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Research Article

Effects of Different Therapeutic Intervention Protocols on Clinical Indicators and Oxidative Damage Parameters in Patients on Mechanical Ventilation

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Abstract

This study evaluated the effect of different physical therapy protocols on clinical indicators and oxidative damage parameters in patients exposed to mechanical ventilator (MV). A total of 40 patients were divided into 4 groups: control (n = 12), passive exercise (n = 10), electrical stimulation (n = 10) and passive exercise plus electrical stimulation (n = 8). Clinical data and blood samples (3-mL) were collected from each patient twice a day, at the beginning of the implementation of protocols (baseline), and at the end of the treatment. Concerning patient characteristics and about clinical data no major significant differences were observed between groups either on baseline or on their last observation carried forward (LOCF). We observed significant differences on thiol content variation from baseline (H = 2.904, p = 0.406), xylenol variation was significant between groups (H = 19.521, p = 0.0002). Post hoc analysis indicated significant differences between protocol 1 and 4 (q = 3.912) as well as 1 and 3 (q = 3.430). Observed variation on xylenol content is not explained by protocol length (p = 0.943). The results on the effects of the protocols used were inconclusive. However, there is evidence that the therapeutic routines can reduce oxidative damage.

Keywords: *Mechanical ventilation; exercise; electrical stimulation; oxidative damage; oxidative stress*

Introduction

Mechanical ventilation (MV) is an essential ventilatory support in patients with acute respiratory insufficiency in intensive care units (ICU) [1,2]. Long periods of exposure to MV can lead easily to disuse and atrophy of skeletal muscle; a scenario that may promote several changes in the response of blood flow distribution [3,4] as well as in several clinical and metabolic factors [5]. Additionally; these patients present dysfunction of vital organs; sepsis; hypoxemia; acidosis; or drug toxicity that negatively affect the cardiovascular system [6,7]; leading to a clinical picture of high instability and subsequent disability [8].

Problems in weaning patients on MV and prolonged hospitalization have been widely reported; resulting from immobility; respiratory muscle weakness and peripherals as well as poor physical fitness [9]. However; numerous other factors may contribute to delayed weaning; and it is believed that a common cause between patients is muscle weakness and deficits of respiratory resistance [10,11]. In such cases; physical therapy appears to help reduce the problem [6,9,12] but the best physiotherapeutic procedure and the possibility that early physiotherapy increases benefits are issues that remain to be addressed.

Although early mobilization and exercise have been used to improve clinical condition of ICU patients [13-15]; there is a paucity of published information about these effects in patients on MV. In addition; several issues associated with the effects of physiotherapy on clinical indicators and oxidative stress parameters in patients exposed to MV remain unanswered; deserving further investigation; among which: 1) the exposure time to MV has a relationship with oxidative damage; 2) the most effective therapeutic method to reduce the duration on MV and improve the clinical condition of MV patients 3) the minimization of the oxidative damage with early mobilization and physical therapy. In this sense; this study investigates exposure to MV regardless of disease; sex or age. Based on these assumptions and questions; we evaluate the effects of different physical therapy protocols (electrical stimulation and passive exercise) and the effect of these protocols on clinical indicators and oxidative damage parameters in MV patients.

Materials and Methods

Patients

Study inclusion criteria were patients admitted to the ICU of São José Regional Hospital; Criciúma; Santa Catarina; Brazil; age > 18 yrs; both genders and who received invasive mechanical ventilation for more than 2 days; in the ICU for at least 5 days. Patients with restlessness; confusion; impaired response or absence thereof to simple orders; in shock (systolic blood pressure < 90 mmHg or need for ongoing vasopressors); persistent respiratory failure (defined as respiratory rate 35 breaths/min and/or ratio of P_{aO2} to fraction of inspired oxygen (F_{IO2}) < 200 mmHg and/or P_{aCO2} > 50 mmHg; and/or pH < 7.30); ongoing renal replacement therapy; ongoing intravenous sedation; scheduled extubation; increased intracranial pressure; with implantation of cardiac pacemakers; brain death; technical barriers that do not allow the implementation of the electrodes (i.e. skin burns; and fractures); degenerative neuromuscular diseases; spinal cord injury and pregnancy were excluded. After selection; 40 patients were initially randomized into four groups and classified as control (n = 12 – standard care); passive exercise (n = 10); electrical stimulation (n = 10); and electrical stimulation plus passive exercise (n = 8). This study was approved by the local ethics committee under protocol 127/2010. Parents or custodians of the patients signed an informed consent form.

