Effect of N-acetylcysteine in hearts of rats submitted to controlled hemorrhagic shock

Efeito da N-acetilcisteína em corações de ratos submetidos ao choque hemorrágico controlado

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DOI 10.5935/1678-9741.20140097

RBCCV 44205-1629

Abstract

Introduction: **Pharmacological therapy is a strategy for the prevention of complications associated with ischemia and reperfusion injury that occurs after volume replacement in the treatment of hemorrhagic shock.**

Objective: **The aim of this study was to evaluate the effect of N-acetylcysteine associated with fluid resuscitation in cardiac injury in a rat hemorrhagic shock model.**

Methods: **Mice Wister male rats were randomly and subjected to controlled hemorrhagic shock for 60 min. and then, subjected to resuscitation with Ringer lactate. In a group of six animals, 150mg/kg of N-acetylcysteine were added to fluid volume replacement. The animals were observed for 120 min and after this period, were euthanized and cardiac tissue was collected for histopathological analysis and measurement of thiobarbituric acid reactive substances and pro-and anti-inflammatory interleukin.**

Results: **Cardiac tissue of the group treated with N-acetylcysteine showed lower concentrations of thiobarbituric acid reactive substances (0.20±0.05 vs. 0.27±0.05, *P*=0.014) and reduced histopathological damage and edema when compared to the group whose volume replacement occurred only with Ringer lactate. There was no difference in the expression of cytokines interleukin 6 (2,138.29±316.89 vs. 1,870.16±303.68, *P*=0.091) and interleukin 10 (1.019,83±262,50 vs. 848.60±106.5, *P*=0.169) between the treated groups.**

Conclusion: **The association of N-acetylcysteine on volume replacement attenuates oxidative stress in the heart, as well myocardial damage and edema, but does not modify the expression of inflammatory cytokines.**

Descriptors: **Shock, Hemorrhagic. Heart. Acetylcysteine. Oxidative Stress. Inflammation.**

Resumo

Introdução: **A terapia farmacológica é uma estratégia de prevenção das complicações associadas à lesão de isquemia e reperfusão tecidual que ocorre após a reposição volêmica no tratamento do choque hemorrágico.**

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No financial support.

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Article received on February 17th, 2014
Article accepted on July 24th, 2014

Abbreviations, acronyms & symbols	
CONCEA	Council for the Control of Animal Experimentation
IR	Reperfusion injury
MAP	Mean arterial pressure
NAC	N-acetylcysteine
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid reactive substances
TNF-α	Tumor necrosis factor alpha

Objetivo: **O objetivo deste estudo foi avaliar a repercussão da N-acetilcisteína associada à reposição volêmica na lesão cardíaca em modelo de choque hemorrágico em ratos.**

Métodos: **Ratos Wistar, machos, foram randomizados e submetidos ao choque hemorrágico controlado por 60 minutos e, depois, submetidos à reposição volêmica com Ringer Lactato. Em um grupo de seis animais, foram adicionados 150 mg/Kg de N-acetilcisteína ao fluido de reposição volêmica. Os animais foram observados por 120 minutos e após este período foram submetidos à eutanásia e coleta do tecido cardíaco para análise histopatológica e dosagem de substâncias reativas ao ácido tiobarbitúrico e interleucinas pró e anti-inflamatórias.**

Resultados: **Foi observada menor concentração de substâncias reativas ao ácido tiobarbitúrico (0,20±0,05 *vs.* 0,27±0,05, *P*=0,014) e menor dano histopatológico e edema no tecido cardíaco do grupo tratado com N-acetilcisteína em relação ao grupo cuja reposição volêmica ocorreu somente com Ringer Lactato. Não foi observada diferença da expressão das citocinas interleucina 6 (2.138,29±316,89 *vs.* 1.870,16±303,68, *P*=0,091) e interleucina 10 (1.019,83±262,50 *vs.* 848,60±106,5, *P*=0,169) entre os grupos tratados.**

Conclusão: **A associação da N-acetilcisteína na reposição volêmica atenua o estresse oxidativo no coração, assim como dano e edema miocárdicos, porém, não modifica a expressão de citocinas inflamatórias.**

Descritores: **Choque Hemorrágico. Coração. Acetilcisteína. Estresse Oxidativo. Inflamação.**

INTRODUCTION

Trauma is the third death cause in the world, compromising mainly young and adult people.Bleeding is the major cause of the early death related to trauma.Additionally, deaths will occur due to severe injury to internal organs in the next hours, or due to multi-organ failure and sepsis, lately[1].

