Coconut water as a resuscitation fluid and their effect on colon anastomosis healing in rats with hemorrhagic shock

Água de coco como fluido de ressuscitação e seu efeito na cicatrização de anastomoses do cólon em ratos com choque hemorrágico

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ABSTRACT

Background/purpose: The hemorrhage and shock are important predictors of mortality and a serious complication particularly found in the perioperative colorectal surgery. Currently, there is no ideal fluid for volume replacement in shock. Relevant knowledge on the composition of coconut water (CW) is available, but studies on its use for resuscitation in shock are scarce. The aim of this study is to examine the influence of CW as a resuscitation fluid on the healing of colonic anastomosis in a model of hemorrhagic shock induced in rats. **Methods**: We used three groups of Wistar rats (6 each), weighing 251±18g. In group 1, rats were shocked and treated with Ringer lactate (RL); in Group 2, shocked rats were treated with modified CW (3% Na); Group 3 rats were not shocked and untreated (control). **Results:** The average rupture pressures of anastomoses were higher in the group submitted to hemorrhagic shock and treated with CW (190.5±7.5 mmHg) than in rats subjected to shock and treated with RL (159.2±6.6 mmHg). This difference was significant ($p<0.05$); in the control group the rupture pressure was 186.8±5.6mmHg. Comparing with the group treated with CW, the difference was not significant ($p>0.05$). Histopathological analysis showed better healing wounds in the group treated with CW, although the differences were not statistically significant comparing with the other groups. **Conclusion**: The data from this study showed that the volume replacement with CW with 3% sodium influenced positively the healing of colonic anastomoses in rats submitted to hemorrhagic shock model.

Key words: Hemorrhagic shock. Ressuscitation. Coconut water. Healing. Colon anastomosis.

RESUMO

Objetivo: A hemorragia e choque são importantes preditores de mortalidade e uma complicação grave particularmente encontrada no perioperatório de cirurgia colorretal. Atualmente, não há um fluido ideal para reposição volêmica em estado de choque. Há conhecimento relevante sobre a composição nutricional de água de coco (AC), masestudos sobre sua utilização para reanimação no choque são escassos. O objetivo deste estudo é analisar a influência da AC como fluido de ressuscitação na cicatrização de anastomose colônica em modelo de choque hemorrágico em ratos. **Métodos:** Foram utilizados três grupos de ratos Wistar (6 em cada), pesando 251±18g. No grupo 1, os ratos chocados foram tratados com Ringer Lactato (RL); no Grupo 2, os ratos chocados foram tratados com AC modificada (3% de Na); Os ratos do grupo 3 não eram chocados e não foram tratados (controle). **Resultados:** As pressões médias de ruptura das anastomoses foram maiores no grupo submetido a choque hemorrágico e tratados com AC (190,5±7,5 mmHg) do que em ratos submetidos ao choque e tratado com RL (159,2±6,6 mmHg). Esta diferença foi significativa (p<0,05); no grupo de controle a pressão de ruptura foi 186,8±5.6mmHg que, em comparação com o grupo tratado com AC, a diferença não foi significante (p>0,05). A análise histopatológica mostrou melhor cicatrização das anastomoses no grupo tratado com AC, com diferenças não significantes em comparação com os outros grupos. **Conclusão:** Os dados deste estudo mostraram que a reposição volêmica com AC com 3% de sódio influenciou positivamente a cicatrização de anastomoses colônicas em ratos submetidos a modelo de choque hemorrágico.

Descritores: Choque hemorrágico. Ressuscitação. Água de coco. Cicatrização. Anastomose. Cólon.

INTRODUCTION

The shock is characterized by the inability of the body to maintain an adequate organ perfusion[1] leading to tissue hypoxia, which can be secondary to several factors such as blood volume, which can be seen in hemorrhagic shock. Thus, the impairment of the gastrointestinal tract mucosa performs an important role in the breakdown of homeostasis, leading to systemic effects, allowing the release of inflammatory mediators that can interfere with quality of intestinal anastomosis[2].