Treatment Protocol

Control: Besides usual care; conventional respiratory physiotherapy (re-expansion pulmonary and tracheal aspiration) was conducted twice a day throughout the period that the patient remained in the ICU.

Passive Exercise: Included passive mobilization of the patient using repeated involuntary movements of the arms and legs (knee flexion; abduction and hip extension of the lower limbs and extension and shoulder abduction and elbow flexion of upper limbs). One session per day was performed for 20 to 30 minutes as three series of approximately 10 repetitions/series.

Electrical Stimulation: Before the protocol was performed; sites were cleansed and the skin was carefully shaved to facilitate electrode attachment (90 × 50 mm) over the motor points of the quadriceps muscle of both legs. The stimulator (KW trademark; manufactured by Industrial Electronic Technology Ltda; Brazil) was set at a 6-second contraction and 12 seconds of relaxation at a frequency of 2500 Hz. Patients received daily sessions of 20 minutes; progressing to 30 minutes for some patients; including 5 minutes to warm-up and 5 minutes for recovery. The sessions continued until extubation.

Electrical stimulation plus passive exercise: The association used included the protocols described above. Electrical stimulation was applied initially; and followed by passive exercise.

Data collection

The clinical data of gas analysis; heart rate; respiratory rate; blood pressure; hemoglobin and hematocrit were collected daily and written on the patient´s medical record. Blood samples (3-mL) were collected from each patient twice; at the beginning of the implementa-

tion of protocols (baseline) and at the end of treatment. Blood was obtained by venipuncture (antecubital vein) and collected in heparin tubes. Plasma was obtained by centrifugation of blood samples for 10 min at 5000 rpm. The samples were stored at -70˚C for subsequent analysis.

Assays

Oxidation xylenol orange (Xylenol): This method detects hydroperoxides (ROOHs) that are the products of lipoperoxidation. The xylenol orange assay is based on oxidation of ferrous ions to ferric ions by ROOHs under acidic conditions. Tissues were homogenized (30 mg/mL) and aliquots (90 µL) were transferred to microcentrifuge vials (1.0 mL). Ten microliters of 10 mM TPP in methanol were added into the vials to reduce ROOHs. All the vials were then vortexed and incubated at room temperature for 30 min prior to the addition of 900 ml of specific buffer. Absorbance of the supernatant was measured at 560 nm using an Ultraspec 2000 spectrophotometer (Pharmacia Biotech; Uppsala; Sweden) as previously described by [16].

Total thiol content: The total thiol content was determined using the 5;5` -dithioibis (2-nitrobenzoic acid) method (DTNB) (Sigma). The reaction was started adding 30 µL of 10 mM DTNB stock solution in PBS. Control samples did not include DTNB. After 30 min of incubation at room temperature; absorbance at 412 nm was measured and the amounts of TNB formed were calculated (equivalent to the amount of SH groups) according to the Aksenov technique [17].

Protein content: Protein content was assayed using bovine serum albumin as standard [18]. A folin phenol reagent (phosphomolybdic-phosphotungstic reagent) was added to bind the protein. The bound reagent was slowly reduced and changed from yellow to blue. Absorbance was read at 750 nm.