Hypoperfusion, following hemorrhagic shock, generates a global hypoxia that promotes the release of inflammatory cytokines and neutrophils activated from the splanchnic territory, notably from the liver and intestine, which via the bloodstream or lymphatic circulation promotes injuries to distant organs. This acute phase response of the trauma is characterized by the production and release of cytokines such as the alpha tumor necrosis factor alpha (TNF-α), interleukins 1β, 6 and 8[2].Although oxygen is essential for the survival of the tissues, during the restoration of perfusion, the cells suffer a harmful effect, characterizing the reperfusion injury[3].

Alterations induced by ischemia and reperfusion injury (IR) can be related to two different mechanisms. One of them, characterized by excessive production and subsequent release of reactive oxygen species (ROS), highly cytotoxic during the reperfusion phase, whose oxidative state biochemical markers are the end products of lipid peroxidation, among which the thiobarbituric acid reactive substances (TBARS)[4]; the other, by the interaction of polymorphonuclear and capillary endothelial cells, mediated by inflammatory cytokines and cell adhesion molecules[5].

In an attempt to minimize the damages caused by ROS, cardiac myocytes use antioxidant systems – substances that slow down or inhibit oxidative aggression. The most important endogenous antioxidants are the superoxide dismutase, catalase, glutathione peroxidase, and vitamin E. These systems are overloaded after ischemia and reperfusion[6].The damage to cardiac myocytes can happen, then, by cell-to-cell contact (neutrophils – myocyte) with the release of oxidative cytokines and proteolytic enzymes. This accumulation and infiltration of neutrophils in the organ's parenchyma is a fundamental step for development of the trauma's secondary injury[7]. The cardiac dysfunction established contributes to aggravate the hypoperfusion injury in other organs during the shock and may result in death.

Associated with fluid replacement therapy, the pharmacological therapy has gained prominence in the reduction of deleterious effects of immune-inflammatory phenomena of bleeding and the volume replacement therapy[8]. Among the antioxidant drugs, the N-acetylcysteine (NAC) – a low-

cost, highly available, low-adverse effects substance – must be highlighted. Widely used in a number of medical science fields, it was initially used as a mucolytic agent. Its use was then extended to antidote for acetaminophen poisoning and prevention of contrast-induced nephropathy[9].

The *in vivo* NAC is metabolized in cysteine, which is a precursor of glutathione.In its reduced and oxidized forms, the glutathione participates – together with the glutathione peroxidase – in the ROS degradation cascade, removing free radicals. Thus, NAC can help restoring depleted glutathione reserves, replenishing cellular thiols during the IR process[10].

On IR injury, the NAC mechanism of action occurs by direct reaction with nitric oxide. This effect seems to occur after ROS release, protecting endothelial cells and subsequent activation of Kupffer cells. Its action through the sulfhydryl groups prevents the reaction of nitric oxide with the superoxide radical, hydrogen peroxide, and hydroxyl radical, preventing the formation of peroxynitrite and its consequences, such as lipid peroxidation, protein denaturation and DNA damage[11].

Despite of being widely used in medical practice and experimental models of IR injury, the literature about the use of NAC in the treatment of hemorrhagic shock and its possible protective effect in cardiomyocytes is scarce. As satisfactory results were observed with the use of NAC as protective drug of lung and liver tissue in experimental studies with controlled hemorrhagic shock models[12,13], as well as in other studies that used tissue IR injury models[14-16], the aim of this study was to assess the possible cardioprotective effect of adding NAC to volume replacement solution after induction and maintenance of controlled hemorrhagic shock.

METHODS

Animals

Male Wistar rats (RattusnorvegicusAlbinus), with ages between 90 and 120 days, and average weight of 319±26g, were used.

