



Original Article

Comparison of the effects of volemic reposition with 7.5% NaCl or blood in an experimental model of muscular compression and hemorrhagic shock[☆]



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ABSTRACT

Objective: Crush syndrome is characterized by traumatic muscular injuries with severe systemic clinical repercussions. The systemic inflammatory reaction characterized acutely by infiltration of neutrophils in the lungs has been studied as part of the spectrum of crush syndrome. Experimental research may demonstrate alternative treatments for crush syndrome. The authors studied the hypothesis that hypertonic saline solution (7.5% NaCl) could minimize the local and systemic effects in a model of muscular compression and hemorrhagic shock.

Methods: Rabbits were submitted to a new model of muscle compression associated with hemorrhagic shock. Compression was applied through an Esmarch bandage, used for 1 h on the entire right lower limb. Hemorrhagic shock was induced for 1 h by dissection and catheterization of the carotid artery. Blood replacement or hypertonic saline solution was used to treat the shock. Biochemical analysis of plasma, quantification of muscular edema, and infiltration of inflammatory cells in the lungs were carried out.

Results: Animals treated with hypertonic solution presented the same hemodynamic response as the blood treated patients, less water in the compressed muscles and less infiltration of inflammatory cells in the lungs. The blood group presented hypocalcemia, a facet of crush syndrome.

Conclusions: The proposed model was effective for the study of crush syndrome associated with hemorrhagic shock. The treatment with hypertonic solution showed benefits when compared with blood volume replacement.

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Comparação dos efeitos da reposição volêmica com NaCl 7,5% ou sangue em um modelo experimental de compressão muscular e choque hemorrágico

R E S U M O

Palavras-chave:

Síndrome de esmagamento
Choque hemorrágico
Coelho
Solução salina hipertônica

Objetivo: A síndrome de esmagamento é caracterizada por lesões musculares traumáticas com graves repercussões clínicas sistêmicas. A reação inflamatória sistêmica, caracterizada agudamente por infiltração de neutrófilos nos pulmões, tem sido estudada como parte do espectro da síndrome de esmagamento. A pesquisa experimental pode demonstrar opções de tratamento para a síndrome de esmagamento. Os autores estudaram a hipótese de que solução salina hipertônica (NaCl 7,5%) pudesse minimizar os efeitos locais e sistêmicos da síndrome de esmagamento em um modelo de compressão muscular e choque hemorrágico. **Métodos:** Coelhos foram submetidos a um novo modelo de compressão muscular associado ao choque hemorrágico. A compressão foi feita por uma faixa de Esmarch aplicada por uma hora em todo membro inferior direito. O choque hemorrágico foi induzido durante uma hora por dissecação e cateterização da artéria carótida. O choque foi tratado com reposição de sangue ou solução salina hipertônica. Foram feitas análises bioquímicas do plasma, quantificação do edema muscular e infiltração de células inflamatórias nos pulmões.

Resultados: Os animais tratados com solução hipertônica apresentaram a mesma resposta hemodinâmica observada naqueles tratados com sangue, menor quantidade de água nos músculos comprimidos e menor infiltração de células inflamatórias nos pulmões. O grupo tratado com sangue apresentou hipocalcemia, característica da síndrome de esmagamento. **Conclusões:** O modelo proposto mostrou-se efetivo para o estudo da síndrome de esmagamento associada ao choque hemorrágico. O tratamento com solução hipertônica apresentou benefícios quando comparado com a reposição volêmica com sangue.

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Introduction

Traumatic muscle compression is part of the spectrum of a syndrome with important clinical repercussions: crush syndrome (CS).¹ CS was described in World War II when the clinical evolution of patients rescued from debris and the sequence of systemic alterations that accompanied lower limb crush syndrome were observed.² In the year following the publication of these results, those authors developed an experimental model to study the pathophysiology of the disease.³

Injury to muscle cells massively releases ions into the intra or extracellular environment, releases proteins (especially myoglobin) into the circulation, and is associated with extracellular fluid retention (edema). All these factors can cause heart and renal alterations, as well as hypovolemic shock.¹