Statistical analysis

Statistical analysis was conducted using SigmaStat 3.1 (Systat; Point Richmond; CA; USA) using only the samples that had more than 90% of the variables reviewest (arbitrary cutoff). We performed the Kolgomorov-Smirnov test with Lilliefors' correction and Levene median test to assess normality and equal variance; respectively. Categorical data was compared using chi-square tests (identified by χ^2). Continuous data was compared with one way ANOVA (identified by F value) or Kruskal-Wallis one way ANOVA (identified by H value). Parametric ANOVAs were followed by the Holm-Sidak test and non-parametric ANOVAs were followed by Tukey test when sample sizes between groups were equal or Dunn's test when sample sizes between groups were different (e.g. missing data). Linear regressions were used in our exploratory investigation. Statistical significance was claimed when $p < 0.05$.

Results

Epidemiological data is presented in Table 1. No major significant differences were observed between groups; although death during protocol presented a borderline association ($F = 7.296$; $p = 0.048$). We had 33.33% females among subjects submitted to protocol 1 while a lower proportion (12.50%) of female subjects were enrolled in protocol 4. There was no significant difference between groups; either at baseline (Table 2) or on their Last Observation Carried Forward (LOCF) (Table 3). We did not obtain major significant results after subjects were submitted to any of the four protocols. Though we did not observe significant differences in thiol content variation from baseline ($H = 2.904$; $p = 0.406$); xylenol variation was significant between groups ($H = 19.521$; $p = 0.0002$) (Figure 1). Post hoc analysis indicated significant differences between protocol 1 and 4 ($q = 3.912$) as well as 1 and 3 ($q = 3.430$). Observed variation on xylenol content was not explained by protocol length ($p = 0.943$).

Parameter	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Statistics	
	Mean (SD)[n] or %	Mean (SD)[n] or %	Mean (SD)[n] or %	Mean (SD)[n] or %	F or χ^2	p
Age (years)	56.42 (10.75)[12]	51.7 (16.76)[10]	57.6 (14.59)[10]	49.25 (19.57)[8]	0.619	0.607
Females (%)	33.33	30.00	20.00	12.50	1.378	0.711
Glasgow score	7.34 (1.23)[9]	7.15 (3.19)[7]	6.25 (2.13)[8]	7 (3.17)[6]	0.309	0.818
Rass score	-3.84 (1.61)[6]	-3.4 (2.31)[5]	-3 (2.92)[5]	-3.6 (1.68)[5]	0.143	0.933
Mechanical ventilation (days)	9.84 (4.97)[12]	10.3 (7.61)[10]	14.9 (7.51)[10]	14 (9.08)[8]	1.288	0.293
Protocol length (days)	7.09 (4.93)[12]	8.6 (8.89)[10]	10.7 (6.9)[10]	9.38 (7.88)[8]	0.486	0.694
Death during protocol (%)	33.33	0.00	40.00	0.00	7.296	0.048

Table 1: *Baseline data and characteristics of the subjects involved in the study. Data are presented in mean and standard deviation or percentage. 1 = standard treatment; 2 = passive exercise; 3 = electrical stimulation; 4 = passive exercise plus electrical stimulation; * p <0.05.*

Parameter	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Statistics	
	Mean (SD)[n] or %	Mean (SD)[n] or %	Mean (SD)[n] or %	Mean (SD)[n] or %	F	p
Bicarbonate	22.2 (6.22)[12]	25.69 (5.09)[10]	23.58 (4.9)[10]	24.98 (4.21)[8]	0.929	0.437
pH	7.36 (0.13)[12]	7.38 (0.09)[10]	7.38 (0.09)[10]	7.4 (0.08)[8]	0.353	0.787
Carbon dioxide pressure	41.63 (17.16)[12]	44.39 (9.45)[10]	40.75 (7.62)[10]	41.25 (8.93)[8]	0.185	0.906
Oxygen pressure	99.58 (39.74)[12]	125.25 (48.88)[10]	108.68 (46.55)[10]	94.93 (53.56)[8]	0.793	0.506
Oxygen saturation	94.13 (6.49)[12]	97.04 (3.83)[10]	94.85 (7.87)[10]	93.72 (6.67)[8]	0.527	0.667
Hematocrit	32.65 (7.56)[12]	31.13 (7.16)[10]	30.93 (5.31)[10]	27.12 (3.92)[7]	1.118	0.355
Hemoglobin	10.65 (2.29)[12]	10.16 (2.11)[10]	10.23 (1.77)[10]	8.6 (0.96)[7]	1.737	0.177
Heart rate	91.92 (17.22)[12]	80.7 (21.6)[10]	91.5 (19.93)[10]	82.38 (16.71)[8]	0.980	0.413
Respiratory rate	16.09 (6.15)[12]	17.8 (8.02)[10]	17.2 (3.77)[10]	16.25 (2.5)[8]	0.208	0.890
Systolic pressure	131.09 (23.5)[12]	137 (25.19)[10]	127.4 (31.18)[10]	134.13 (28.07)[8]	0.233	0.873
Diastolic pressure	74.25 (23)[12]	77.5 (15.48)[10]	70.9 (18.24)[10]	76.25 (16.1)[8]	0.229	0.875

