EXPERIMENTAL STUDY

Effects of hypertonic saline replacement on colonic anastomosis in experimental hemorrhagic shock model in rats

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ABSTRACT

BACKGROUND: Inadequate intestinal perfusion resulting from hemorrhagic shock negatively affects wound healing. In this experimental study, we aimed to evaluate the effects of resuscitation with hypertonic saline on colonic anastomosis in rats with controlled hemorrhagic shock.

METHODS: A total of 24 male Wistar albino rats weighing between 200 and 250 g were used in this study. The rats were divided into four groups as: Control, hypotonic, isotonic, and hypertonic. Median laparotomy, colon resection, and colocolonic anastomosis were performed to the rats in the control group. After creating controlled hemorrhagic shock to rats in other three groups, resuscitation was achieved with hypotonic, isotonic, and hypertonic saline. After resuscitation, median laparotomy, colon resection, and colocolonic anastomosis were performed on rats in these three groups. On the 5th post-operative day, a median laparotomy was applied to the rats in all groups and anastomosis lines were evaluated. Anastomotic bursting pressure, tissue hydroxyproline level, and tissue fibrosis degree were compared between the groups.

RESULTS: There was no statistically significant difference between the groups in terms of anastomotic bursting pressure, tissue hydroxyproline level, and tissue fibrosis degree (respectively; p=0.320, p=0.537, p>0.05).

CONCLUSION: In rats with controlled hemorrhagic shock, resuscitation with hypertonic saline does not differ significantly from isotonic or hypotonic saline in terms of healing of colonic anastomosis.

Keywords: Colonic anastomosis; fluid resuscitation; hemorrhagic shock; hypertonic saline.

INTRODUCTION

Shock is defined as inadequate perfusion at the cellular level and its most common types are hypovolemic/hemorrhagic shock.[1,2] The mortality rate in patients with hemorrhagic shock secondary to trauma is 30–40%.[1,3] In this period which is regulated by neuroendocrine, metabolic, and immune responses, loss of intravascular volume causes an increase in vascular resistance and thus leading to a decrease of blood flow in the skin, and both gastrointestinal and renal systems.[3,4]

There is no consensus about the ideal fluid type to be used in fluid resuscitation in patients with hemorrhagic shock, but crystalloids are considered to be the first in fluid preference.[5] It is known that hypertonic saline, which reduces the incidence of acute respiratory distress syndrome and multi-organ failure and has an immune-modulating effect by reducing free oxygen radicals, can provide resuscitation with a much smaller volume.[5–7]

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The risk of mortality was further increased in patients who were clinically in shock in the first presentation, had blunt and penetrating colon injury.[8] Anastomosis after resection of the damaged segment in extended colon injuries is an increasingly common practice and the issue of anastomosis safety is a priority problem for surgeons.

Numerous local and systemic factors such as ischemia, hypovolemia, local infections, surgical technique, steroids, chemotherapeutic agents, malnutrition, and diabetes mellitus effect colonic anastomosis healing.[9–13] There are few studies in the literature regarding which fluid has a favorable effect on colonic anastomosis healing during shock resuscitation.

In this experimental study, we aimed to evaluate the effects of resuscitation with hypertonic saline on colonic anastomosis in rats with controlled hemorrhagic shock.

MATERIALS AND METHODS

The present study was performed at Istanbul University University Aziz Sancar Experimental Medicine Research Institute after the approval of İstanbul University Animal Experiments Local Ethics Committee. 24 Wistar-albino male rats weighing 200–250 g were used for the study. The rats were randomized into four groups as follows:

Group 1: Control Group

Following median laparotomy and colon resection under general anesthesia, a single layer of end to end colocolonic anastomosis was performed with 5/0 polypropylene suture. No controlled hemorrhagic shock was created during the procedure.

Group 2: Hypotonic Replacement Group

Under general anesthesia (2 ml/100 g) of blood was taken from the tail vein through 24G cannula to create controlled hemorrhagic shock. Then 6 ml/100 g of hypotonic saline (0.45% NaCl) was replaced and resuscitation was achieved. After resuscitation median laparotomy and colonic anastomosis was performed with the same standards.

Group 3: Isotonic Replacement Group

Under general anesthesia (2 ml/100 g) of blood was taken from the tail vein through 24G cannula to create controlled hemorrhagic shock. Then 4 ml/100 g of isotonic saline (0.9% NaCl) was replaced and resuscitation was achieved. After resuscitation median laparotomy and colonic anastomosis was performed with the same standards.

Group 4: Hypertonic Replacement Group

Under general anesthesia (2 ml/100 g) of blood was taken from the tail vein through 24G cannula to create controlled hemorrhagic shock. Then 1 ml/100 g of hypertonic saline (7% NaCl) was replaced and resuscitation was achieved. After resuscitation median laparotomy and colonic anastomosis was performed with the same standards.