In addition to the known myoglobinuria, which evolves to renal failure, in experimental models, CS also appears to be associated with a systemic inflammatory response mediated by neutrophils and cytokines, the main shock organ being the lungs.⁴⁻⁸

In accident victims, the interaction of external agents acting on the body can lead to multi-system injury and acute hemorrhage. Volume replacement with isotonic crystalloid solutions (used in an infusion equivalent to three times the estimated hemorrhage volume) is recommended in treatment protocols.⁹ Hypertonic saline solution (NaCl 7.5%)

has been studied in experimental laboratory studies and in clinical protocols as an alternative in the treatment of hypovolemic shock.^{10,11} Hypertonic solution has an important anti-inflammatory effect that could be related to the improvement in the survival of the animals that received this infusion in experimental protocol.^{10,12}

Among all aspects of muscle compression injury, the present study focused on muscle edema, plasma electrolyte alterations, and neutrophil infiltration in the lungs (which may be associated with a distant inflammatory reaction). The authors hypothesized that large muscular lesions can course with an inflammatory reaction in the lungs, thus contributing to the onset of CS and that hypertonic saline solution can reduce the edema of the crushed limb and may reduce the pulmonary inflammatory reaction.

Methods

Surgical procedures

The experimental protocols were approved by the Research Ethics Committee. The animals were provided by the Vivarium Center and kept for at least three days in the vivarium for observation and adjustment to the new environment.

In this study, 24 male New Zealand rabbits weighing between 2500 and 3000 g were used. Surgical procedures were done under aseptic conditions.

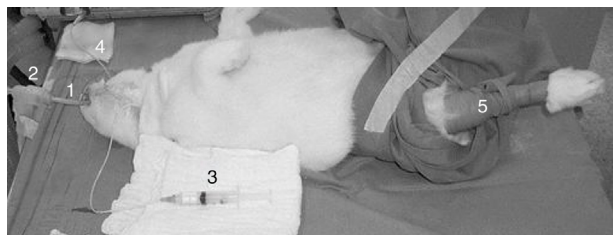


Fig. 1 – Photograph of the model. The animal was intubated (1) and connected to a mechanical ventilation device (2). The common jugular vein was dissected and cannulated with an intracath connected to a syringe with an anesthetic drug (3). The carotid artery was also dissected and cannulated (4). Esmarch bandage applied over the right hind paw (5).

The animals were pre-anesthetized with a veterinary analgesic and muscle relaxant (Rompum[®]) at a dose of 5 mg/kg intramuscularly. General anesthesia was induced with ketamine hydrochloride (Ketalar[®]) at a dose of 35 mg/kg intramuscularly). Subsequently, trichotomies of the anterior cervical region and of the right hind paw were conducted.

Orotracheal intubation was performed with the animals in the supine position. Controlled mechanical ventilation was initiated with a respiratory rate of 25–35 per minute, a tidal volume of 10 mL/kg, and an increase in the partial pressure of inspired oxygen.

Under aseptic conditions, a cervical incision was made approximately 2 cm on the right paramedian sector for the identification, dissection, and preparation of the external jugular vein. It was cannulated with a child-sized intracath[®], which was kept filled with heparinized saline solution. Then, the right common carotid artery was identified, isolated, and cannulated in the same way as the jugular vein. Arterial catheterization was used for the continuous measurement of mean arterial pressure (MAP), induction of hypotension, and collection of samples for arterial blood gas analysis. Venous access was used for anesthesia supplementation, when necessary, and volume replacement. The surgical wound was sutured to minimize insensible water losses. General anesthesia was supplemented, when necessary, with a diluted solution of sodium pentobarbital (1 mL of 3% sodium pentobarbital in 3 mL of saline; Fig. 1).

Experimental groups

After randomization, the animals were divided into three groups (n = 5 per group):

Group I: Control (mock surgery). Animals from this group were only submitted to all stages of surgical preparation; they were kept in controlled mechanical ventilation for 180 min (equivalent to the total time used in the experiments with the animals from the other groups).

Group II: Compression of the lower limb musculature, hemorrhagic hypotension, and resuscitation of hemorrhagic shock with 7.5% hypertonic saline solution at 4 mL/kg of body weight.

