Rabbit model of uncontrolled hemorrhagic shock and hypotensive resuscitation

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Abstract

Clinically relevant animal models capable of simulating traumatic hemorrhagic shock are needed. We developed a hemorrhagic shock model with male New Zealand rabbits (2200-2800 g, 60-70 days old) that simulates the pre-hospital and acute care of a penetrating trauma victim in an urban scenario using current resuscitation strategies. A laparotomy was performed to reproduce tissue trauma and an aortic injury was created using a standardized single puncture to the left side of the infrarenal aorta to induce hemorrhagic shock similar to a penetrating mechanism. A 15-min interval was used to simulate the arrival of pre-hospital care. Fluid resuscitation was then applied using two regimens: normotensive resuscitation to achieve baseline mean arterial blood pressure (MAP, 10 animals) and hypotensive resuscitation at 60% of baseline MAP (10 animals). Another 10 animals were sham operated. The total time of the experiment was 85 min, reproducing scene, transport and emergency room times. Intra-abdominal blood loss was significantly greater in animals that underwent normotensive resuscitation compared to hypotensive resuscitation (17.1 ± 2.0 ml/kg vs 8.0 ± 1.5 ml/kg). Antithrombin levels decreased significantly in normotensive resuscitated animals compared to baseline (102 ± 2.0% vs 59 ± 4.1%), sham (95 ± 2.8% vs 59 ± 4.1%), and hypotensive resuscitated animals (98 ± 7.8% vs 59 ± 4.1%). Evidence of re-bleeding was also noted in the normotensive resuscitation group. A hypotensive resuscitation regimen resulted in decreased blood loss in a clinically relevant small animal model capable of reproducing hemorrhagic shock caused by a penetrating mechanism.

Key words: Hemorrhagic shock; Animal model; Resuscitation

Introduction

Severe hemorrhage accounts for approximately 40% of all trauma-related deaths (1). Hemorrhage is the leading cause of preventable death and the first following admission to a hospital, particularly among patients in need of urgent surgery, with the death of 80% of those who die during an operation being due to hemorrhage (2). Therefore, reducing early mortality caused by hemorrhage is a major objective of research both at the experimental and clinical levels (3-5).

Animal models have played a crucial role in hemorrhagic shock research. Almost all advances and interventions in hemorrhage have been initially or concomitantly described in such models. They have become more sophisticated and capable of addressing hemostatic and physiological responses to hemorrhage (6-10). However, a major challenge faced by all experimental models is to fully mimic the human condition and being able to address many aspects of traumatic hemorrhagic shock in a clinically relevant manner (6-10).

There are basically three major types of experimental models for hemorrhagic shock: fixed volume hemorrhage, fixed pressure hemorrhage and uncontrolled hemorrhage. Some models use a combination of strategies even though they provoke distinct hemodynamic and hemostatic responses (11,12). Most experts agree that an ideal and clinically relevant model of traumatic hemorrhagic shock should incorporate severe uncontrolled hemorrhage and tissue injury. In addition, the model’s fluid resuscitation strategy should be in keeping with current clinical practice where either normotensive resuscitation - for rapid restoration of end-organ perfusion - or the more recent approach of hypotensive resuscitation for penetrating torso injuries could be applied (3,13). Another important aspect of a model of traumatic hemorrhage is to be consistent, as closely as
possible, with the transport times and fluid infusion rates of emergency medical systems (14,15).

The purpose of the present study was to develop a clinically relevant rabbit model of uncontrolled hemorrhagic shock that simulates penetrating torso trauma. The model also involved normotensive and hypotensive resuscitation regimens.

Material and Methods

The study was approved by the Animal Research Committee of Universidade Federal de Minas Gerais (CETEA/UFMG, Protocol #203/2007), Belo Horizonte, MG, Brazil. Animal experiments were performed according to a protocol approved by the University Ethics Committee in Animal Experimentation. Male New Zealand rabbits (2200 to 2800 g), 60-70 days old, were maintained at 25°C on a 12-h light/dark cycle. The animals were housed individually in an authorized facility of the Faculty of Medicine (UFMG) and were given rabbit chow (Nutricolho, Purina, Brazil) and water ad libitum. All animals were acclimated for 2 weeks before the experiment.

