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# A new model for the study of secondary intra-abdominal hypertension in rats

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ABSTRACT

*Background:* To build a new and appropriate model of secondary intra-abdominal hypertension (IAH) in rats.

*Methods:* A total of 32 female Sprague–Dawley rats were randomized into four groups. Group I: the rats were hemorrhaged to a mean arterial pressure (MAP) of 40 mm Hg for 1 h and portal hypertension was induced by partial ligation of the portal vein 1 h later; Group II: after inducing portal hypertension, hemorrhagic shock of MAP of 40 mm Hg was induced and maintained for 1 h; Group III: after inducing portal hypertension, hemorrhagic shock of MAP of 40 mm Hg was induced and maintained for 2 h; Group IV: after inducing portal hypertension, hemorrhagic shock of MAP of 40 mm Hg was induced and maintained for 2 h, and a specially designed abdominal restraint device was used. After these procedures, respectively, the collected blood was reinfused and lactated Ringer solution was continuously infused until the secondary IAH model was established.

*Results:* No models were built in Groups I, II, and III. One rat died in Group IV after portal vein ligation, and all the remaining rats successfully developed IAH; the success rate was 87.5%. During the resuscitation period, the average time was 5.26 ± 0.59 h and the average total infusion volume was 665.5 ± 86.04 mL/kg.

*Conclusion:* A rat model of secondary IAH was successfully established by resuscitation after a combination of inducing portal hypertension, hemorrhaging to a MAP of 40 mm Hg for 2 h, and using an abdominal restraint device. All these criteria mimic key etiological factors for the development of secondary IAH.



## 1. Introduction

Intra-abdominal hypertension (IAH) is defined as a sustained pathologic elevation of intra-abdominal pressure (IAP) of 12 mm Hg or more [1]. When the IAP is more than 25 mm Hg and associated with new organ dysfunction, it is defined as abdominal compartment syndrome (ACS), a life-threatening condition [1]. A raised IAP has a direct or indirect impact on every organ system in the body [2,3]. Malbrain *et al.* [4] found that the cumulative incidence of IAH in a mixed population of critically ill patients was 32.1%, and the occurrence of IAH during the intensive care stay was also an independent predictor for mortality (mortality rate 30%–50%).

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Building an appropriate animal model is an integral part to further study the pathophysiological mechanism of IAH/ACS. Several approaches have been chosen to induce IAH/ACS. These include the instillation of liquid (such as Ringer lactate solution [5,6], saline [7], or gelatin [8]), insufflation of gas (such as carbon dioxide [9], air [10], or nitrogen [11]), or intra-abdominal balloon [12] to increase IAP. However, rapidly increasing the IAP and using animal models that lack pathogenesis for the induction of IAH are disadvantageous, as they differed from the natural development of secondary IAH in patients [13].

In 2010, Shah *et al.* [14] first created a new porcine model of ACS, in which ACS was induced by hemorrhagic shock, partial occlusion of the portal vein, large-volume resuscitation, and the development of new organ dysfunction. This led to organ edema and fluid translocation, which contributed to increased IAP. This animal model of ACS incorporated the pathophysiological elements responsible for most ACS cases. However, swine were used in this experiment [14], which bore the disadvantages of high expense, large animal size, complicated surgical procedures, and the need for an entire team. Moreover, this animal model is primarily used to study the diagnosis and the management of IAH and ACS (especially surgical management), and is not suitable for studying the correlation between different resuscitation fluids and IAP or for a large sample size experiment. Therefore, the aim of this study was to create a new, natural, simple, and easily reproducible small animal model of secondary IAH.

## 2. Material and methods

All procedures were approved by the Ethics Committee of the Third Military Medical University, and all animals were provided by the Experimental Animal Center, Daping Hospital, Third Military Medical University.

### 2.1. Anesthesia

Female Sprague–Dawley rats (230–240 g) were given free access to water and were housed in a 12-h light–dark cycle. The animals were pretreated with atropine sulfate (0.1 mg/kg, intramuscular) and anesthetized with an intraperitoneal injection of 30 mg/kg sodium pentobarbital (30 mg/mL). The anesthesia was administered using pentobarbital sodium (15 mg/mL) as required throughout the experiment.

### 2.2. Surgical preparation

The rats were immobilized in supine position with the forearm in abduction at 90° to the body, and later extended. The right femoral vein was cannulated using polyethylene tubing (PE-50) for the infusion (ZNB-XB infusion pump; Beijing Kelly Med Co, Ltd, Beijing, China). The left femoral artery was cannulated for mean arterial pressure (MAP) monitoring (mercury manometer), and the left femoral vein was cannulated 3.5 cm to the inferior vena cava for inferior vena cava pressure (IVCP) monitoring (water manometer, zeroed at the level of the midaxillary line, 1 mm Hg = 13.6 mm $H_2O$). All the cannulae contained normal saline with heparin (5 U/mL). Another catheter (outside diameter, 0.8 mm) was inserted into the bladder for the measurement of urine output and the collection of urine samples. The rats were stabilized for 15 min before blood withdrawal and surgical procedure. To maintain body temperature at 37°C, the rats were placed on a warming plate.

### 2.3. Shock and resuscitation protocol

Hemorrhagic shock was induced by withdrawing arterial blood (0.5 mL/min) to a MAP of 39–42 mm Hg, and MAP was maintained by blood withdrawal or infusion. The hemorrhagic shock period was determined according to the experimental design. At the end of shock, the collected blood (anticoagulated with 100 U/mL heparin) was reinfused. After 15 min of stabilization, infusion of lactated Ringer solution (30 mL/h) continued until the development of IAH was achieved.

### 2.4. Induction of portal hypertension protocol

An extensively studied prehepatic portal hypertensive animal model was used [15,16]. After a 3-cm midline abdominal incision was made, the portal vein was freed from the surrounding tissues. A ligature (silk 4-0) was placed around a 21-gauge blunt-tipped needle lying alongside the portal vein. Subsequent removal of the needle yielded a calibrated stenosis of the portal vein, and the incision was then closed.

### 2.5. Use of the abdominal restraint device

To mimic decreased abdominal wall compliance, a specially designed device (circumference 15 cm, width 6 cm, soft, and non-ductile) was loosely modeled to the abdominal wall of an anesthetized rat under physiological conditions with normal abdominal volume and IVCP. This device did not compress the abdomen and affect physiological parameters for circulation and ventilation. This device was used for all rats in Group IV.

### 2.6. Experimental design

The rats were randomly divided into four groups (Fig. 1). Group I ($n = 8$): the rats were left in shock for 1 h. Portal hypertension was induced when shock ended. Group II ($n = 8$): portal hypertension was induced 1 h before shock, and the rats were left in shock for 1 h. Group III ($n = 8$): portal hypertension was induced 1 h before shock, and the rats were left in shock for 2 h. Group IV ($n = 8$): portal hypertension was induced 1 h before shock, and the rats were left in shock for 2 h, and a specially designed abdominal constraint device was used in this group.

### 2.7. Determination of the starting and end points

After blood reinfusion, the IVCPs of the rats were different. The IVCP after blood reinfusion was set as the starting point (when the IAP was 0 mm Hg), and the end point was determined by an elevation of 12.5 mm Hg (170 mm $H_2O$) of IVCP, a point at which the development of IAH was achieved [1]. If IAH was not achieved, death was considered to be the end point