Group III: Compression of the lower limb musculature, hemorrhagic hypotension, and resuscitation from the hemorrhagic shock with reinfusion of the drawn blood.

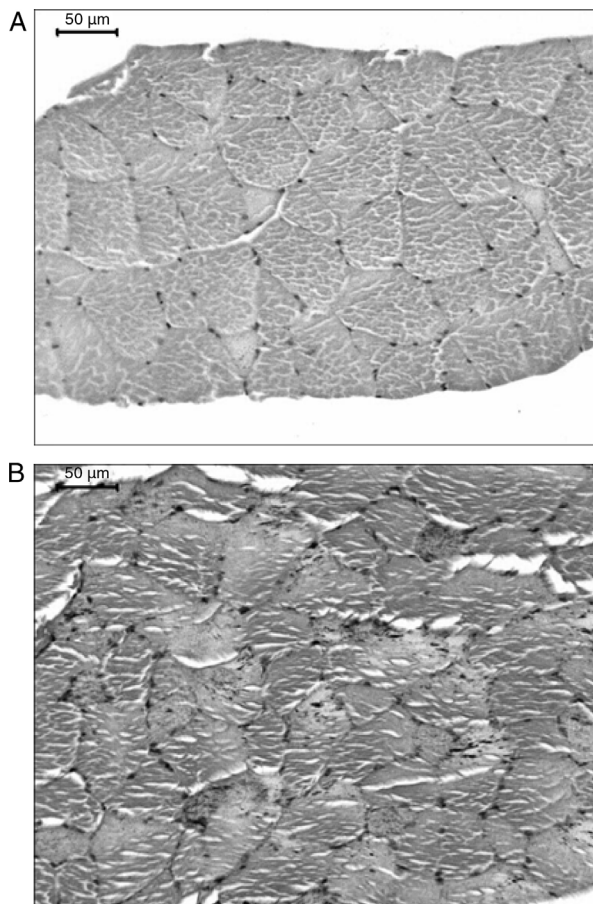


Fig. 2 – Histology of a rabbit muscle compressed for 1 h with Esmarch bandage at 300 mmHg pressure. (A) Normal muscle. (B) Muscle compressed for 1 h. Hematoxylin/eosin staining. 200× magnification.

Compression of the lower limb musculature

The muscle layers of the right hind paw were compressed with a 5 cm wide Esmarch bandage. Compression was standardized based on the number of 'winding turns with the Esmarch bandage (ten winding turns in the first limb segment and ten winding turns in the distal segment of the right hind paw) with an increase of pressure on the muscle compartment (300 mmHg).⁷ The muscle compartment pressure was measured using a variation of the Whitesides method, with the needle placed between the Esmarch bandage and the animal's skin.¹³ During the pilot experiment phase, it was observed that the compression method used caused important structural changes in the muscular architecture (Fig. 2). The right hind paw muscle compression was maintained for 1 h. After that period, the Esmarch bandages were removed. The animals were maintained under general anesthesia, controlled mechanical ventilation, and monitoring for an additional hour. After that time, the anesthetic level was deepened and controlled mechanical ventilation was suspended for euthanasia of the animals.

Hemorrhagic hypotension induction

After 15 min of initiation of compression, hemorrhagic hypotension was induced by active blood drawing through the carotid artery catheter. The collected blood was stored in a sodium citrate solution (0.15 mL for each mL of collected blood) to prevent coagulation and preserve the blood, which was reinfused in the animals from group III. The induction of hemorrhagic hypotension was interrupted after one of two criteria: a decrease of MAP values to 40 mmHg or the maximum removal of 40% of the calculated blood volume (blood volume was estimated as 50 mL for every 1000 g of body weight).

Treatment of hemorrhagic shock

Hemorrhagic hypotension was maintained for 60 min; after that period, the animals were treated with 7.5% hypertonic saline infusion at 4 mL/kg body weight (group II) or reinfusion of the total volume of blood drawn (group III).

Biochemical plasma analysis

Samples were collected for arterial blood gas analysis and biochemical blood tests. The arterial blood gas assessment aided in the correction of respiratory parameters. Arterial blood gas and laboratory tests were performed at previously defined moments (15 min after surgical preparation, immediately before hemorrhagic hypotension induction, immediately before treatment of hemorrhagic hypotension, immediately before and 15 min after the release of the Esmarch bandage, and 15 min after treatment). In the control group, samples were collected every 45 min.