Animal preparation

Animals were anesthetized with 60 mg/kg ketamine and 8 mg/kg xylazine (Fort Dodge Animal Health, USA) administered intramuscularly. Additional doses were administered intravenously as needed (15 mg/kg ketamine and 2 mg/kg xylazine). The operative sites were prepared with 10% povidone iodine solution. A tracheotomy was performed and a 3-cm segment of a polyethylene catheter (4.7 mm in inner diameter) was partially inserted into the trachea. The right internal jugular vein and the right carotid artery were cannulated with polyethylene tubing (PE10; Clay Adams, USA) previously filled with lactated Ringer’s (LR) solution. A 5-cm midline laparotomy was performed under sterile conditions, and 2-0 nylon continuous full thickness running sutures were placed through the edges of the laparotomy for later abdominal closure.

Baseline blood samples

Immediately after the laparotomy phase, blood samples (a total of 3 mL) were obtained from the carotid artery for the following baseline tests: arterial blood gas (Siemens, Germany) in a 1-ML sample in a heparinized syringe, complete blood count and platelet count (STKS Coulter, USA) in a 1-ML sample in a vacuum blood collection tube containing ethylenediaminetetraacetic acid (Vacuette, Greiner Bio-one, Brazil), and activated partial thromboplastin time (aPTT) (Sysmex CA-540, USA) and antithrombin activity (STAGO, USA) in a 1-ML sample in a vacuum blood collection tube containing 3.8% sodium citrate.

Experimental groups

Thirty animals were randomly divided into three groups of 10 animals each. Sham group animals underwent the same preparation and baseline blood sampling as the other two groups, but not hemorrhagic shock. The normotensive resuscitation (NR) group received an intravenous LR solution to maintain mean arterial pressure (MAP) at baseline (pre-shock) levels. The hypotensive resuscitation (HR) group received LR to maintain MAP at 60% of baseline levels.

Hemorrhagic shock procedure

All animals were placed on a heating pad, which was not heated until intravenous fluid infusion was started and baseline rectal temperature was assessed. The right carotid artery tubing was connected to a Pro-Paq (Protocol Systems, USA) for continuous MAP monitoring. Ten minutes were allowed for stabilization of the parameters before baseline MAP was obtained. During this time the abdomen was covered with a plastic film to reduce insensible fluid losses. Hemorrhagic shock was then induced by a standardized single-puncture injury to the left side of the infrarenal aorta, 2 mm below the lower pole of the left kidney, with a 16-gauge needle (16 G Jelco® Medex, USA). The abdomen was immediately closed by pulling the previously placed sutures. For the next 15 min no fluids were infused to simulate the average time emergency medical services (EMS) would take to arrive at the scene (Figure 1). Fifteen minutes after the beginning of shock, LR solution at 39°C was infused continuously through the right jugular vein catheter with an infusion pump (Stoelting Co., USA). The infusion rate was set to maintain MAP at the predetermined levels for each group, with a 5 mL/min maximum rate. Also at this time the heating pad was turned on to maintain rectal temperature above 35°C. Fluid resuscitation was continued until 85 min after the aortic injury to simulate average transport and emergency room times. The infusion rate was reduced every time goal MAP was achieved and total LR volume was recorded for each group. A new set of blood samples for the previously described laboratory tests were obtained after 85 min, i.e., at the end of the experiment. In addition, the abdomen was opened and total intra-abdominal blood loss was calculated as the difference between blood-soaked sponges minus the weight of pre-weighed dry sponges. Animals were killed at the end of the experiment with a ketamine and xylazine overdose and oblique section of the abdominal aorta.

Statistical analysis

Data are reported as means ± SEM. Statistical analysis consisted of one-way analysis of variance (ANOVA) with post hoc analysis using the Tukey test for multiple comparisons between experimental means. The unpaired t-test was used to analyze comparisons between two groups. The level of significance was set at P < 0.05 for all analyses.

Results

Mortality

The overall mortality of animals subjected to hemor-
Hemorrhagic shock was 20% (5 of 25 animals). All 5 deaths occurred before the beginning of fluid resuscitation. Three deaths occurred within 5 min after injury to the aorta and 2 at approximately 14 min after the injury; all dead animals were replaced. A total of 20 animals survived the injury to the aorta and consequently the hemorrhagic shock. There were no deaths among sham-operated animals (N = 10).