The bleeding and shock are important predictors of mortality. Concerning to trauma, the exsanguination is involved in 44% of pre-hospital deaths, in 55% of deaths after hospital admission and 82% of intraoperative trauma deaths[3].

In patients with hemorrhagic shock is preferable to avoid surgery of the gastrointestinal tract[4] like colon anastomosis, because they are more likely than most other gastrointestinal anastomosis for leaks and breaks-up[5]. The anastomotic dehiscence is a serious complication in colorectal surgery. The failure in the healing of anastomosis is associated with increased length of hospital stay, morbidity and mortality and is therefore an important socio-economic problem.

Among the colorectal surgeries, at least one third of the deaths can be attributed to anastomotic dehiscence. The failure of colon anastomosis healing is related to factors involving surgical techniques either as a complex interaction between growth factors and peptide residues of collagen, through the phases of inflammation, fibroplasia and maturation[6].

Healing is a complex process influenced by many factors, among these the general state of the patient, the blood supply and tissue oxygenation, the quality of the local vasculature, blood volume, surgical technique and the site of anastomosis[7]. Therefore, hemorrhagic shock will directly harm the healing process as the major bleeding results in decreased oxygen supply, lack of energy supply and tissue injury[3].

Given the emergence of hemorrhagic shock, administration of different types of fluids is of paramount importance in the treatment of hypovolemia, frequently observed in the perioperative period. Among the types, isotonic solutions were, for many years, the main fluid used in volume replacement. However, sometimes its use promoted satisfactory results especially in the treatment of severe shock. Considering the decrease in tissue perfusion observed in hemorrhagic shock, fluid therapy aims to restore tissue oxygenation in order to normalize body functions as a whole including the healing of anastomosis[8].

From this perspective, the control of hemorrhagic shock is done by the bleeding control and by the rapid restoration of blood volume. Thus, there are several solutions that can be administered for fluid replacement. The current fluids include the crystalloid solutions, plasma, albumin or synthetic colloids such as gelatin solutions, dextran, and more recently hydroxyethyl starch being the most used solutions for initial resuscitation in hemorrhagic shock, according to the ATLS® guide lines. Among the crystalloid solutions with higher clinical applicability are sodium chloride 0.9%, also known as normal saline, Ringer's lactate and glucose solution associated with the sodium chloride solution[9].

A few clinical studies have evaluated the influence of different fluids administered in the perioperative period. Studies evaluating the effect of different reposition fluids in the healing of intestinal anastomosis are scarce[10].

Harlak et al observed that anastomotic safety seems to be affected by the type of fluid used: lactated Ringer's (LR), hydroxyethyl starch (HES) and hypertonic saline solution 7.5% (HS) all seemed to enhance healing as effectively as transfused blood[10].

Experimental studies are needed to test new resuscitation fluids, and in the present protocol we introduced the coconut water to treat hemorrhagic shock and influence colon anastomosis healing. The justification of the original use of this fluid is based on growing scientific evidence supporting the role of coconut water in applications for health. This fact is due to its chemical composition of sugars, antioxidants, vitamins, electrolytes, minerals and amino acids[11]. We worked with the hypothesis that coconut water has a favorable biochemical composition which, when used in hemorrhagic shock, may contribute to a good volume resuscitation and influence the healing of intestinal anastomosis.

The aim of this study is to analyze the effect of modified coconut water, as resuscitation fluid in a model of hemorrhagic shock, in the wound healing of colon anastomosis in rats.

METHODS

Male Wistar rats weighing 251±18 g were maintained in the animal facilities of the Nucleus of Experimental Surgery from Federal University of RN, Brasil on a 12:12 light-dark cycle with free access to standard laboratory feed (Presence®) and water. All animal experiments were performed according with the requirements of the Brazilian law nº 11794/08, which completely accord with the CONCEA guidelines for the care and use of laboratory animals. This protocol was approved by the institutional Ethics Committee on the use of Animals.