Monitoring of MAP

Continuous MAP monitoring and electrocardiographic recordings were performed using the Biopac[®] modular system during the entire experiment.

Histological analysis of the lungs

Histology with hematoxylin and eosin staining was used to quantify the infiltration of polymorphonuclear neutrophil cells in the lungs. The slides were evaluated under light field microscopy. The digital images were acquired with a video camera and projected onto the monitor, for analysis using Leica *Ò* software, or onto a screen, for morphometric analysis with a 100-point grid. With the Leica *Ò* software, under 400× magnification, five microscopic fields from each experimental group were assessed to calculate the relation between the septal area, greatest septal dimension, and cellular infiltration of the alveoli. Under 400× or 1000× magnification, ten microscopic fields were chosen at random and projected onto a screen. A 100-point grid (area of 1000 mm² at 400× or 100 mm² at 1000×) was superimposed on the screen. The points on the lung tissue were counted and used to estimate the lung tissue area. The polymorphonuclear cells found at the edges of the morphometry grid were counted and used to calculate the cell

infiltration index (number of polymorphonuclear cells/mm² of lung tissue).

The dry weight of lower limb musculature

Samples of the muscles of the two hind paws (right and left) were collected to quantify the formation of muscle edema. The muscle fragments were weighed, standardized at 5 g (damp weight), and then placed for 24 h in a dehydration oven (dry weight). The volume of water in muscles was calculated by the formula:

$$\% \text{ of water} = \frac{(\text{damp weight} - \text{dry weight}) \times 100}{\text{damp weight}}$$

Results

Mortality

The authors aimed at studying 15 animals, five per group. As nine deaths were observed, a total of 24 animals were used. During the experiment, five deaths occurred during the procedures. These deaths were attributed to protocol learning and standardization (anesthesia, controlled mechanical ventilation, and induction and duration of hemorrhagic hypotension) during the initial phases of the experiment. In the animals selected for treatment or control, four deaths were observed: two occurred during hemorrhagic hypotension and the other two occurred after blood therapy. All animals ($n=9$) that died during application of the protocol were excluded.

MAP

The graphs in Fig. 3 represent the variations in MAP values.

Biochemical plasma analysis

Blood potential of hydrogen (pH)

Variations in blood pH were similar in the two study groups that underwent hypotension and muscle crushing and, at the end of the experiment, a decrease in their values was observed.

Partial oxygen pressure (PaO₂) and hemoglobin saturation (SAO₂)

Considering all experiments together, the median PaO₂ before volume replacement was 96.6 mm Hg. After volume replacement with blood or hypertonic solution, the median values obtained were 96.7 mmHg and 162.4 mmHg, respectively. No statistical difference was observed between the groups.

SAO₂ did not vary between groups when the baseline values were compared with those after volume replacement with blood or hypertonic solution. The mean initial value was 95.1 ± 1.21%. After reinfusion of blood, the saturation was 98.2 ± 0.6%; using hypertonic solution, the value was 96.2 ± 1.24% (ANOVA, not statistically significant).