Table 2: *Baseline clinical data of the subjects involved in the study. Data are presented in mean and standard deviation. 1 = standard treatment; 2 = passive exercise; 3 = electrical stimulation; 4 = passive exercise plus electrical stimulation; * p < 0.05.*

Parameter	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Statistics	
	Mean (SD)[n] or %	Mean (SD)[n] or %	Mean (SD)[n] or %	Mean (SD)[n] or %	F	p
Bicarbonate	25.55 (8.21)[12]	28.51 (4.87)[10]	24.33 (7.32)[10]	25.58 (5.49)[8]	0.691	0.564
pH	7.41 (0.11)[12]	7.41 (0.08)[10]	7.34 (0.15)[10]	7.39 (0.08)[8]	1.144	0.345
Carbon dioxide pressure	41.24 (12.46)[12]	45.54 (9.78)[10]	46.3 (13.86)[10]	44.7 (18.12)[8]	0.309	0.819
Oxygen pressure	110.87 (37.85)[12]	99.2 (36.3)[10]	103.67 (37.42)[10]	77.54 (35.53)[8]	1.366	0.269
Oxygen saturation	97.11 (2.3)[12]	95.69 (5.19)[10]	93.45 (8.77)[10]	88.73 (12.72)[8]	2.086	0.119
Hematocrit	31.34 (6.43)[12]	30.58 (7.67)[10]	27.98 (5.36)[10]	27.35 (4.12)[7]	0.935	0.434
Hemoglobin	10.2 (2.14)[12]	9.88 (2.37)[10]	9.15 (1.96)[10]	8.42 (1.15)[7]	1.370	0.268

Heart rate	84.67 (21.03)[12]	91.6 (22.72)[10]	91.8 (16.52)[10]	95.88 (16.05)[8]	0.583	0.630
Respiratory rate	21.42 (3.48)[12]	23.4 (7.83)[10]	21.7 (4.91)[10]	22.88 (6.11)[8]	0.286	0.835
Systolic pressure	149.25 (31.04)[12]	155 (26.24)[10]	129.8 (24.16)[10]	139.63 (15.11)[8]	1.884	0.150
Diastolic pressure	84.5 (18.81)[12]	86 (22.81)[10]	71.5 (14.31)[10]	78.5 (10.16)[8]	1.440	0.247

Table 3: *Last Observation Carried Forward (LCOF) of the basal clinical data. The data are presented in mean and standard deviation. 1 = standard treatment; 2 = passive exercise; 3 = electrical stimulation; 4 = passive exercise plus electrical stimulation; * p <0.05.*

