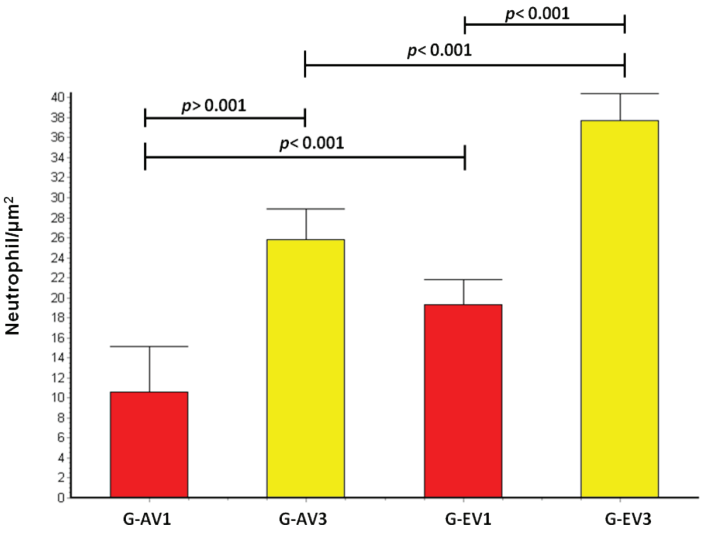

FIGURE 4 - Effects of mechanical ventilation on neutrophil counting in lung tissue at the end of 1-h and 3-h MV. Data are expressed as mean ± SD.

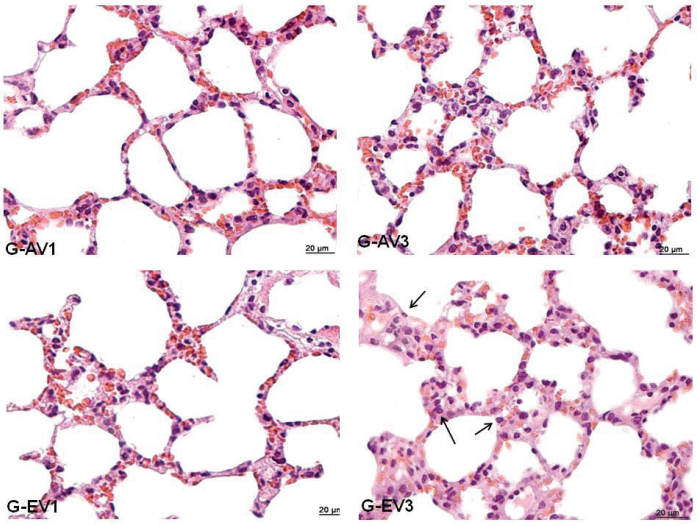

FIGURE 5 - Representative photomicrographs of rat lung histological sections in G-AV1; G-EV1; G-AV3 and G-EV3. Presence of inflammatory cells (arrows) in the animals ventilated for 1 h compared with the 3-h ventilated groups. (HE, 400x).

Discussion

We investigated whether bronchoalveolar levels of malondialdehyde, total protein and neutrophil influx in elderly rats submitted to mechanical ventilation for 1 hour and 3 hours.

In a recent study, Nin *et al*.[28] ventilated young and elderly animals during 60 min using respiratory rate 70 cpm, FiO_2 0.35, and a protective lung strategy (V_T = 9 mL/kg and PEEP = 5 cmH_2O) or with an injurious strategy (V_T = 35 mL/kg and PEEP = 0 cmH_2O, overventilated). In this study elderly rats showed increased susceptibility to injurious mechanical ventilation-induced pulmonary injury, vascular dysfunction, and systemic inflammation in lungs using a lower FiO_2. The higher FiO2 in our study was chosen because the high FiO_2 used under certain circumstances may help to cause redistribution of pulmonary blood flow and to decrease vasoconstriction in atelectatic or non-ventilated areas[29]. Our study showed that higher FiO_2 promoted an increase of Pa_{O_2} in adult and elderly animals ventilated for 1 hour, but Pa_{O_2} decreased and Pa_{CO_2} increased after 180 min in elderly compared with adults mechanically ventilated.

The use of higher FiO_2 was determinate by unclear whether such high FiO_2 will increase oxygen free radical activity. The higher FiO_2 can induce a concomitant increase in free radical activity and the lipid peroxidation causes tissue damage[21,30-33]. Moreover, lipid peroxide end-products of tissue damage can disrupt cellular function by formation of disulphide bridges across nucleotide or amino acid chains[32] and inhibits lymphocytic activity [33]. Whether MV *per se* initiates pulmonary inflammation in patients with non-injured lungs is still unclear[34], although we have shown that a lung protective MV strategy attenuates intrapulmonary coagulation caused by a more conventional MV strategy[35].

Some studies have assessed the changes occurring in lungs of aged rats. First, the older lungs might be subjected to greater mechanical stress due to a difference in the alveolar septae, which may result in a greater susceptibility to stretch-induced lung injury.

Second, for any given mechanical stress, the aged lung may have a more vigorous inflammatory response. This potential mechanism would be in line with studies showing that, after certain stimuli, aged animals and humans show a more marked response after an inflammatory challenge[29,36]. Although oxygen toxicity is a well known entity[37], FiO_2s up to 0.5 are commonly considered 'safe' by physicians[20]. It appears that physicians are more concerned about avoiding hypoxia and ischaemia than about the risks of hyperoxia. In The Netherlands, no formal guidelines for oxygenation targets are available. This may be related to the fact that the influence of oxygenation targets has never been studied making it impossible to provide evidence-based recommendations. Based on other observational studies, it may well be that also in other countries actual PaO_2s in ICU patients are higher than recommended[38,39].

Our study, using protective strategy and higher FiO_2, expanded the results to demonstrate the increase in total protein and malondialdehyde in BAL in elderly animals ventilated for 180 min when compared with adults. In 1-h ventilated elderly animals, the differences in the biochemical analyses were augmented when compared with adults, but this effect was neither followed by arterial oxygen decrease nor was the alveolar structure affected.

This study has limitations. Animals submitted to MV and PEEP were ventilated with a 100% inspired oxygen fraction, although subsequent hyperoxia may have influenced lung results. However, this protocol was used to avoid any possibility of hypoxia. In fact, hypoxia has been associated with rapid increases in vascular permeability and in leukocyte adherence[40]. Secondly, in our study the tracheal peak pressure was not evaluated. Thirdly, ventilatory strategies using a moderate tidal volume of 10 mL/kg and PEEP levels from zero up to 10 cmH_2O have been used with surgical patients[41,42].

Conclusions

The present study showed that the association of a protective tidal volume and high FiO_2 may be harmful to microcirculation in the setting of elderly normal lungs during a short time of MV. Our findings indicate that aged rats ventilated for 3h and high inspired oxygen concentration developed more marked local changes compared with their adult, younger counterparts. This finding may have relevance in understanding the development of complications related to MV and the use of high fraction of inspired oxygen in elderly subjects.

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