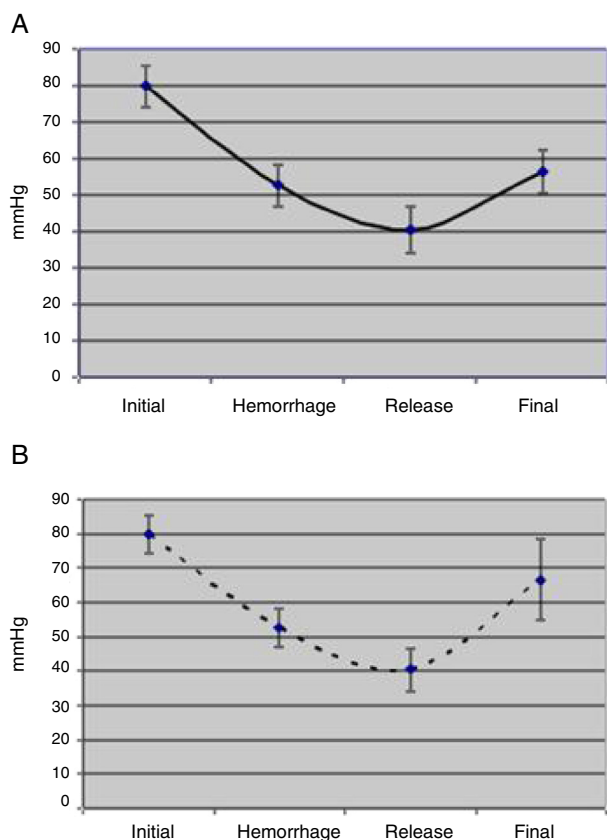


Fig. 3 – Mean arterial pressure variations in the protocol. There was a decrease in mean arterial pressure with the hemorrhage, which became more pronounced after the release of the Esmarch bandage. The return to initial values was similar in the groups treated with blood (A) and 7.5% hypertonic solution (B).

Hematocrit

The mean initial hematocrit value of the samples was $29.1 \pm 1.19\%$. After hemorrhage induction, the mean value was 23.3 ± 0.71 (Student's t-test, $p=0.002$). Before treatment, the median hematocrit was 23%; after blood treatment, it increased to 30%. After treatment with a hypertonic solution, the median hematocrit was 23%. When comparing hematocrit values after blood or hypertonic treatment, a statistically significant difference was observed (Dunn's method, $p < 0.05$).

Plasma potassium

No changes in plasma potassium were observed after the release of the Esmarch bandage or after the two methods of volume replacement.

Plasma calcium and sodium, lactate

The results are shown in the graphs of Figs. 4-6.

Dry weight – percentage of water in muscle

A statistically significant difference was observed in the water percentages of the muscles among all groups (Kruskal-Wallis, one way ANOVA on ranks, $p=0.04$). Regarding the percentage of water, a statistically significant difference was

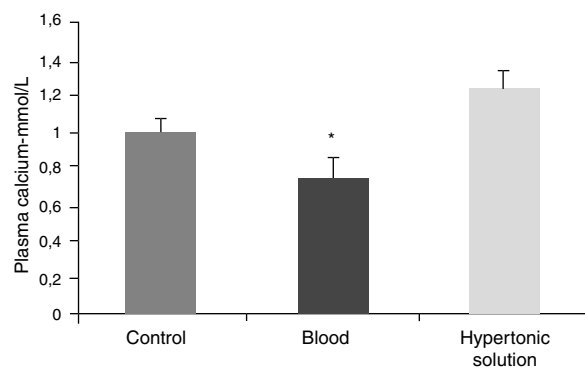


Fig. 4 – Variations in plasma calcium according to the volume replacement method. At the end of the experiments, before the euthanasia, the group treated with blood presented lower values than the group treated with 7.5% hypertonic solution (* $p < 0.05$).

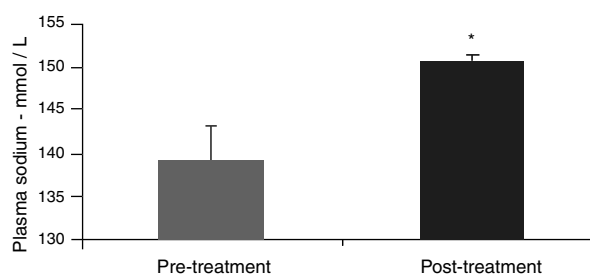


Fig. 5 – Increased plasma sodium levels after volume replacement with 7.5% hypertonic saline solution (* $p < 0.05$).