All surgical procedures were performed between 09:00 and 12:00, to overcome the possible effects of diurnal hormonal changes on rats using sterile surgical instruments. The rats were anesthetized with an intramuscular injection of 10 mg/kg xylazine (Alfazyne, 2%, Alfasan, Woerden, Holland) and 80 mg/kg ketamine (Ketalar, Pfizer Pharma, GMBH Germany).

Rats were housed in groups of three in cages with a temperature of 21 ± 1°C and 12 h of light/dark cycles, fed with standard laboratory diet and tap water ad libitum, and visited every day. On the 5th post-operative day, a median laparotomy was applied to the rats in all groups under anesthesia. Colon lumen was closed with silk suture 2 cm distally and proximally from the colonic anastomosis line. The pressure sensor mercury manometer was placed in the lumen. Methylene blue was slowly infused into the lumen, and the pressure at which methylene was leaked from the anastomosis was recorded as bursting pressure. A tissue sample taken from the anastomosis line was sent to the biochemistry laboratory to measure tissue hydroxyproline level (ng/mg). Tissue hydroxyproline concentration was determined using the Chloramine-T spectrophotometric method as previously described by Reddy and Enwemeka.[14] Another sample taken from the anastomosis line was evaluated histopathologically by a single expert pathologist using a conventional light microscope. The grade of tissue fibrosis was evaluated according to a scale defined by Hooker et al.[15] It was scored and recorded as 0: Nil, 1: minimal fibrosis, 2: moderate fibrosis, and 3: high grade fibrosis.

Statistical Analysis

Average, standard deviation, median lowest, and highest frequency values were used in the descriptive analysis of the data. The distribution of variables was measured by the Kolmogorov–Smirnov test. One-way ANOVA test, Chi-square test, Fisher's test, and Mann–Whitney U test were used to compare the groups. The statistical significance lev-

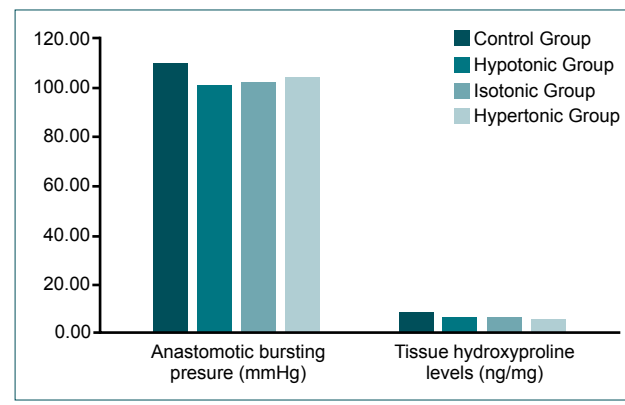

Figure 1. Comparison of anastomotic bursting pressures and tissue hydroxyproline levels of the groups.

Table 1. Comparison of anastomotic bursting pressures and tissue hydroxyproline levels of the groups

	Control Group	Hypotonic Group	Isotonic Group	Hypertonic Group	p
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Anastomotic bursting pressure* (mmHg)	110.2±8.4	101.3±9.3	102.5±7.2	104.5±9.3	0.320
Tissue hydroxyproline levels** (ng/mg)	9.4±6.8	7.0±2.1	6.8±3.6	6.0±2.3	0.537

*Mann-Whitney U test; **One-way ANOVA test. SD: Standard deviation.

Table 2. Comparison of anastomosis line tissue fibrosis levels of the groups

Tissue fibrosis level*	Control Group (n)	Hypotonic Group (n)	Isotonic Group (n)	Hypertonic Group (n)	p
0	–	–	–	–	
1	2	5	3	4	
2	3	1	3	2	>0.05
3	1	–	–	–	

*Chi-square test.

el was set as p<0.05 for all analyses. All statistical analyses were performed with SPSS version 20 (IBM Inc., Chicago, USA).

RESULTS

The mean anastomotic bursting pressure and tissue hydroxyproline levels of the groups are shown in Table 1 and Fig. 2a, b.

Although the mean anastomotic bursting pressure of the hypertonic group was significantly higher than the isotonic and hypotonic groups, this difference was not statistically significant (p=0.320).

The median tissue hydroxyproline level was highest in the control group and lowest in the hypertonic saline group. There was no significant difference between the groups in terms of the tissue hydroxyproline level (p=0.537).

