

Resuscitation with modified gelatin causes higher bacterial translocation in experimental sublethal hemorrhagic shock

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Summary

The effect of colloidal solutions on bacterial translocation was studied. Sublethal hemorrhagic shock was established by blood withdrawal until the mean arterial pressure fell to 40 mmHg within 15 min on 36 adult Wistar Albino rats. Resuscitation was performed using four different solutions with the same amount of blood. Group I (n = 9) 0.9% NaCl, Group II (n = 9) 10% dextran 40, Group III (n = 9) 6% hydroxyethyl starch, Group IV (n = 9) 4% modified fluid gelatin. Before resuscitation and after anesthesia blood samples were drawn to analyze pH, P_{CO₂}, P_{O₂}, SaO₂, HCO₃ and ABE values. Twenty-four hours after anesthesia laparotomy was performed to obtain tissue samples of the liver, spleen and mesenteric lymph nodes. Samples were cultured on EMB and blood agar media. Results were analyzed with the one-way ANOVA and Post-hoc test (Tukey's HSD).

The translocated bacteria were mainly *Escherichia coli* and three grew in Group I, two in Group II, three in Group III and six in Group IV. Although there was a trend in difference in bacterial translocation rates among groups, statistical analyses revealed no difference among groups (p < 0.05).

It can be concluded that resuscitation with modified gelatin causes higher bacterial translocation in an experimental sublethal hemorrhagic shock model.

Key words: Hemorrhagic shock; Resuscitation; Bacterial translocation.

Introduction

In trauma patients surviving initial resuscitation and operation, infections and organ failure are the most common causes of death or serious complications [1]. Intestinal barrier function is compromised following severe hemorrhage which may allow bacterial translocation to occur and subsequently initiate a systemic response leading to multiple system organ failure. Following hemorrhage, Kupffer cell and splenic macrophages, and peritoneal macrophage antigen presentation function were significantly depressed [2]. Coliform bacteria are the most frequently reported bacteria to translocate after hemorrhage [3]. It was shown that fluid resuscitation in an experimental peritonitis and hemorrhagic shock model increased survival [4]. However the most appropriate solution for volume replacement is controversial. In light of this knowledge we designed an experimental study to find out if colloidal solutions would have any effect on bacterial translocation in sublethal hemorrhagic shock.

Materials and Methods

Adult Wistar Albino rats (250-285 g) (from the Animal Laboratory Unit of SDU) were used in the study. All of "the guiding principles in the use and care of laboratory animals" were strictly adhered to throughout the study. Animals were housed under conventional environmental conditions at an ambient temperature with free access to pellet rodent chow and tap water (drinking bottle) ad libitum until the time of the experiment.

Under general anesthesia with ketamine (40 mg/kg IP) + xylazine (8 mg/kg IP), the anterior neck regions of the animals were shaved and a transverse incision was made to isolate the carotid artery. After right carotid artery cannulation with a 22-gauge angiocatheter, rats were monitored to calculate mean arterial pressure (MAP). Hemorrhagic shock was initiated by blood withdrawal until the MAP rose to 40 mmHg within ten min. Blood was collected and put in 0.1 ml citrate/ml to prevent clotting. After a hypotensive period of 20 min, animals were resuscitated with four different solutions in a volume equal to blood loss: Group I (n = 9) 0.9% NaCl, Group II (n = 9) 10% dextran 40, Group III (n = 9) 6% hydroxyethyl starch and Group IV (n = 9) 4% modified fluid gelatine. In order to emulate the operative procedures of trauma events, inhalation anesthesia with isoflurane (2 MAC/1 hour duration with 6 lt./min O₂) was applied after resuscitation. Before resuscitation and after anesthesia peripheral blood samples were drawn to analyze total protein, albumin, pH, pCO₂, pO₂, SaO₂, HCO₃ and ABE values.

Twenty-four hours later, the rats were re-anesthetized with the same anesthetics and the abdomen was opened with a midline incision under sterile conditions. Tissue samples from the mesenteric lymph nodes, spleen and liver were collected aseptically. Tissue samples were washed in sterile saline (0.9% NaCl) and homogenized. Tissue homogenates were placed on EMB and blood agar culture media. All culture plates were incubated for 24 h at 37°C and bacteria were identified by using standard techniques. Animals were sacrificed five days after the experiment.

All quantitative measurements were expressed in mean values (± standard deviation) and statistically analyzed with the one-way ANOVA and Post-hoc test (Tukey's HSD). The incidence of bacteria presence was analyzed by using the chi-square test. Statistical significance was accepted as p < 0.05.