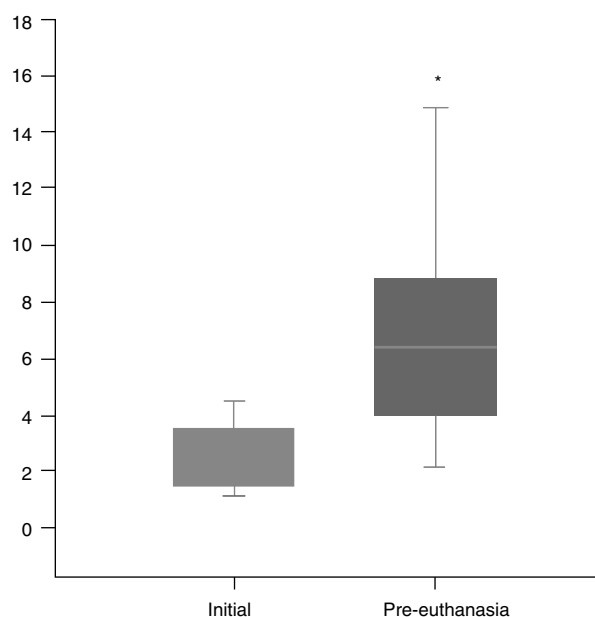


Fig. 6 – Plasma lactate increased in all groups when baseline and pre-euthanasia values were compared (* $p < 0.05$).

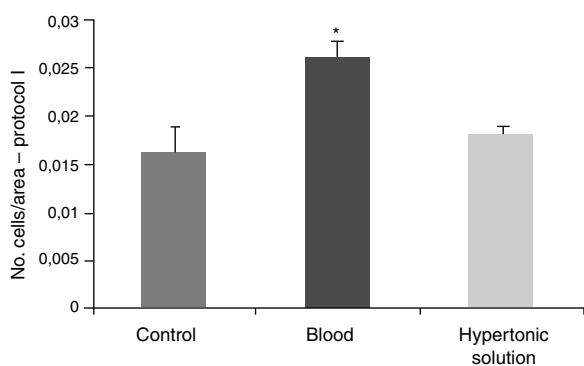


Fig. 7 – Differences between the number of neutrophils found in the alveolar septa. The highest value was found in the group that underwent blood treatment when compared with the control group and the group treated with 7.5% hypertonic saline solution (* $p < 0.05$).

observed between the control group, whose median was 79.1% (78.6–80%), and the group treated with a hypertonic solution, whose median was 71.9% (70.2–75%; Dunn's method, $p < 0.05$).

Lung histology

Ratio between cells (neutrophils)/area in the alveolar septa.

The comparison of the means of cellularity in the alveolar septa is shown in the graph of Fig. 7.

Discussion

Orthopedic surgeons involved in the care of polytrauma patients and disaster victims should be familiar with the systemic and local repercussions of muscle crushing.⁴ Although much studied after earthquakes with buried victims,^{14,15} any form of prolonged muscle compression may lead to the development of these symptoms.¹ Systemic repercussions may be observed within just an hour after muscle compression.^{1,16}

The present study was aimed at assessing traumatic muscular compression and CS through a method of intense compression of the entire musculature of a limb.⁷ The authors expected to reproduce all components of a crushing injury at maximum values for a short period. Hemorrhagic hypotension was included, aiming at reproducing the scenario observed in trauma emergencies. The analysis of the histology of the compressed muscle in pilot experiments (Fig. 1), as well as the mortality observed after the protocol was established, reflects the local and systemic aggression of this model.

Several models have been proposed to study muscle compression and CS.^{3,5,7,17,18} The present model has the advantage of using two 5 cm Esmarch bandages, a material of low cost and easy application. Muscle compression models in rats lead to local and systemic alterations in periods ranging from three to 6 h after the compression is released.^{5,7,17} As hemorrhagic shock was associated, compression was carried out for only 1 h, due to the high mortality rate observed in a pilot study in which a longer compression was associated with shock.

Muscle compression, as that observed in CS, can evolve with the onset of compartment syndrome.^{1,19} Recent studies

have demonstrated the possible positive effects of resuscitation of rats and rabbits with a hypertonic saline solution on CS models.^{20,21} In the present model, the mechanisms used for compression led to an active removal of free water from the tissues. After compression release, the tissues returned to perfusion conditions, and then rehydration occurred. The authors expected to observe an increase in the volume of water in the crushed paws, which would suggest edema. Surprisingly, in all cases, a smaller amount of free water was observed in the crushed muscles when compared with their controls. It is likely that the fact that the animals suffered euthanasia 1 h after the release of compression did not allow sufficient time for the development of edema and that the corrected hypovolemic shock may have influenced this response. Muscle edema is assumed to be dependent on the total compression time of the limb, requiring several hours for its development.^{3,22} Animals treated with the hypertonic solution presented a smaller amount of retained water in the injured muscles when compared with those treated with blood replacement.