As a result of the histopathological evaluation done for determination of fibrosis grade, in the control group minimal grade fibrosis was observed in two rats, moderate in 3, and high grade in 1. In the hypotonic saline group, minimal fibrosis was observed in five rats and moderate in 1, in the isotonic group minimal fibrosis was observed in three rats and moderate in 3, and in the hypertonic group, minimal fibrosis was observed in four rats and moderate in 2 (Table 2 and Fig. 2a, b). No high grade fibrosis findings were observed in any of the groups except the control group. There was no statistically significant difference between the groups in terms of tissue fibrosis grade (p>0.05).

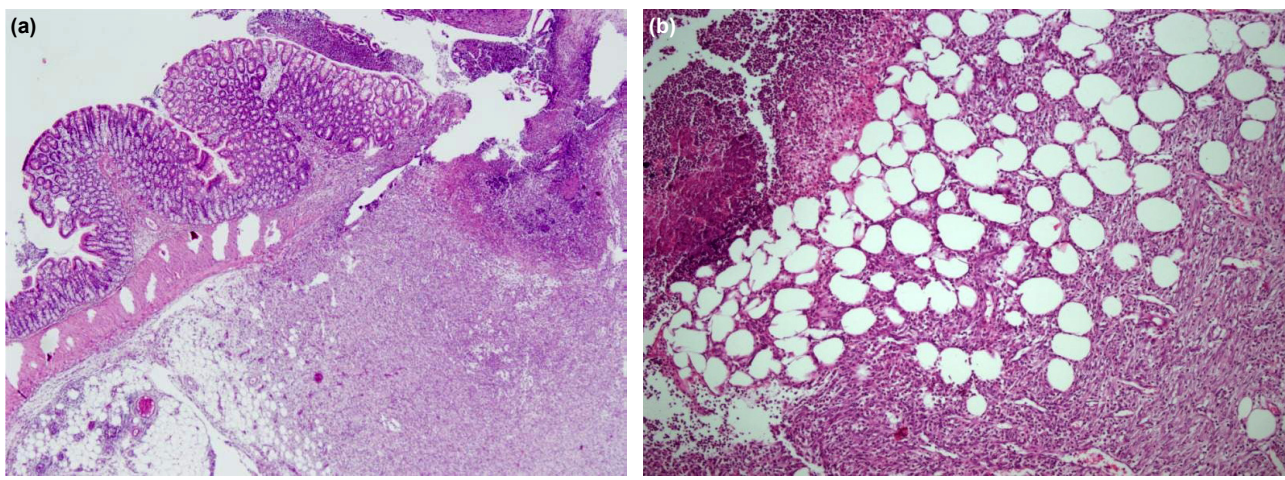

Figure 2. (a) Histopathological examination of the isotonic group demonstrates the minimal fibrosis (HE, ×40). **(b)** Moderate fibrosis findings in a hypertonic saline group rat (HE, ×100).

DISCUSSION

We frequently encounter patients with multi-trauma and penetrating or blunt abdominal injuries in the emergency services. Almost always surgical intervention is need for those patients. In this patient group with high mortality risk and decreased tissue perfusion, appropriate pre-operative and post-operative fluid resuscitation is critical for both ensuring hemodynamic stability and rapid normalization of perfusion and ideal wound healing.

Hypertonic solutions ensure the passage of interstitial fluid into intravascular space, improvement of cardiac contractility, increase of mesenteric oxygenation, and smooth muscle relaxation.[16,17] The increased oxygen and nitrogen products during hemorrhagic shock initiate a series of inflammatory processes. Macrophage and Kupffer cells affected by this process have an important role in liver dysfunction and multi-organ failure. Sharma et al.[18] showed that hypertonic saline replacement preserves liver function, improves hemodynamic parameters, and suppresses the inflammatory process during hemorrhagic shock. Crystalloid solutions pass readily through the capillary membrane and therefore need to be replaced in large volumes to ensure effective intravascular volume. Large volume fluid replacement during shock resuscitation temporarily improves intravascular volume but subsequently causes edema and impairment of oxygen perfusion in the tissues. It has been reported that large volumes of crystalloid fluid replacement negatively affects intestinal anastomosis stability.[19] Chiara et al.[20] showed that small volume replacements of hypertonic saline solutions during hemorrhagic shock regulated both systemic and splanchnic circulations and reported that this does not cause an increase in pulmonary artery pressure, does not lead to tissue edema, and only causes a temporary sodium elevation.

Intestinal ischemia develops after hemorrhagic shock and this affects the healing process of anastomosis following resection. Experimental studies have shown that hypertonic saline significantly reduces intestinal mucosa apoptosis through heme oxygenase-1 mRNA over-expression.[21] Zakaria et al.[22] experimentally showed that hypertonic saline replacement significantly improves intestinal microcirculation in hemorrhagic shock.

