Establishment of an Experimental Mouse Model of Trauma-Hemorrhagic Shock

Yin TANG, Xue-Feng XIA, Yun ZHANG, Bing-Feng HUANG, Tao MA, Wei CHEN, and Ting-Bo LIANG

Department of Hepatobiliary and Pancreatic Surgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, P.R. China

Abstract: This study established an experimental mouse model of trauma-hemorrhagic shock (THS). THS-induced mice (C57BL/6J, n=33) were subjected to femoral fracture, ischemia for 90 min, and resuscitation for 15 min. The sham-operated mice (C57BL/6J, n=33) underwent the same anesthetic and surgical procedures, but neither trauma-hemorrhage nor fluid resuscitation were performed. Mean arterial pressure (MAP) and microvascular tissue perfusion over the small intestine, liver, and left kidney were longitudinally measured in all mice. Blood was collected for analysis at baseline and 3, 6, 12, and 24 h post resuscitation, and the small intestine, liver, and left kidney were resected for hematoxylin and eosin staining 24 h post resuscitation. Compared with the sham group, MAP and microvascular tissue perfusion over the small intestine, liver, and left kidney were all significantly reduced in the THS group at the end of hemorrhage. Following resuscitation, no significant differences were observed between the groups. THS induction was associated with significantly increased plasma concentrations of Cr, AST, CPK, IL-6, IL-10, and TNF-α from the baseline values by two- to three-fold after the hemorrhage phase, and THS-induced mice demonstrated significantly increased histological injury scores. The rapid drop in MAP and microvascular tissue perfusion observed following THS induction, and the gradual recovery post resuscitation, reflects the successful establishment of a THS experimental mouse model.

Key words: arterial pressure, mouse model, tissue perfusion, trauma-hemorrhagic shock

Introduction

Severe traumatic injury and blood loss can lead to a cascade of detrimental signaling events, which can lead to shock, sepsis or multiple organ dysfunction (MOD) [8, 10]. Hemorrhagic shock following critical trauma is associated with high morbidity and mortality [23, 24]. The establishment of a standard experimental model of trauma-hemorrhagic shock (THS) is necessary to understand the pathophysiological effects of traumatic tissue injury and hemorrhagic shock, to investigate the causes of suppressed immune function and detrimental signaling cascades, and to develop more effective ways of helping those patients who suffer from traumatic injuries [15, 17].

Small animal models have many advantages, including the availability of many strains, low cost, ease of care, rapid reproduction, and ease of operation [22, 28]. In recent years, we established a standard experimental model of THS in rats and conducted research using this model [16, 30, 31]. Due to the many available mouse strains, numerous reagents for mice, and the availability of knockout and transgenic animals, this species represents an unparalleled resource for the study of biologic...
responses [28]. Thus, the establishment of a standard experimental model of THS in the mouse has much potential. Due to their small size, THS in mice presents unique technical challenges. In this paper, we describe how we mastered this procedure to create a model that could not only imitate clinical features but could also be performed with relative ease.

Materials and Methods

Animals

Male C57BL/6J mice, aged 8–12 weeks and weighing 20–25 g, were purchased from the Animal Resource Center at Zhejiang University Medical College. All the animal research protocols used in this study were approved by the Institutional Laboratory Review Board and accorded with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication, 1985).

Procedure

Instrument preparation: All surgical procedures were performed using aseptic techniques. All materials and instruments were sterilized before use, including sterile gloves, 3–0 sutures, 6–0 sutures, PE-10 tubing, 30 G needles, 1 ml syringes, 10 ml syringes, cotton tip applicators, gauze, 2-way stopcocks, and instruments.

Prior to the surgical procedure, a right carotid artery murine catheter was inserted, which would be used for withdrawal of blood and resuscitation. Using sterile gloves, a sterile PE-10 tube approximately 100 mm in length was prepared, one end of which was beveled for exiting the artery. A 30 G needle was inserted into the other blunt end of the tubing. A 1 ml syringe was filled with 0.5–1.0 ml of heparinized saline solution and attached to one end of a 2-way stopcock, with the 30 G needle and the catheter attached to the other end. The stopcock, 30 G needle, and PE-10 tube were filled with heparinized saline solution from the 1 ml syringe to prevent blood clotting. A check was made at this point to ensure that no air bubbles were in the system. The 2-way stopcock was then turned off, and the completed catheter was placed on the sterile field dressing with the surgical instruments.