All animals were handled according to the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D.C., 1996) and the animal experimental ethical principles of the National Council for the Control of Animal Experimentation (CONCEA).Study protocol approved by the Research Ethics Committee of Universidade Federal de São Paulo, Protocol No. 1712/11.

Anesthesia and operative procedure

The animals were weighed and anesthetized with ketamine (50 mg/Kg) + xylazine (15 mg/kg) by intraperitoneal injection. They were considered anesthetized after being in deep sleep without reaction to mechanical stimuli, with loss of righting reflexes and member withdrawal after painful stimulus caused by gripping and palpebral reflex. Additional doses of the anesthetic cocktail (half the initial dose) were provided to animals as necessary during the procedure, which were also kept spontaneously ventilating in ambient air.

The right common carotid artery, right external jugular vein, and the right femoral artery were cannulated with Intracath® 22G (Bencton-Dicknson, Sandy, EUA).Heparin and resuscitation fluids were injected with venous catheter, according to the experimental groups; arterial catheters were used to the bleeding that caused the shock and monitoring of the mean arterial pressure (MAP), whose values allowed establishing the effectiveness of the procedures employed.

Experimental groups and induced controlled hemorrhagic shock

After the surgical procedure, the animals were divided into the following study groups:

Control group (GC, n=6): without induction of hemorrhagic shock, suffering euthanasia shortly after the post-operative stabilization period [15 minutes (min)];

Ringer's lactate group (RL, n=6): induced hemorrhagic shock. 33 mL/kg of Ringer's lactate solution (RL) plus 50% of the blood withdrawn were used for volume replacement for 20 min.

Ringer's lactate group combined with NAC (RLNAC, n=6): induced hemorrhagic shock. 150 mg/kg of NAC[17] dissolved in 33 mL/kg of RL solution plus 50% of the blood withdrawn were used for volume replacement for 20 min.

Non-fractional sodium heparin was infused before induction of hemorrhagic shock (100 UI/rat). Next, blood was removed through the arterial catheter for an interval of 10 min, using a 10 mL previously heparinized syringe, until reaching MAP of 35 mmHg. This pressure was maintained for 60 min, removing or reinserting heparinized whole blood, in the case of ±5 mmHg change in MAP.

To control the MAP, the arterial catheter was connected to a pressure transducer, connected to a calibrated preamp and a data acquisition computerized system (Dixtal DX 2020), in which the hemodynamic data (MAP and heart rate) were stored.

After 60 min of the beginning of hemorrhagic shock, the animals were submitted to volume replacement with the treatments specified above. The volume resuscitation was considered successful when the MAP remained above 80 mmHg for at least 5 min. After the shock and resuscitation stages, the animals were monitored for another 120 min; after this period, euthanasia was performed by exsanguination, under anesthesia.

Euthanasia and organ removal

After euthanasia, median thoracotomy was performed and the heart was collected. Part of the left ventricle was

immediately frozen in liquid nitrogen and stored at -70° C. Another fragment was fixed in 10% formaldehyde solution. Next, this fragment was dehydrated in growing ethanol concentrations according to the histological techniques for inclusion in paraffin. The tissue fragment was cut in sections of 4 µm and stained with hematoxylin and eosin solution.

Determination of Lactate and Serum Potassium

In order to assess the metabolic changes caused by hemorrhagic shock and the effectiveness of treatments, arterial blood samples (0.3 mL/animal) were collected for evaluation of lactate and serum potassium, in pre-heparinized syringes, before the shock induction, at the end of the shock period, and at the end of the stabilization after volume reanimationphase (Radiometer ABL 555, Copenhagen, Denmark).

Determination of thiobarbituric acid reactive substances in cardiac tissue

A fragment of the left ventricle was withdrawn after euthanasia and frozen at -70° C; subsequently, it was homogenized in 1 ml of KCl 1.15% with sonicator (PT3100 Polytron) and used to determine the TBARS.