Anastomotic bursting pressure is the best parameter showing anastomosis healing in the early period.[23] Marjanovic et al.[24] showed that bursting pressure was higher in colloid fluids than crystalloids in experimental intestinal anastomosis models. Comparing hypertonic saline, ringer lactate, hydroxyethyl starch (HES), and albumin replacements in their experimental study in rats, Harlak et al.[25] observed no significant statistical difference in terms of anastomotic bursting pressure between the four fluids. In our study, the mean anastomotic bursting pressure of the hypertonic saline group was the closest to the control group but this difference was not statistically significant.

Collagen is the main building protein of the skin, bone, and all living tissues. The collagen molecule is formed from hydroxylation of hydroxyproline and hydroxylysine. In the intestinal anastomosis collagen synthesis starts after 12 h and its synthesis and breakdown continue in a balanced manner. Marjanovic et al.[24] reported that tissue hydroxyproline level was significantly higher in patients with colloid fluid replacement than those with crystalloid replacement. Similar to our study Harlak et al.[25] reported that tissue hydroxyproline level did not differ significantly with the given fluid type.

Wound healing is a process that results in fibrosis which is an indicator of fibroblastic activity in the tissue. The grade of fibrosis in the anastomosis line is important for anastomosis safety. Eroglu et al.[26] in the experimental sublethal hemorrhagic shock model, demonstrated that gelatin significantly increased fibrosis in wound healing compared to HES, dextran, and isotonic. Harlak et al.[25] in their experimental study observed that there was no significant difference between ringer lactate, HES, hypertonic saline, and albumin replacements in terms of tissue fibrosis. In our study, we observed no significant difference between the groups in terms of fibrosis grade.

There are very few experimental studies and data similar to our study in the literature. However, the limited number of rats used in the experiment and single-dose replacement of the fluid rather than continuous infusion is the limitations of our study. Although, the superiority of hypertonic saline in colonic anastomosis healing over other fluids could not be shown statistically in this study, it can be considered as the ideal replacement fluid in the resuscitation of hemorrhagic shock since it has few side effects and can maintain hemodynamic stability with small volume.

Conclusion

The data obtained from this experimental study show that the resuscitation of hypertonic saline in rats with controlled hemorrhagic shock model did not differ statistically from isotonic and hypotonic saline replacement in terms of anastomotic bursting pressure, tissue hydroxyproline level and fibrosis degree. However, it is a fact that there is a need for further experimental and clinical prospective studies with larger numbers.

Ethics Committee Approval: This study was approved by the Istanbul University Animal Experimental Ethics Committee (Date: 28.03.2013, Decision No: 30387).

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DENEYSEL ÇALIŞMA - *ÖZ*

Sıçanlarda deneysel hemorajik şok modelinde hipertonik salin replasmanının kolon anastomozu üzerine etkileri

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AMAÇ: Hemorajik şok sonucu gelişen yetersiz intestinal perfüzyon yara iyileşmesini olumsuz etkilemektedir. Bu deneysel çalışmada kontrollü hemorajik şok oluşturulan sıçanlarda hipertonik salin ile resüsitasyon yapılmasının kolon anastomozu üzerine etkilerini değerlendirmeyi amaçladık.
GEREÇ VE YÖNTEM: Çalışmada, ağırlıkları 200–250 gr arasında olan toplam 24 erkek Wistar albino sıçan kullanıldı. Sıçanlar kontrol, hipotonik, izotonik ve hipertonik olmak üzere dört gruba ayrıldı. Kontrol grubundaki sıçanlara median laparotomi, kolon rezeksiyonu ve kolokolonik anastomoz uygulandı. Diğer üç gruptaki sıçanlara kontrollü hemorajik şok oluşturulduktan sonra hipotonik, izotonik ve hipertonik salin ile resüsitasyon sağlandı. Resüsitasyon sonrası bu üç gruptaki sıçanlara da median laparotomi, kolon rezeksiyonu ve kolokolonik anastomoz yapıldı. Ameliyat sonrası beşinci gün tüm gruplara median laparotomi yapıldı ve anastomoz hatları değerlendirildi. Gruplar arasında anastomotik patlama basıncı, doku hidroksiprolin seviyesi ve doku fibrozis derecesi karşılaştırıldı.
BULGULAR: Anastomoz patlama basıncı, doku hidroksiprolin seviyesi ve doku fibrozis derecesi açısından gruplar arasında istatistiksel anlamlı fark saptanmadı (sırasıyla; p=0.320, p=0.537, p>0.05).
TARTIŞMA: Kontrollü hemorajik şok oluşturulan sıçanlarda, resüsitasyonun hipertonik salin ile yapılmasının kolon anastomozu iyileşmesi açısından izotonik veya hipotonik salinden anlamlı bir farkı yoktur.

Anahtar sözcükler: Hemorajik şok; hipertonik salin; kolon anastomozu; sıvı resüsitasyonu.

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