Surgical procedures: The experimental mouse was anesthetized by administering an intraperitoneal injection of chloral hydrate (300 mg/kg). Once asleep, the mouse was placed on a controlled heating metal surgical plate at a temperature of 36–37°C (TMS-202, Softron, Tokyo, Japan). The mouse was immobilized in the supine position by taping the extremities. Mean arterial pressure (MAP) was longitudinally measured throughout the experimental period by a programmable sphygmomanometer (BP-98A, Softron) using the tail-cuff method as described previously [18, 26, 27].

After immobilization and sterilization, once the animal’s respirations began to slow, a small 10 mm incision was made in the middle of the cervical skin parallel to the trachea. Dissection of the right carotid artery in the experimental mouse followed, making sure not to damage the thyroid gland, the vagus nerve, blood vessels, or muscles. A total of three 6–0 sutures were placed around the right carotid artery. Suture 1 was the most distal in location. This suture needed to be tied off, immediately ligating the vessels. Suture 2 was the most proximal in location, with a more loosely tied knot. The distal and proximal sutures were used to prevent blood loss, to pull the vessel taut, and to lift the vessel to aid in catheter insertion. Suture 3 was a catheter support suture and was placed between the distal and proximal sutures and tied with a loose knot that could be used to secure the catheter inside the vessel following insertion.

Once the sutures were secure, a small incision was made on the top of the right carotid artery using microscissors. This hole was close to the distal suture so that a sufficient amount of artery was available for the initial catheter insertion. Dumonts were used to push the catheter into the lumen, and the middle support suture was lightly tied down to hold the catheter in place. The proximal hemostat was released, and the proximal suture was loosened around the right carotid artery. At this point, pulsating blood was visible in the catheter (Fig. 1).

Trauma-hemorrhagic shock: The experimental mouse was subjected to a mid-diaphyseal transverse fracture in the left femur using a special clamp to induce trauma. The 2-way stopcock was turned on, and the animal rapidly bled into a 1 ml syringe, reducing the MAP to 35 ± 5 mmHg within 10 min [11, 29]. This time was defined as maximum bleed-out, and the volume of withdrawn blood was recorded. The 2-way stopcock was then turned off, and the 1 ml syringe with the shed blood was removed.

Resuscitation: At the end of the shock period (90 min), a sterile 10 ml syringe was filled with 5 ml of Ringer’s lactate solution and attached to the end of the 2-way
stopcock. The mouse was then resuscitated using twice the volume of the shed blood in the form of Ringer's solution, which was infused slowly over 15 min at a constant rate by a microinfusion pump (WZS-50F, Zhejiang University Medical Instrument Ltd., China).

Post resuscitation: The catheter was removed, and the right carotid artery was ligated using the three 6–0 sutures. The incision was sewn up using a sterile 3–0 suture. The animal was placed into a clean cage, which was kept on a circulating heating pad for several hours post recovery. The animal was allowed food and water ad libitum after resuscitation.

The sham-operated mice underwent the same anesthetic and surgical procedures, but neither trauma-hemorrhage nor fluid resuscitation was performed.

Animal grouping
A total of 66 mice were randomly divided into the following two groups: (1) a sham-operated group that underwent the same anesthetic and surgical procedures but without the induction of trauma/hemorrhage or fluid resuscitation (n=33) and (2) a THS and resuscitation group (n=33).

Microvascular tissue perfusion
For both the sham and THS groups, the microvascular tissue blood flow over the small intestine, liver and left kidney was monitored using a laser Doppler probe (Moor Instruments Ltd., UK) [6, 13]. This technique works by scanning a 2 mW helium–neon laser across the surface of the organs. Light that is backscattered from moving erythrocytes undergoes a shift in frequency proportional to their velocity, according to the Doppler principle. The resulting color-coded image represents microvascular tissue blood flow over the scan area. Data is expressed as the mean ± standard deviation (SD) of three animals at every time point.