The lipid peroxidation of cardiomyocytes' cell membranes caused by the formation of free radicals was established by means of the TBARS dosage method[18], which value was expressed as nanomoles per milligram of protein (nmol/mg of protein).For this purpose, after homogenization the aliquots were centrifuged at 10,000 rpm for 20 min at 4° C (5804® Centrifuge Eppendorf, Hamburg, Germany). For reaction, 100 µL of supernatant, 100 µL of 8.1% sodium dodecil sulphate, 750 µL of 20% acetic acid, and 750 µL of 0.8% thiobarbituric acid were added. The mixture was heated for 50 min at 95° C. After the period established, 200 µL samples were analyzed in the 532 mn spectrophotometer (Multiscan Ex, MTX Labsystems, Virginia, USA).The results were expressed as µg/mg of protein. All analyses were performed in duplicate.

Determination of protein Interleukin 6 and 10 (IL-6), (IL-10) in cardiac tissue

The determination of IL-6 and IL-10 in cardiac tissue previously frozen in liquid nitrogen was performed using the Duo-set ELISA method (R & D Systems, Inc., Minneapolis, MN, EUA).Initially, the tissue samples were macerated and homogenized in PBS at a concentration of 1 mg/mL. After this procedure, the samples were centrifuged at 2600 rpm (Eppendorf 5804R Hamburg, Germany) for 15 min at 6° C.The collected supernatant was used in the measurements.

On the 96 well plate, 100 µL/well of capture antibody anti-IL-6 or anti-IL-10 were added. After incubation for one night at 4° C, the supernatant was discarded and the plate was washed three times with wash buffer. Then a block reaction was performed by adding 200 µL/well of 2% bovine serum albumin (BSA) in PBS and incubation for one hours at room temperature (20 to 26° C).The plate was again washed three times with wash buffer. It was added in duplicate 100 µL/well of standard and samples and incubating the plate for two hours at room temperature. For standard curves, recombinant IL-6 or IL-10 were used in the concentrations of 62.50; 125; 250; 500; 1000; 2000; 4000 e 8000 pg/mL. After repeating the plate washing procedure, 100 µL/well of biotinylated detection anti-IL-6 (400 ng/mL) or anti-IL-10 (300 ng/mL) were added, and the plate was incubated for 2 hours at room temperature. At the end of incubation, the plate washing process was repeated and then 100 µL/well of streptavidin peroxidase enzyme were added in the proportion of 1:200 of enzyme: PBS with 0.05% of tween-20 and incubation for an hour at room temperature protected from light. Next, the plate wash cycle was repeated and the reaction revealed by adding 3.3' tetramethylbenzidine in one well and incubation for 60 min at room temperature protected from light. The reaction was blocked by adding 50 µL/well of H_2SO_4 (1N) and the optical density of samples at 450 nm (Multiscan Ex, MTX Labsystems, Virginia, USA) was evaluated immediately after the reaction blocking. All analyses were made in duplicate.

Histopathological Analysis

An experienced pathologist assessed the histology slides qualitatively on light microscopy (Zeiss Axio Image A2, Oberkochen, Germany), blind to the groups. At least twenty cutting areas were randomly chosen and analyzed. The severity of histological lesions was assessed through parameter-based scores: myocardial damage, assessed by the presence of contraction bands and eosinophils; leukocyte infiltration, assessed by the presence of neutrophils, macrophages and lymphocytes; and interstitial edema. Each parameter was assessed by a score using the following scale: 0 – absent; 1 – slight; 2 – moderate; 3 – severe; and 4 - very severe[19].

The total score corresponding to inflammatory lesions was performed by summing the values ascribed to each parameter for each animal (total ranging from 0 to 12).

Statistical Analysis

The data are presented as mean ± standard deviation.

The data were analyzed by means of the SigmaStat Statistical program version 3.1 (Systat Software, San Jose, USA).

The groups were compared by Variance Analysis (One-way Variance Analysis - ANOVA) or ANOVA on ranks (Kruskal-Wallis One-way Analysis of Variance on Ranks), after normality and equality variance tests. In the event of statistical difference (P<or=0.05) the ANOVA was complemented with the appropriate post-hoc test. Differences among groups were considered statistically significant when P< 0.05.

Linear regression analysis was also performed to assess the correlation between the studied TBARS and interleukins' dosages.