The animals were randomly selected and allocated into 3 groups, six rats each, all undergo colonic anastomosis: Group 1 - shocked rats treated with ringer lactate. Group 2 – rats treated with 3% sodium coconut water -. Group 3 (Control) - rats not shocked, untreated.

Surgical procedures

The rats were housed individually in polypropylene cages. Initially the animals underwent a 7 days of acclimatization in the laboratory. One day before the operations, the animals were maintained on a liquid diet. For shock and surgical procedure the rats were anesthetized with intramuscular ketamine 50 mg/kg, associated with xylazine 20 mg/kg body weight and operated under aseptic technique.

The colonic resection was performed with the rats under shock. It included a 2 cm segment of colon at 10 cm from ileocecal valve. Intestinal continuity was restored with end-to-end anastomosis, using 6-0 polypropylene, with a surgical microscope DFV (São Paulo, Brazil), 10x magnification. The postoperative pain was controlled with meperidine 10 mg/kg i.m. once a daily, for the first 3 days).

Water was allowed in first 24 hours after surgery, followed by solid diet until euthanasia. The animals were weighed prior to the operation and the time 7th postoperative day, when euthanasia was performed with an overdose of anesthetic (thiopental 100mg/kg i.p.).

Hypovolemic shock

After stabilization of anesthesia, shock was installed. The right femoral vein and left femoral artery were dissected. 24F silicone gauge cannulae were inserted into the femoral artery for monitoring of mean arterial blood pressure and into the femoral vein for further volume resuscitation. After a stabilization period, blood was drained through the femoral artery until the arterial blood pressure stabilized around 35 mmHg. The hypovolemic shock was maintained for 30 min. After this period, the animals received volume replacement over 10 min. The replacement volume was equal to the blood lost volume. Each infused solution for volume replacement was heated at 37ºC. The rats were kept warm in the microenvironment at a temperature of 37ºC.

Coconut water was obtained from coconut Cocus nucifera L 6 months (period from the inflorescence to the harvest of the fruits). Coconut water was removed with sterile technique immediately before administration to the animals. It was appropriately modified to reach 3% sodium level. The pH of the coconut water was previously adjusted to 7.4 using sodium bicarbonate 20% and the liquid filtered in sterile filter prior to intravenous infusion.

The ringer lactate solution was of from B. Braun, Rio de Janeiro, Brazil. All solutions were infused i.v, using pump infusion (B. Braun, Rio de Janeiro, Brazil). The calculated volume was injected for 10 min at a constant infusion rate. After shock, anastomosis and infusion, the animals were observed for 7 days, when they were euthanized with an overdose of anesthesia (thiopental 100 mg / kg) IP, and colon anastomosis were evaluated.

Anastomotic resistance Assessment

The colonic anastomosis site was subjected to the resistance test pressure on the 7th postoperative day, as follows. After anesthesia a relaparotomy was made. The colon was cut proximal and distal to anastomosis site. A n° 6 Polyethylene catheter was introduced at its proximal end, then tied with No. 00 cotton suture; a second catheter was inserted into the distal end of colon, also tied with 00 cotton suture. This catheters were connected to a digital manometer. The peritoneal cavity was filled with saline solution, keeping the colonic anastomosis zone immersed into liquid. The proof system was inflated with O_2 at continuous flow of 0.5 liters per minute. The maximum rupture pressure of the anastomosis was recorded when appeared bubbling air into the aqueous solution.

Colon healing evaluation.

A segment containing the anastomosis was fixed in 10% formalin and embedded in paraffin. 5 µm sections were stained with hematoxylin-eosin and Masson's trichrome and examined an experienced pathologist, without prior knowledge of their groups. Quantitative analysis was taken as the amount of leukocytes, fibroblasts and collagen fibers, using a scanner system and image analysis. The total area of the microscopic field was observed by optical microscope (Olympus BX50), whose image was captured by Olympus high resolution camera and scanned through the ImageProplus software, (Cybernetics- Media LP, USA). Each digitized field was quantified in pixel. After selected the desired resolution, the images were stored for measuring the density of the inflammatory reaction.