Hemorrhagic hypotension was included in this protocol to reproduce the scenario observed in trauma emergencies. With the use of prolonged periods of hemorrhagic hypotension, a high mortality rate was observed, and the authors were able to observe an effective response using hypertonic solution when compared with the group treated with whole blood. Although hypertonic solutions have not been conclusively proven to improve the response of victims of shock trauma, it may be considered as an option for resuscitation.^{9,23} It is important to discuss some differential aspects of the two treatments for hemorrhagic hypotension: blood replacement or hypertonic solution. MAP recovery to the baseline values was similar, regardless of the form of treatment. Hematocrit, however, presented decreased values in the hypertonic solution group. The decrease in hemoglobin values was not accompanied by a decrease in the supply of oxygen (PaO₂ and SAO₂ results were comparable between the groups). It is important to remember that an extended period of hemorrhagic hypotension may cause the systemic changes that characterize hemorrhagic shock syndrome.²⁴ This syndrome may be observed even after correction of hemodynamic parameters; it appears to be related to decreased perfusion in the microcirculation.^{25,26} In the present study, it was possible to observe some favorable effects related to blood flow in the microcirculation when a hypertonic solution was used; for example, MAP recovery was accompanied by a lower blood viscosity (low hematocrit) with an adequate supply of oxygen to the microcirculation.

Serum lactate was analyzed and presented a significant increase in its plasma concentration after hemorrhagic hypotension. This fact corroborates a systemic condition compatible with hemorrhagic shock. No difference was observed in the final values of plasma lactate, regardless of treatment methods (blood or hypertonic solution). The authors stress that the elevation of plasma lactate can be a sign of severity in patients with hemorrhagic shock.^{27,28}

The major electrolytes (Na⁺, K⁺, and Ca²⁺) and plasma osmolarity were assessed in the different phases of the experiments; it was observed that, acutely, the plasma K⁺ concentration was not altered. As might be expected, sodium concentrations and plasma osmolarity showed a significant

increase in their values after infusion of the hypertonic solution when compared with the animals treated with whole blood. Hypertonic solutions of 7.5% NaCl are known to be hyperosmotic; this property has been extensively studied in patients with intracranial hypertension secondary to traumatic brain injury.^{9,29}

A decrease in plasma calcium concentration was observed in the group whose hypotension was treated with whole blood. Numerous studies on CS have shown that hypocalcemia, when found, is related to poor clinical prognosis, and that these patients often evolve to death.^{1,15} The accepted hypotheses take into account that, in these cases, the calcium is retained intracellularly and, therefore, it can activate catalytic enzymatic systems or be associated with alterations of cellular metabolism.³⁰

Hyponatremia was assessed in a study of earthquake victims, and it was suggested that low sodium levels be included as another prognostic factor for CS.³¹ The benefits of volume replacement with a hypertonic solution, as in this study, could be a new field for research.

Systemic inflammatory response syndrome may be part of the spectrum of major muscle crushing, CS, and shock.^{4,5,7} The effects of the hypertonic saline solution on inflammatory response and shock have been well investigated in experimental models.^{32,33} The final step of the study was the assessment of the variations of the morphometric data obtained in lung histology, a simple method to quantify a possible systemic inflammatory response. Regarding neutrophils, it was observed that the animals treated with the hypertonic solution presented infiltration indexes of inflammatory cells comparable to those of the mock surgery group. However, this value was increased in the blood group. In this model, treatment of hemorrhagic hypotension with blood infusion was associated with a greater infiltration of inflammatory cells in the lung, indicating a possible benefit of the hypertonic solution in modulating the inflammatory response.³⁴

Conclusion

The proposed model allowed the assessment of some of the complex pathophysiological mechanisms in the association of hemorrhagic shock and muscle compression. The hypertonic saline solution presented positive results regarding muscle edema, plasma electrolyte changes (especially calcium), and neutrophil infiltration in the lungs.

Conflicts of interest

The authors declare no conflicts of interest.

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