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Results

Bacterial translocation was seen in all groups: three animals in Group I, two animals in Group II, three animals in Group III and six animals in Group IV (Table 1). Besides *Escherichia coli*, *Enterobacter* and *Klebsiella* species were also isolated in some cases. Although there was a trend in the difference of bacterial translocation rates among groups, statistical analyses revealed no differences among groups ($p > 0.05$).

In analyses of blood samples which were drawn before resuscitation and after anesthesia there were statistically significant differences among groups; pH and pO_2 values were increased in all groups after resuscitation and a decrease was seen in pCO_2 values. There were however no statistical differences in pH, pO_2 and pCO_2 values among groups after resuscitation (Table 2). On the other hand there were significant differences in HCO_3 and ABE and SaO_2 levels after resuscitation. HCO_3 , ABE and SaO_2 values were significantly decreased in Group II ($p < 0.05$).

Discussion

It has been shown that nonlethal hemorrhage causes bacterial translocation in various experimental models [5-7]. The time of resuscitation is also important in that longer periods of hypotension before resuscitation cause higher mortality rates and a higher incidence of bacterial translocation [7]. Factors that promote bacterial translocation from the gut include disruption of the indigenous intestinal microflora [8], impaired host immune defenses [9, 10] and physical disruption of the intestinal mucosa

[11]. It has also been reported that animals in hemorrhagic shock could not mount a normal inflammatory response [12] and that the peritoneal clearance of bacteria was impaired [13]. In addition to animal studies the rate of bacterial translocation in blunt abdominal trauma has been shown to increase with presence of hemorrhagic shock [14].

Traditionally, gut ischemia has been regarded as the cause of intestinal injury after hemorrhagic shock [15]. However it has also been shown that increased local protease activity [16] and oxygen free radicals [17], decreased TNF and IL-6 release [18] were associated with significantly increased mucosal injuries. Fluid resuscitation in hemorrhagic shock decreases the incidence of bacterial translocation [4, 19, 20]. In a study by Behrman *et al.* it was indicated that resuscitation in hemorrhagic shock alters blood flow in the superior mesenteric artery [21]. Synthetic colloids are widely used for replacement of blood loss during major surgical procedures. The main reason for using colloidal volume replacement is to maintain the circulating blood volume by stabilizing plasma oncotic pressure in the perioperative period [22].

Conclusion

It can be concluded that resuscitation with modified gelatin causes higher bacterial translocation in an experimental sublethal hemorrhagic shock model.

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Table 1. — Presence of bacterial translocation in tissue samples.

	n	Mesenteric lymph node	Spleen	Liver	Isolated bacteria
Group I	9	3/9	2/9	2/9	E. Coli, Klebsiella
Group II	9	2/9	0/9	0/9	E. Coli
Group III	9	3/9	0/9	0/9	E. Coli, Enterobacter
Group IV	9	6/9	3/9	3/9	E. Coli, Enterobacter

Table 2. — pH, pCO_2 , pO_2 , HCO_3 , ABE and SaO_2 values of groups before resuscitation (b) and after anesthesia (a) (mean \pm standard deviation).

	Group I	Group II	Group III	Group IV
pH-(a)	7.18 \pm 0.05	7.17 \pm 0.05	7.12 \pm 0.05	7.21 \pm 0.02
pH-(b)	7.20 \pm 0.11	7.13 \pm 0.09	7.23 \pm 0.06	7.22 \pm 0.11
pCO_2 -(a)	71.3 \pm 10.6	67.94 \pm 13.57	79.53 \pm 14.46	64.08 \pm 8.60
pCO_2 -(b)	63.15 \pm 11.96	53.76 \pm 16.52	54.36 \pm 13.30	55.25 \pm 12.69
pO_2 -(a)	74.63 \pm 18.70	61.05 \pm 20.91	45.35 \pm 12.87	55.60 \pm 6.98
pO_2 -(b)	94.71 \pm 17.87	70.78 \pm 28.80	64.87 \pm 25.90	73.55 \pm 19.31
HCO_3 -(a)	26.00 \pm 2.10	24.07 \pm 3.98	24.88 \pm 2.83	25.57 \pm 3.13
HCO_3 -(b)	24.30 \pm 3.08	17.31 \pm 4.87	22.01 \pm 3.59	22.37 \pm 4.94
ABE-(a)	-3.94 \pm 2.10	-5.82 \pm 3.45	-6.71 \pm 2.75	-3.32 \pm 2.96
ABE-(b)	-4.70 \pm 5.40	-12.38 \pm 5.44	-5.75 \pm 2.31	-5.84 \pm 6.49
SaO_2 -(a)	88.70 \pm 5.90	78.75 \pm 12.30	61.65 \pm 18.85	81.25 \pm 6.26
SaO_2 -(b)	94.58 \pm 3.11	82.20 \pm 11.95	83.46 \pm 9.92	88.41 \pm 8.50

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