Statistics

Statistical analysis was performed using SPSS 17.0 software for the analysis of variance (ANOVA) followed by Tukey's test, considering significant differences at $p<0.05$.

RESULTS

Table 1 - Descriptive results of weight variables, colon anastomotic resistance and their inferential statistical tests.

Variables	Groups			P-value
	Shock+anast+coconut water	Shock+anast+ringer	Anastomosis	
Weight (g)	275.3±8.7	290.5±22.2	285.2±9.6	0.232[1]
Pressure (mmHg)[2]	190.5±7.5[¥]	159,2±6,6[¥]†	186.8±5.6†	< 0.001[1]

Mean ± Standard deviation
1 – p-value analysis of variance (ANOVA).
2 - Values - at line - followed by similar symbols have statistically significant differences between groups, referenced by the Tukey test.

The rats were weighed at the end of the observation period and the statistical analysis showed no significant difference ($p>0.05$) between groups. The similarity was expected, as the rats were randomly chosen and were undergoing the same diet and environment conditions, in a short period of time (7 days).

The average of the anastomosis burst pressure was higher in the group submitted to hemorrhagic shock and treated with the modified coconut water (190.5+7.5) than in group shocked and risen with Ringer lactate solution (159.2+6.6); the group subjected only to anastomosis showed an intermediate value in the burst pressure averages rating (186.8+5.6).

According to the statistical analysis performed by the Tukey test, we observed that the shocked rats treated with coconut water showed differences in anastomoses burst pressure that were statistically significant compared to the shocked rats and raised with ringer lactate ($p<0.001$). However, the rats group treated with coconut water, when compared to rats submitted only to intestinal anastomosis, showed similar burst pressure results, with no statistically significant difference between these samples. Comparing the anastomosis only animals, with the group anastomosis+shock+treatment with ringer, the difference was statistically significant ($p<0.001$).

The colon incision site was easily identifiable from the surrounding normal tissue in histological sections, because of the changes observed in colonic structure. Complete wound healing was observed in the blades of animals submitted only to anastomosis. The new mucosal epithelium was thinner than the normal epithelium around, in the three groups. The submucosa and muscle layers of the colon lost its normal structure and were mostly replaced by a repair tissue with newly formed collagen fibers, especially in the group of animals treated with coconut water and anastomosis group. (Figures 1 and 2).

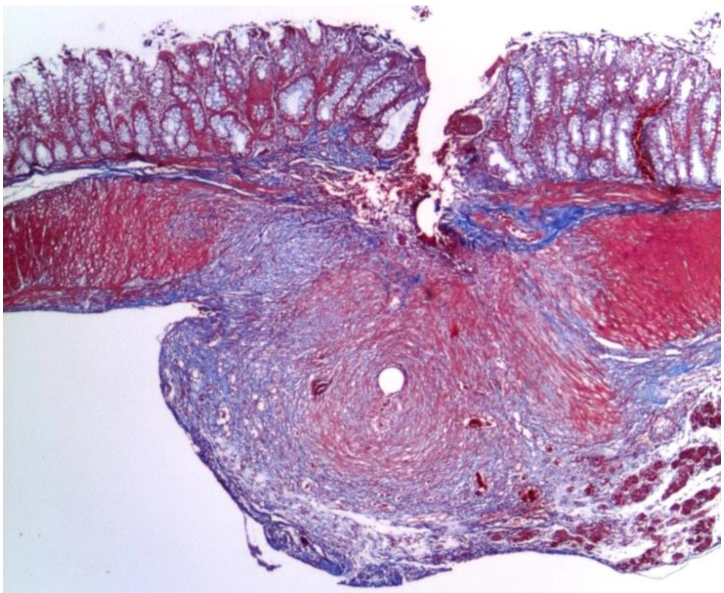

Figure 1 – Chronic inflammatory process exacerbated + giant cell foreign body reaction + initial fibrosis + fibrin.Masson's Trichrome Staining 200x