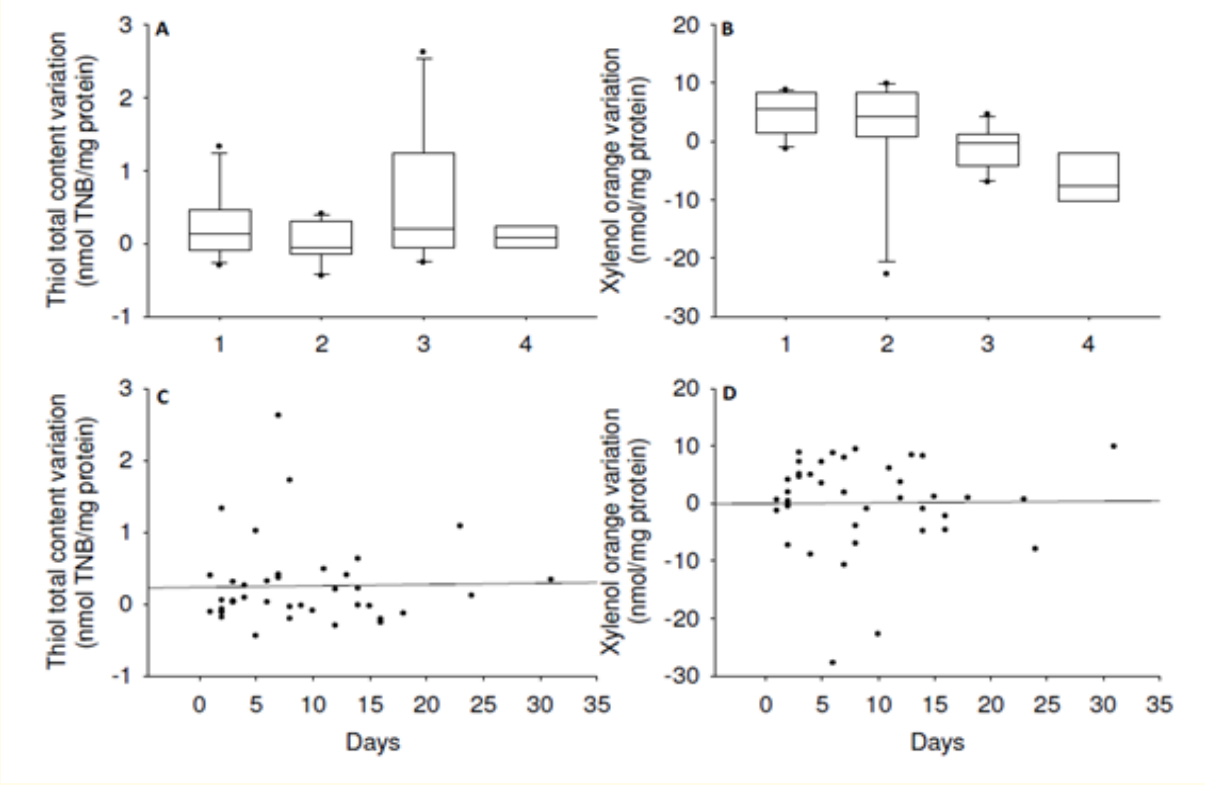

Figure 1: *Change in Xylenol (figure 1 A/C) and total content of thiols (Figure 1 B/D) (LOCF - baseline). The data are presented in mean and standard deviation. The normality test was performed followed post hoc of Dunn's test. Linear regressions also were used for analysis of oxidative damage. Statistical significance in all tests was claimed when p < 0.05. 1 = standard treatment; 2 = passive exercise; 3 = electrical stimulation; 4 = passive exercise plus electrical stimulation.*

Discussion

Advances in the management of MV are needed to obtain better results to shorten weaning times and improve survival rates. This is because although mechanical ventilation is a rescue measure; the longer a patient remains on this life support mode; the higher the odds of complications such as tracheal injuries; infection; cardiovascular and respiratory failure [19]. For example; muscle weakness is a frequent complication associated with disease severity as well as with immobilization duration. Conventional treatments often address the maintenance of circulatory; respiratory and renal function to ensure patient survival. Therefore; most patients on mechanical ventilation

receive sedatives and painkillers to reduce stress and oxygen consumption; a strategy that may result in long periods of unconsciousness and immobility [12]. Although physiotherapy is a common practice in most ICUs; few data have been published on its current practice; feasibility; and benefits. Therapeutic actions that may help reduce the harmful effects caused by mechanical ventilation appear to be crucial and necessary in ICUs. Thus; to evaluate the effects of different physiotherapy practices on clinical parameters and systemic oxidative stress in patients on MV; this study used two different protocols; passive exercise and electrical stimulation. The results indicate that physiotherapeutic intervention reduced oxidative damage; though it may not lead to conclusive outcomes regarding protocol length effects given our sample size. In this sense; this parameter should be investigated in future studies.