RESULTS

Metabolic Analysis

At the end of the shock period, the RL and RLNAC groups showed significant lactate levels increases compared to the control group (7.23±1.03 vs 6.85±1.03 vs 1.15±0,25 mmol/L respectively; *P*=0.002).There were no significant differences at the end of the stabilization after volume reanimationphase in lactate levels between the three groups (2.89±0.94 vs 2.75±0.99 vs 1.75±1.09 mmol/L, respectively; *P*=0.101).

Serum potassium levels also showed significant increase in groups RL and RLNAC when compared with the control group after the shock period (6.68±0.44 vs 6.86±0.84 vs 4.95±0.39 mmol/L, respectively; *P*<0.001).However, at the end of the experiment, group RL presented the highest potassium level in comparison with the RLNAC group (5.95±0.75 vs 5.02±0.59 mmol/L, respectively; *P*=0.026).

Oxidative stress in cardiac tissue

Figure 1 shows the results concerning the quantification of TBARS in cardiac tissue for study groups. The TBARS dosage in cardiac tissue at the end of the stabilization after volume reanimation presented statistically significant increases in RL groups (0.27±0.05 nmol/mg protein) and RLNAC (0.20±0.05 nmol/mg protein) in relation to the control group (0.03±0.02 nmol/mg protein); however, TBARS values decreased in RL-NAC group in relation to the RL group (*P*=0.014).

Protein dosage of pro- and anti-inflammatory interleukins in cardiac tissue

Figures 2 and 3 show the results concerning the quantification of IL-6 e IL-10 in cardiac tissue for study groups.

It may be seen that the IL-6 dosages at the end of the post-treatment stabilization period were higher in RL (1.870±303.68 pg/mg protein) and RLNAC (2.138±316.89 pg/mg protein) groups, in relation to the control group (GC) (462.28±70.24 pg/mg protein), without any differences among treated groups (*P*=0.091). Likewise, IL-10 dosages presented increases in treated groups (848.58±106.48 and 1.019±262.51 pg/mg protein, respectively) in relation to the GC (247.31±39.82 pg/mg protein), without any differences among treated groups (*P*=0.169).

The linear regression analysis suggests positive association between dosages of TBARS and IL-6 (r2=0.744, *P*<0.001) and TBARS and IL-10 (r2=0.638, *P*<0.001).

Histopathology of heart tissue

Animals in the RLNAC group presented significantly lower myocardial damage when compared with the RL group (score 1 (1-2) vs. 2.5 (2-5), *P*=0.049), as well as for edema scores (score 0 (0-1) vs. 2 (1-2), *P*=0.016).There were no differences on edema scores between the RLNAC groups and the control group (*P*=0.935) (Figure 4 A-C).

The evaluation of myocardial inflammatory infiltrate showed similarities between the three groups (*P*=0.427).

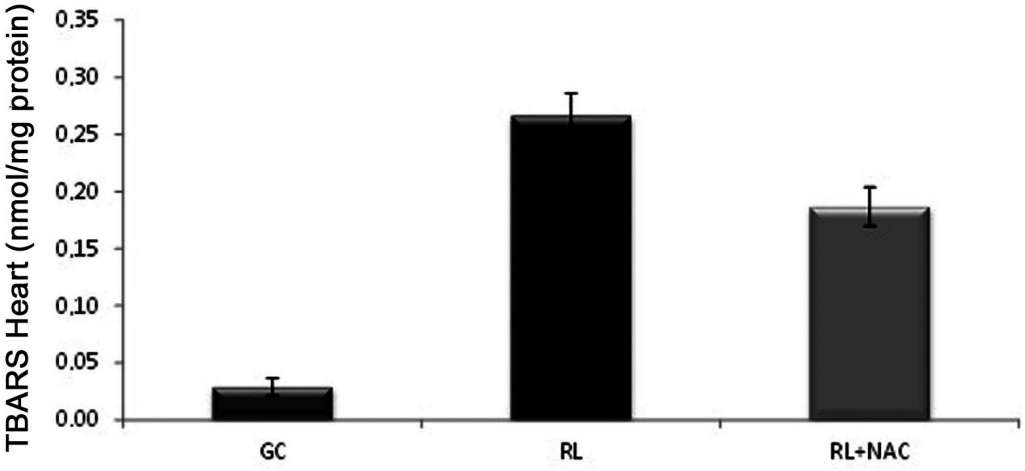

Fig. 1 - Thiobarbituric acid reactive substances (TBARS) values in cardiac tissue in the control group (GC), Ringer Lactate (RL) and Ringer lactate with N-acetylcysteine group (RLNAC). GC < RL, RLNAC; RLNAC < RL, P< 0.05.