Sample collection
Blood was collected from both groups for analysis at baseline and 3, 6, 12, and 24 h post resuscitation, and the small intestine, liver and left kidney were resected at the same time point.

Plasma analysis
Blood was obtained via inferior vena cava puncture, placed in heparinized syringes, and centrifuged at 2,500 × g for 10 min. The plasma was collected and stored at −80°C until analysis [11]. Plasma potassium (K⁺), creatinine (Cr), glucose, creatine phosphokinase (CPK), alanine transaminase (ALT), and aspartate transaminase (AST) were measured using a biochemical analyzer (C800, Abbott Laboratories, Chicago, IL, USA) [31]. The plasma levels of IL-6, IL-10, TNF-α, and TGF-β1 were measured by enzyme-linked immunosorbent assay (ELISA) using mouse ELISA kits (Dakewe, Shenzhen, China) according to the manufacturer’s protocols.

Histology
Samples of the small intestine (ileum, 10 mm length, 20 mm distal of the cecum), liver (third segment) and left kidney were fixed in 10% formaldehyde for 24 h and then embedded in paraffin. Sections 5 µm thick were stained with hematoxylin and eosin (HE) and examined using light microscopy [1]. The histological injury evaluation of the HE stains was in accordance with special rating scales for each organ [21].

Small intestine specimens were analyzed following the method of Chiu et al. [3], who evaluated mucosal damage (grade, 0–5). The histological score for liver damage established by Suzuki et al. [25] was applied based on degrees of congestion, necrosis, and formation of vacuoles (score, 0–12). Finally, kidney samples were rated as described by Dobyán et al. [5] and Helmcen and Thurau [9], considering swelling and flattening of
epithelial cells, tubular dilatation, edema, microthrombosis, and acute tubular necrosis (score, 0–18).

Statistical analyses

Data are expressed as means ± SD. Differences between the sham-operated group and the THS group at each time point were evaluated using the Student’s t-test, and P<0.05 was considered statistically significant.

Results

Establishment of the THS model

In our THS model, mice were exposed to a bone fracture and continuous bleeding in order to induce THS. MAP and microvascular tissue perfusion over the small intestine, liver and left kidney were longitudinally measured in all mice (Fig. 2). Compared with the sham-operated group, the THS-induced mice demonstrated a significant reduction in MAP (31.57 ± 3.10 vs. 63.55 ± 8.49 mmHg, P<0.01) and microvascular tissue perfusion over the small intestine (86.11 ± 12.27 vs. 420.19 ± 67.47 PU, P<0.01) (A), liver (91.94 ± 13.35 vs. 284.69 ± 59.90 PU, P<0.01) (B), and left kidney (71.62 ± 9.82 vs. 267.36 ± 33.71 PU, P<0.01) (C) at the end of hemorrhage. At the end of resuscitation, no significant differences were observed between the THS group and sham-operated group. Data are expressed as means ± SD of three animals at every time point. *: P<0.05 and #: P<0.01 vs. the sham-operated group, respectively.
Effects of THS on plasma concentrations of $K^+$, Cr, glucose, CPK, ALT, and AST

THS induction significantly increased the plasma Cr ($P<0.01$), AST ($P<0.05$), and CPK ($P<0.01$) concentrations from the baseline values by two- to three-fold immediately after the hemorrhage phase (Fig. 3B, 3D, and 3F). These increases were transient, and 12 h after complete resuscitation with Ringer’s solution, the concentrations dropped once again to normal levels. In contrast, the plasma glucose concentration decreased in the THS-induced mice 6 h after resuscitation (Fig. 3E) ($P<0.05$).

Plasma $K^+$ and ALT concentrations did not differ significantly between the THS-affected mice and sham-operated mice at any time point (Fig. 3A and 3C).

Effects of THS on plasma levels of IL-6, IL-10, TNF-$\alpha$, and TGF-$\beta$1

Compared to the sham-operated group, the THS-induced group had significantly increased plasma levels of IL-6 ($P<0.01$), IL-10 ($P<0.05$), and TNF-$\alpha$ ($P<0.05$) (Fig. 4A, 4B, and 4C). The plasma level of TGF-$\beta$1 did not differ significantly between the THS-affected mice and sham-operated mice at any time point (Fig. 4D).