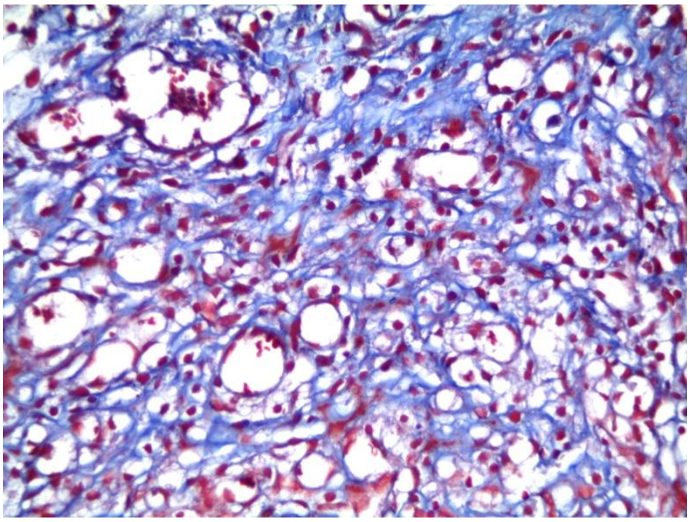

Figure 2 - Repair tissue with fresh collagen fibers stained in blue with Masson's trichrome. Histological section of anastomosis rat group + shock treatment with coconut water. 400x.

The rats submitted to hemorrhagic shock and treated with ringer had predominant chronic transmural inflammation exacerbated sometimes with abscess, giant cell foreign body reaction, initial fibrosis and fibrin deposition. (Figures 3 and 4).

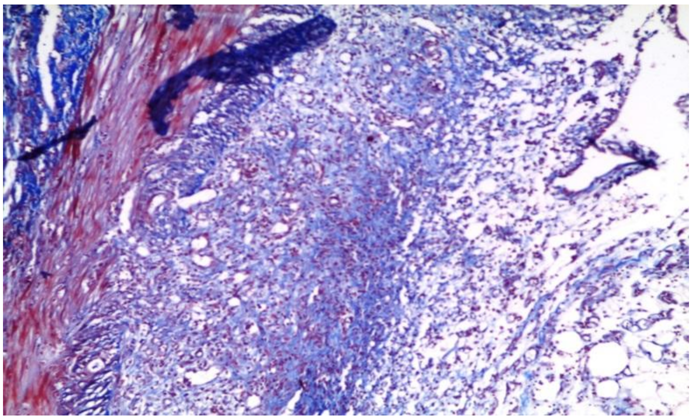

Figure 3 - Transmural intense inflammatory reaction and in mucosa. Masson's trichrome. 200x.

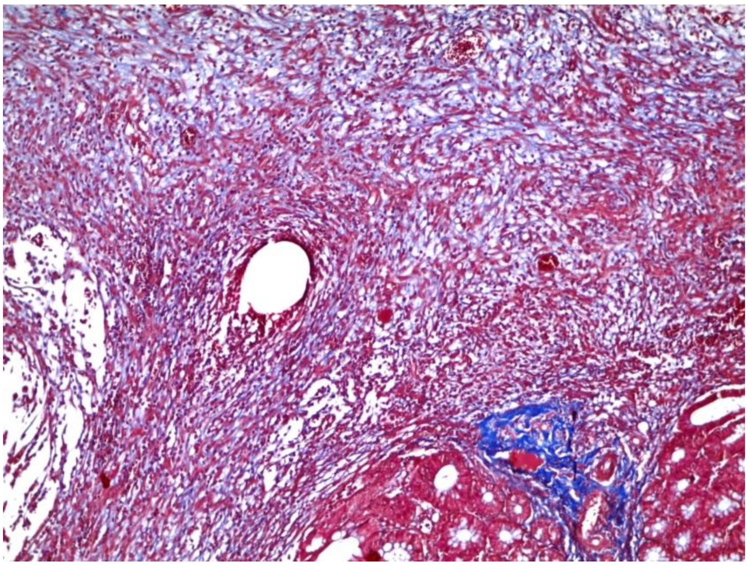

Figure 4 - Transmural inflammatory reaction, giant cells and fibrosis. Masson's trichrome, 200x.