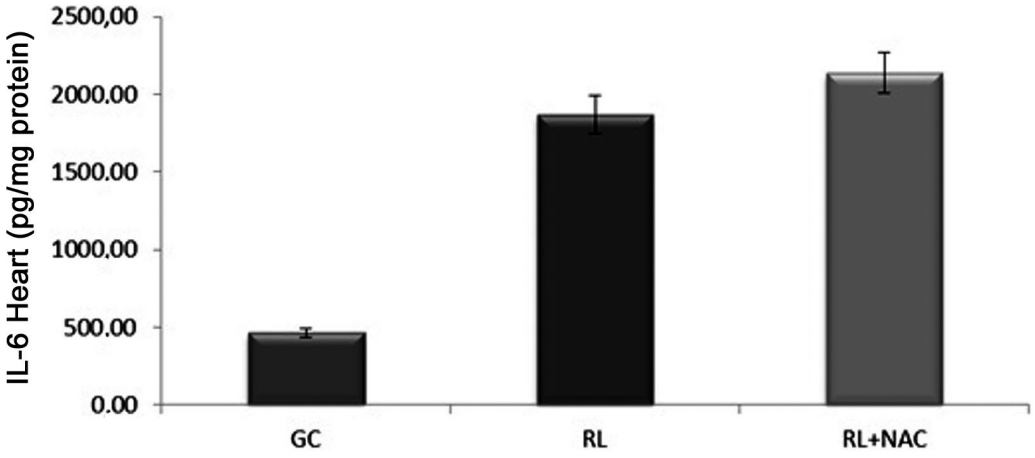

Fig. 2 - Interleukin 6 expression in cardiac tissue for the control group (CG), Ringer Lactate group (RL) and Ringer lactate with N-acetylcysteine group (RLNAC).
** Significant difference when compared to CG, (P<0.05) .*

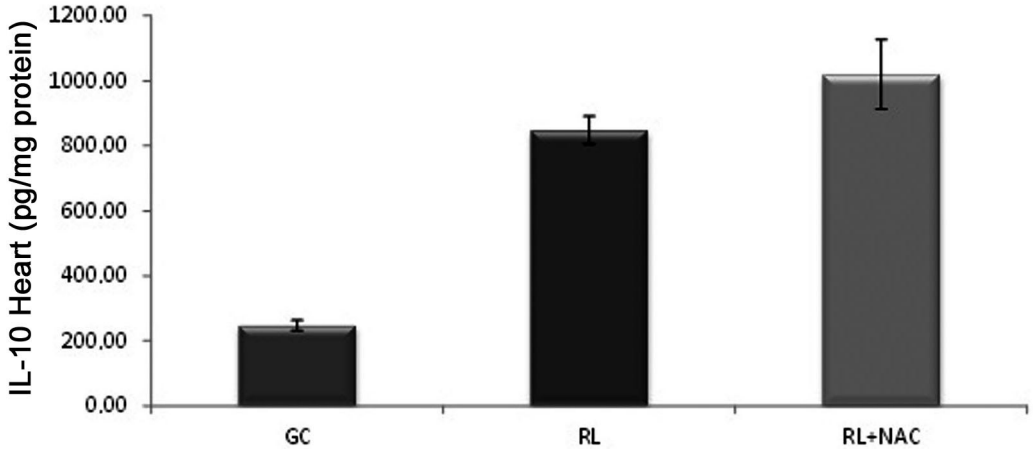

Fig. 3 - Interleukin 10 expression in cardiac tissue for the control group (CG), Ringer Lactate group (RL) and Ringer lactate with N-acetylcysteine group (RLNAC).
** Significant difference when compared to CG, (P<0.05).*