Physiological variables and intra-abdominal bleeding

There were no statistically significant differences in baseline MAP between groups: 66.5 ± 1.6 and 65.1 ± 1.3 mmHg in the sham and NR groups, respectively, and 67.8 ± 4.2 in the HR group. Five minutes after aortic injury MAP was similarly reduced in the NR group (17.3 ± 1.2 mmHg) and HR group (15.4 ± 1.4 mmHg; P > 0.05) to a significantly lower level in both groups compared to sham-operated animals (63.7 ± 2.0 mmHg; P < 0.05). The MAP of the NR group was restored to levels similar to those of sham-operated animals at 30 min after the beginning of shock (50.2 ± 4.5; P > 0.05) with the infusion of LR solution. However, as expected, the MAP of the HR group remained at 60% of baseline, a significantly lower level than in the other two groups, throughout the resuscitation phase up to the 85th min (41.4 ± 2.8; P > 0.05; Figure 2).

Baseline rectal temperature varied between 37.5 ± 0.2 and 37.9 ± 0.1 (P > 0.05). At the end of the experiment (85th min), the rectal temperature of sham-operated animals was 36.9 ± 0.1°C compared to 36.0 ± 0.3°C for shocked animals (P > 0.05).

Animals that underwent normotensive resuscitation bled significantly more from the aortic injury than animals that underwent hypotensive resuscitation (17.5 ± 2.8 vs 8.1 ± 1.8 mL/kg; P < 0.05; Figure 3).

Fluid infusion

Significantly more intravenous fluid was required by the NR group animals to maintain their MAP at baseline levels (123.2 ± 10.1 mL/kg) compared to sham-operated animals
(21.0 ± 4.3 mL/kg) and to the HR group (59 ± 1.2 mL/kg; P < 0.05; Figure 4). Furthermore, over time, the NR group required significantly more fluid than the other two groups, as particularly noticed after the 50th min of the experiment, during the resuscitation phase (Figure 5).

**Laboratory tests**

Baseline hemoglobin, hematocrit, and arterial blood gases were equivalent among groups (Table 1). As expected, because of hemorrhagic shock and resuscitation, hemoglobin decreased significantly in the NR (6.9 ± 2.0 g/dL) and HR (7.2 ± 1.2 g/dL) groups compared to sham-operated animals.

**Figure 5.** Fluid volume during time of lactated Ringer infusion through the right internal jugular vein catheter. The arrow indicates the time when re-bleeding possibly begins. Sham = no hemorrhage (N = 10 rabbits); NR = normotensive resuscitation to baseline values (N = 10 rabbits); HR = hypotensive resuscitation to 60% of baseline mean arterial pressure (N = 10 rabbits). Data are reported as means ± SEM. *P < 0.05 compared to sham; †P < 0.05 compared to sham and hypotensive resuscitation (one-way ANOVA followed by the post hoc Tukey test for multiple comparisons).

<table>
<thead>
<tr>
<th>Test</th>
<th>Sham</th>
<th>NR</th>
<th>HR</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>85 min</td>
<td>Baseline</td>
</tr>
<tr>
<td>Hg (g/dL)</td>
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<td>12.2 ± 0.2</td>
<td>12.3 ± 0.5</td>
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<td>HCT (%)</td>
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<td>PaCO₂ (mmHg)</td>
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<tr>
<td>PaO₂ (mmHg)</td>
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<td>Platelets (mm³)</td>
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</tr>
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<td>Antithrombin (%)</td>
<td>99.8 ± 2.0</td>
<td>95.2 ± 2.8</td>
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</table>

Hg = hemoglobin; HCT = hematocrit, PaCO₂ = partial carbon dioxide pressure in arterial blood; PaO₂ = partial oxygen pressure in arterial blood; BE = base excess; aPTT = activated partial thromboplastin (time ratio vs control value); sham = no hemorrhage; NR = normotensive resuscitation group after hemorrhage; HR = hypotensive resuscitation group after hemorrhage. *P < 0.05 vs baseline of same group and sham group at baseline and 85 min; †P < 0.05 vs HR at baseline and 85 min (one-way ANOVA followed by the post hoc Tukey test for multiple comparisons).
animals (12.2 ± 0.2 g/dL, P < 0.05). Similar results were obtained for hematocrit, even though HR animals received significantly less fluid (Table 1). Regarding arterial blood gases at the 85th min of the experiment, mean arterial pH was 7.1 ± 1.1 in the NR group and 7.2 ± 1.5 in the HR group. Data also showed evidence of compensatory hyperventilation with a significant decrease in PaCO2 levels among shocked animals (Table 1). As expected, base excess was significantly worse in the shock groups compared to baseline and to sham-operated animals (Table 1).