THS-induced mice demonstrated increased histological injury scores

THS-induced mice were subjected to trauma-hemorrhagic shock (MAP of $35 \pm 5$ mmHg) for 90 min followed by resuscitation. The THS-induced mice demonstrated significantly increased histological injury scores 24 h post resuscitation compared with the sham-operated group (Fig. 5A and 5B).

Discussion

In recent years, many studies have been performed in mice following trauma and hemorrhagic shock. Although there are numerous methods to induce trauma and hemorrhagic shock in mice, there are still many drawbacks associated with these methods. According to the method of Chaudry et al. [12], a 20 mm midline laparotomy was performed and then closed in order to induce soft tissue trauma. This method may not be relevant to the clinical setting if, for example, the patient is injured in a road accident.
traffic accident. According to the method of Matsutani et al. [20], combined trauma (fracture and laparotomy) and hemorrhagic shock is performed in mice in order to induce multiple trauma. However, if the burden of multiple trauma is severe enough, the animals may die immediately. According to the method of Hsieh et al. [11], both femoral arteries and a femoral vein of the mouse are catheterized for monitoring MAP, bleeding and fluid resuscitation, respectively. The technique reported by Zhang et al. involves catheterization of both femoral arteries of the mouse [29]. One catheter is used for monitoring MAP, and the other is used for bleeding and fluid resuscitation. However, these surgical procedures are technically very difficult to perform in mice. Moreover, ischemia may occur in the legs of THS-induced mice, although collateral flow can prevent this. Until now, there has not been a standard mouse experimental model of THS. As a result, we established a model in this study that was not only able to imitate clinical features but was also relatively easy to perform.

The THS model in this article is a fixed pressure method. However, the procedures can be used with either a fixed pressure or fixed volume hemorrhage approach, and the animal can remain in THS for 0.5–3 h, which has excellent potential for the investigation of pathophysiological conditions in mice [2]. Our THS model could also be an important model for studying cellular communication patterns and the responses of systems such as hormonal and inflammatory mediator systems, and danger signals, as it elicits distinct responses that differ from other forms of shock [7, 15]. Moreover, the development of knockout and transgenic murine strains and the induction of biologic agents to inhibit specific signaling have presented valuable opportunities to further elucidate our understanding of the up- and down-regulation of signal transduction after THS [14, 19].

THS patients tend to be at risk of developing hypovolemia, with subsequent tissue malperfusion and organ dysfunction. Although blood volume therapy can help to maintain hemodynamic stability and diminish the risk of developing hypovolemia and its effects, THS has been shown to induce histological injury, which is an impor-

**Fig. 4.** Effects of THS on plasma levels of IL-6, IL-10, TNF-α, and TGF-β1. Blood samples were obtained before THS (baseline) and after THS at 3, 6, 12, and 24 h post resuscitation. Data are expressed as means ± SD of three animals at every time point. * and #: $P<0.05$ and $P<0.01$ vs. the sham-operated group, respectively.
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tant contributor to cell dysfunction, organ failure, and mortality [4]. We examined the effects of ThS on hemodynamics, microvascular tissue perfusion (Fig. 2), vital organ function, and histological injury in various tissues in an induced ThS mouse model that mimicked a clinical condition with subsequent resuscitation of the animals using Ringer’s lactate solution.

In our model, we observed that immediate resuscitation after ThS rapidly restored hemodynamic stability. However, ThS induction was associated with a significant increase in plasma Cr, AST, CPK, IL-6, IL-10, and TNF-α concentrations after the hemorrhage phase, and ThS-induced mice demonstrated a significant increase in histological injury scores 24 h post resuscitation compared with the sham-operated group. In conclusion, this ThS mouse model was proven to be effective when the effects of ThS were evaluated and compared with the sham-operated group.

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References


patocellular carcinoma in heavy drinkers with liver cirrhosis. *Alcohol Alcohol. Suppl.* 1B: 109–114. [Medline]