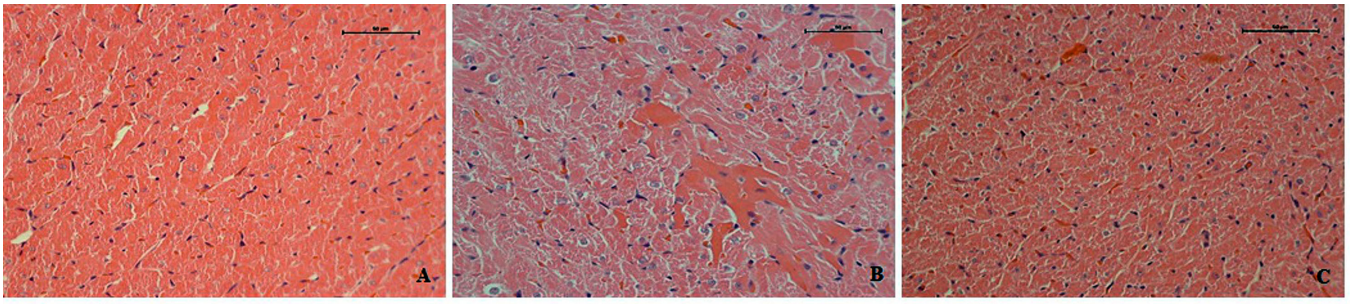

Fig. 4 - Photomicrographs of heart tissue stained with HE of animals subjected to hemorrhagic shock and fluid resuscitation with Ringer's lactate (RL Group, photomicrograph B) or Ringer Lactate associated with N-acetylcysteine (RLNAC Group, photomicrography C), compared to a control group (Group GC, A photomicrograph). It is observed in A, normal myocardium; B, myocardial damage by the presence of cardiomyocyte hipereosinophilic (more intense pink); C, myocardial preserved.

DISCUSSION

The results suggest that the NAC plays a promising role in the pharmacological therapy combined with fluid replacement in treating hemorrhagic shock, reducing tissue damage, edema, and oxidative stress on the cardiac tissue. To the extent of our knowledge, this is the first study that assessed the NAC effect on heart injury in a controlled hemorrhagic shock model in rats.

With regard to biochemistry data, the lactate – an important tissue stress predictor – presented a significant increase during the shock, followed by normalization after volume reanimation, although without NAC's intervening. Nevertheless, the treatment with NAC reduced the potassium levels.

After the beginning of the ischemia that follows the shock, the oxidative phosphorylation is exhausted and the anaerobic metabolism becomes the primary source of ATP production. Such break down in the cell's energetic condition leads to an accumulation of extra-cell potassium. The mechanism that causes potassium accumulation is not fully explained. The Na-K pump is inhibited in ischemic muscle cells models, contributing to reduce the K influx parallel to ATP-sensitive potassium channels, and it may be the main mechanism through which potassium efflux increases during muscle cell ischemia[20].

In an experimental study assessing secondary systemic changes to a prolonged hemorrhage hypertension condition, Torres et al.[21] noted that the potassium increase was related to mortality and could explain sudden and early death of some animals during the experiment. While evaluating the role potassium plays as a marker of tissue hypoxia in an experimental model, Rocha Filho et al.[20] noted that the increase in potassium serum levels complied with hemodynamic deterioration, finding a strong correlation between potassium and lactate levels. NAC, by acting on microcirculation and improving tissue perfusion, may take part in potassium washout restoring the aerobic metabolism. However, an in-depth evaluation is necessary to clarify whether this findings may be ascribed or not to the NAC'S protection role. No data have been found in literature to corroborate such fact.

In this study, it was noted that the myocardium damage and edema induced by hemorrhagic shock were lessened by volume replacement reanimation and NAC. Although the hemorrhagic shock was maintained for 60 minutes, no leucocitary infiltrated in the cardiac tissue was noticed. Such results agree with the studies performed by Meurs et al.[22], who evaluated the neutrophil recruitment in several organs in hemorrhagic shock protocols. The authors pointed out that, in the heart, the early expression of adhesion molecules in the microvascular bed was not accompanied by the leucocitary recruitment, different from lungs, liver, and kidneys, in which the expression of adhesion molecules was accompanied by an expressive leucocyte migration to tissues.