Baseline aPTT ratios, platelet count and antithrombin levels were similar among groups (Table 1). There were no significant differences in aPTT ratios after hemorrhagic shock. Platelet count decreased equivalently in both hemorrhagic shock groups compared to baseline and to sham-operated animals (Table 1). However, antithrombin level was only significantly reduced in the NR group compared to baseline, to the HR group and to sham-operated animals (P < 0.05).

Discussion

The current trauma literature has repeatedly called for better clinically relevant experimental models that more closely mimic hemorrhagic shock in humans (6,9,10,15). We have devised a small animal model that reproduces many aspects of hemorrhage caused by a penetrating injury to the torso. The model also simulates fluid resuscitation prior to definitive surgical bleeding control. The lag time between vessel injury and the beginning of fluid resuscitation permits blood to clot at the site of injury. This is extremely important in terms of clinical relevance in any hemorrhagic shock model related to trauma (15,16). Clot formation can be determined by whole blood clotting time and takes an average of 10 min to occur (16). Consequently, any experimental model in which fluid infusion begins before clot formation interferes with the physiological process of hemostasis and jeopardizes clinical relevance.

The present model also simulates the average time urban EMS take to arrive at the scene of an accident (approximately 15 min), and this time is also allotted in our model before fluid resuscitation is started (14). Furthermore, we also consider a total time of 85 min for EMS transport and emergency room times from the time of injury. An audit of time to emergency trauma laparotomy in the United Kingdom showed a median time from emergency call to operation of 127 min (140 min for blunt and 86 min for penetrating injuries), which is similar to the time allotted in our model (17).

Most trauma patients in severe hemorrhagic shock also require blood product transfusion. However, the time to start the first transfusion varies considerably. A recent study showed that the beginning of transfusion of the first unit of red blood cells occurred on average 20 min after trauma, and the time to first fresh frozen plasma transfusion ranged from 24 to 350 min (median 93 min) (18).

Overall, the duration of the experiment in our model is consistent with the timing of events that take place in an urban setting with a trauma victim who sustains a penetrating torso injury. Enough time was allotted to simulate emergency room exams, intubation, anesthesia, operating room set-up, incision, and vascular control of a bleeding site (14,15). The model, however, was not intended to simulate the timing of events of an emergency room thoracotomy, a scenario of a patient in extremis.

Our model causes a severe hemorrhagic shock as shown by the significant decrease in MAP, hemoglobin and hematocrit, by abnormal base excess, and by a 20% mortality rate within the first 15 min. The average intra-abdominal blood loss of 30 mL represents approximately 20% of the total blood volume of the animal, and corresponds to a class II shock as per the Advanced Trauma Life Support protocol of the American College of Surgeons (13). With the addition of tissue injury provoked by laparotomy, a severe shock is achieved in the model, to the extent that, in a pilot study previously performed by us, there was 100% mortality within 30 min after aortic injury if no resuscitation fluid at all was given to the animals, underscoring the importance of the infusion of at least some fluid during the treatment of severe hemorrhagic shock.

Other experimental models involving tail amputation or hemorrhage by controlled blood withdrawal do not reproduce faithfully the events of major penetrating torso trauma and the resulting uncontrolled hemorrhage. Additionally, such models produce only minimal tissue injury (11,19-21). In our model, however, significant tissue injury was created by both the invasive pre-shock surgical procedures and, more importantly, by the laparotomy. The latter was closed swiftly, immediately after the aortic injury to mimic the tamponade effect of the abdominal wall and provided an adequate environment for clot formation in abdominal injury.

Acute fluid management of the bleeding trauma patient plays a major role in the hemostatic response. Therefore, a clinically relevant animal model of hemorrhagic shock should be in keeping with the current resuscitation practice. The primary resuscitation strategy of severe hemorrhage is to administer large volumes of crystalloids and packed red blood cells. In our model, fluid resuscitation reproduced the average crystalloid infusion by EMS during transport, approximately 1.7 mL·kg⁻¹·min⁻¹ or a maximum of 5 mL/min of LR infusion in the case of a 2.5-kg rabbit (14,15,22).