Baseline data in Table 1 show similar characteristics among the subjects evaluated. Only the interruption of death was statistically significant in MV; and; interestingly enough; it was observed in patients exposed to protocols 1 and 3. These results are possibly associated with the patient's health. The causes that lead patients to MV are the most diverse possible; and outcomes are likewise quite varied. The interruption of MV caused by death occurs due to severity of disease; and not necessarily due to therapeutic intervention protocols. The average period on MV represents an important parameter when applying intervention protocols to accelerate the weaning outcome. In the present study we did not observe significant differences in this parameter. However; in a study by Ely and colleagues [16] showed that patients on MV who were exposed to the intervention of respiratory therapy MV spans by 1.5 days; with lower morbidity [20] showed that the group under physiotherapy intervention reduced time on MV and increased the rate of successful weaning. Yet; hospital mortality was similar between the groups.

As for baseline clinical data; no differences were observed among groups (table 2); as well as the LOCF (table 3). Although our results showed no significant changes in clinical variables analyzed; possibly due to differences between subjects concerning time and the cause that led to VM; it was seen that electrical stimulation and/or passive mobilization (passive exercise) is a safe intervention; feasible and well tolerated but also contributes substantially to the improvement of muscle strength [21]. Studies using electrical stimulation reported satisfactory results in chronic patients [22,23]. Comparing a protocol of active exercises and electrical stimulation in patients with severe chronic obstructive pulmonary disease long time exposed to MV; it was possible to found that the group receiving electrical stimulation achieved a significantly greater increase in muscle strength when compared to control group participants [23]. Chiang and colleagues [24] also found significant increase in limb muscle strength and respiratory function in patients on MV exposed to passive exercise.

ROS generation during MV causes significant damage to biomolecules. The main mechanisms involved in ROS production are directly linked to the oxidation of diaphragmatic myofibrils; proteolysis and activation of xanthine oxidase [25-28]. It is known that more than two weeks in bed is enough for changes in the redox state occur; leading to oxidative damage [29]. Although the time on MV in this study was less than two weeks (Table 1); oxidative damage was observed in all groups. These results are probably due to the patient's condition itself; and not necessarily to MV time. The results reported here suggest a positive effect of passive exercise performed alone or associated with electrical stimulation against oxidative damage in lipids and proteins; respectively. MV itself can directly contribute to lung injury generating oxidative stress and inflammation in healthy mouse lungs [30].

In recent research; some patients were analyzed on MV for a period similar to ours; and also observed elevated levels of lipid peroxidation [31]. Similarly; other studies suggest that systemic and tissue oxidative stress arises from muscle atrophy induced by MV; particularly diaphragmatic atrophy; and this increases the degradation of muscle proteins [19]. The association of oxidative stress in patients on MV is marked by a lower content of glutathione [32]. Although data in the literature are not consistent; similar studies in humans and animal models also suggest that high metabolic activity as well as the vascular endothelial dysfunction in patients on MV [33] can be determinant for the systemic oxidative damage observed. Therefore; even systemic oxidative damage may have important implications; compromising the health of the patient on MV.

Given the limitations of the present study; it was not possible to provide conclusive results about the effects of the protocols used. However; there is evidence that the therapeutic routines that enhance muscle activity assist in patient recovery on MV and help reduce oxidative damage. However; future studies with greater control of the variables should be carried out; with more representative stratification of subjects.

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Conflict of Interest

The authors declared no conflict of interest.

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