However, in our study, the TBARS dosage in the cardiac tissue at the end of the stabilization after volume reanimation presented significant increases, describing the lipid peroxidation injury, which was attenuated by NAC.

NAC effects on IR injuries were dose-dependent. While studying lung pre-conditioning with different doses of NAC to prevent IR injury after liver injury by reflow, Weinbroum et al.[17] noted that the 100 mg/kg dose attenuated the liver injury but not the lung one. High doses, such as 225 mg/kg, could imply a suppression of the properties that protect macrophages and monocytes residing in lungs, resulting in a decrease in lungs defense. The authors have concluded that the 150 mg/kg dose was more effective to reduce accumulation of xanthine oxidase in the liver tissue, reducing the tissue damages caused by ROS.

Although this study shows the protecting effect of NAC on the oxidative stress in cardiac tissue, and that there is a positive correlation between oxidative stress and increase in the inflammatory cytokines, it did not show tissue reduction of pro-inflammatory IL-6.

Experimental studies have shown that the expression of the ribonucleic acid messenger of IL-6 (RNAm IL-6) is increased based on hypoxia conditions, mainly in the lungs, liver, and intestines of rats submitted to hemorrhage, inducing the cardiomyocytes to produce IL-6.Kupffer cells are the most important producers of systemic IL-6 after the shock[23]. Such increase in the genic expression and IL-6 levels in the cardiomyocytes occurs mainly two hours after the hemorrhagic shock has begun and is correlated to the cardiac dysfunction[24].

The mechanism whereby IL-6 promotes cardiac dysfunction has not been completely explained. Studies[24,25] suggest that IL-6 could act in activating the κB (NF- κB) nuclear factor that, in turn, would activate the transcription of inflammatory cytokines, chemotaxins, and adhesion molecules, notably the ICAM-1 in the heart. Such cascade of events would favor neutrophils adhesion and migration processes through the endothelial barrier to the interstitial space and parenchymatous tissue, with consequent myocardial damage.

Despite the increased levels of IL-6 noted in the hearts of both groups submitted to hemorrhagic shock, there was no difference in the scores for leucocitary infiltration for all study groups, including the GC group. The experimental protocol follow-up of this study is considered short to be able to verify myocardium infiltrate, because the increase in interleukins dosages takes place before inflammatory cells are present in the tissue.

In this study, we noted that the shock protocol activated the inflammatory cascades with significant increase of IL-6 and IL-10; nevertheless, there was no interference in the modulation with NAC in reducing IL-6 and increasing the expression of the IL-10.Mukherjee et al.[26] reported that the NAC treatment caused decreased dosage of serum IL-6 and

increased plasma dosage of IL-10 in neonatal rats after two hours of induced septic shock. However, the authors state that, after 4 hours from the beginning of the experiment, the serum levels of IL-6 and IL-10 were similar in the groups, showing that the effect of the administration of NAC on interleukins expression is time-dependent. Therefore, they suggested once again that longer experimental protocols are needed to elucidate the effect of NAC on the expression of interleukins in hemorrhagic shock.

CONCLUSION

NAC showed a protective role in the cardiac tissue of rats submitted to hemorrhagic shock, mainly in lessening oxidative stress and histologic injury. Nevertheless, new studies must be performed that should consider the use of larger NAC doses associated with longer observation protocols in order to allow analyzing data regarding the late stage of the shock.

Authors' roles & responsibilities	
LDOF	Analysis and/or interpretation of data, final approval of the manuscript, design and study design, operations and/or experiments conduct
KRS	Final approval of the manuscript, conception and design of the study, operations and/or experiments conduct, manuscript writing or critical review of its content
PFS	Analysis and/or interpretation of data, final approval of the manuscript, study design, manuscript writing or critical review of its content
MKK	Analysis and/or interpretation of data, statistical analysis, final approval of the manuscript, manuscript writing or critical review of its content
SMS	Analysis and/or interpretation of data, final approval of the manuscript, operations and /or experiments conduct
EFSM	Analysis and/or interpretation of data, final approval of the manuscript, study design, manuscript writing or critical review of its content

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