Another resuscitation regimen considered in our model is HR, a strategy known since World War I but only recently applied to the clinical management of trauma patients with penetrating torso injuries (3,13,23-25). Previous experimental models of hemorrhagic shock have demonstrated that HR leads to less blood loss and ultimately lower mortality when compared to NR (8), although in the present study there were no deaths among animals receiving either resuscitation regimen.
HR addresses at least two important factors that affect the hemostatic response: reduces dilution of pro-coagulants and dislodgment of the clot from the site of injury (3,8,13,23-25). A study using a computer-based model of uncontrolled hemorrhagic shock and resuscitation concluded that a rapidly bleeding injured patient bleeds for approximately 5.5 min before intrinsic hemostasis is achieved (15). Thus, by the time EMS arrives at the scene, in most cases bleeding increased significantly and “self-resuscitation” has already begun (15). However, the recently formed clot is fragile and vulnerable to blood pressure changes caused by overzealous fluid resuscitation. Thus, an increase in blood pressure can easily dislodge the clot from the vessel causing rebleeding, a phenomenon known as the “leaky bucket” theory (15).

As mentioned previously, the present data support the concept of ongoing self-resuscitation, especially noticed between five and 15 min after injury to the aorta. Furthermore, our data also support the fact that rebleeding increased after the 50th min in normotensive resuscitated animals, and intra-abdominal blood loss was significantly greater compared to HR animals. Interestingly, another study comparing no fluid, small volume infusion of hypertonic saline dextran (7.5% NaCl/6% dextran70), and a large volume of LR solution demonstrated no difference in blood loss (19). In that study, however, the intra-abdominal blood loss was assessed after only 20 min from the start of the resuscitation phase and may not have allotted enough time to detect rebleeding, and consequently a difference in blood loss between groups (19).

Even though it was not the purpose of this study to investigate the coagulopathy related to hemorrhagic shock, we believe that the model described herein sets the stage for such an investigation (4,9). Using basic routine laboratory coagulation assays, we demonstrated that hemorrhagic shock is associated with a significant decrease in platelet count independent of the resuscitation strategy used. We speculate that platelets were less dysfunctional among animals undergoing permissive hypotension resuscitation, arguably contributing to the reduced intra-abdominal blood loss when compared to the NR group. The low levels of antithrombin also suggest the role of dilutional coagulopathy particularly among the NR group animals. The fact that the aPTT remained within normal limits in both groups coincides with recent clinical observations demonstrating that this test is a poor indicator of the hemostatic disturbances that take place in traumatic hemorrhage (26,27).

Bleeding is among the easiest forms of insults that can be reproduced in experimental models and has been studied for more than 80 years. However, all animal models, including ours, have important limitations, the need for anesthesia being one of them. Although the cardiovascular effect of xylazine has been shown to be negligible in conscious large animal experiments, ketamine undoubtedly compromises mean arterial pressure, oxygen consumption, as well as cardiac and pulmonary performance (28,29). Therefore, the pattern of response to hemorrhagic shock with either hypotensive or normotensive resuscitation could have been different if no anesthesia had been used.

Although the present model has the advantage of using a small animal such as the rabbit, which is more cost-effective and easier to manage, larger animals have cardiovascular and hemodynamic responses to shock more similar to those of humans. Nonetheless, a recent report concluded that the hemostatic response of the rabbit is physiologically one of the most similar to the human response (30).

Our study has another limitation related to most animal models, which is the difficulty to reproduce the late complications of shock and resuscitation of trauma patients (21). The total time period of this experiment (85 min) enabled us to study only the acute phase of hemorrhagic shock, which represents a small window in a very complex chain of events. Finally, even though a model of uncontrolled bleeding is considered to be more clinically relevant than controlled hemorrhage models, it tends to produce inconsistent results with respect to the extent of bleeding, reduction of blood pressure, and survival (31).

Our rabbit model reproduces the sequence of events from pre-hospital to early in-hospital care of penetrating trauma and uncontrolled intra-abdominal hemorrhage caused by an arterial injury in a clinically relevant fashion. Using this model, we were able to demonstrate that hypotensive resuscitation results in less intra-abdominal blood loss than normotensive resuscitation, possibly by preventing re-bleeding episodes.

Acknowledgments

Research supported by CAPES (#0017/08-6) and CNPq.

References

5. Moore FA, McKinley BA, Moore EE. The next generation in...