The measurement results showed better healing of wounds in the group treated with coconut water, although not statistically significant differences when comparing the groups.

DISCUSSION

It is known that the hypovolemic shock causes tissue damage and increases hypoxia and the pro-inflammatory activity in the body. Thus, a rapid fluid resuscitation by suitable fluids is essential to minimize damage and to improve the healing process. Therefore, the present study shows an encouraging alternative to reduce the damage caused by hypovolemia using the modified coconut water, which showed positive results compared to the traditional fluid resuscitation (ringer lactate) in addition to match qualitatively with the anastomoses repar that did not suffer the process of hypovolemia and consequent hypoxemia.

In the present study, we compared the quality of healing in colonic anastomosis in three groups: rats with induced hemorrhagic shock, resurrected with conventional crystalloid (Ringer lactate); rats with induced shock, resurrected with modified coconut water and not shocked rats, submitted a colon anastomosis only.

Hemorrhagic shock is responsible for promoting a systemic inflammatory response, which contributes to the development of future organic complications such as dehiscence and infection of surgical sites.Studies show that the decreased blood flow to a certain organ after hemorrhagic shock causes a decrease O_2 in tissue and, therefore, there is an increased anaerobic metabolism and the reduction of ATP levels and intracellular calcium. The reduction of ATP stimulates release of inflammatory cytokines such as TNF, IL - 1, IL - 6, and an increase in prostaglandin E2.[13] The fluid resuscitation strategy besides reduce mortality, is directly related to the response variation to systemic inflammation and cytokine production. [14] Therefore, the choice of a particular fluid resuscitation of hemorrhagic shock was directly related to tissue injury and the intensity of tissue inflammation.

TNF is considered the major inflammatory agent in hemorrhage and plays a crucial role in enhancing the other pro-infamatórias cytokines (IL-1, IL-6) and self destructive excessive inflammation.[15] Farias et al (2013) showed a statistically significant reduction in the expression of pro inflammatory cytokines in rats raised with modified coconut water instead of other fluids used for volume replacement in mice under study (saline and fresh blood).[14]

Therefore, it would be the resuscitation fluid widespread during hemorrhagic shock a key to the healing process in colonic anastomoses?

In our study, we observed a direct reduction in the healing process in rats shocked raised with ringer lactate compared to the anastomosis group. Thus, it is evident in a practical way the aforementioned studies, attributing this result to likely tissue damage resulting from hypovolemic shock. We still found a higher burst pressure of the anastomoses in the shocked group and treated with coconut water, compared to the ringer lactate group, indicating a higher resistance of the anastomosis and probably more effective healing process. The anastomosis + shock + coconut water group obtained results equivalent to the anastomosis without shock group, possibly infer a reduction of tissue damage caused by hypovolemic shock in the group that had the coconut water as resuscitation fluid.

These results obtained from the infusion of coconut water, are possibly related to their physicochemical properties. Published studies have increasing scientific evidence of the use of coconut water in practices and medical applications, which can be justified by its unique chemical composition of sugars, vitamins, minerals, amino acids, organic acids, fatty acids.[11,16] It has also been demonstrated by spectrometry the presence of selenium as a constituent of coconut water, indicating that this can also be used as a source of the mineral, which is an essential element. [17] In addition, also presents an important antioxidant, protecting the body from oxidative damage[18], caused for example by hypoxia due to hemorrhagic shock.

The literature reveals significant changes in depression of nonspecific immunity due to bleeding and hemorrhagic shock, such as decreased opsonization and phagocytic activity. Moreover, the reduction of cellular immunity and decrease in B cell function were also observed.[20] Thus, hemorrhagic shock increase the susceptibility to infection of the wound which could favor the disruption of anastomoses[19]. However, surgical infection was not observed in our study, a factor that could affect the results.[20]

CONCLUSION

Data from this study showed that the volume replacement with coconut water content of Na 3% had a positive influence in the development of colon anastomosis healing in rats submitted to hypovolemic shock model.

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