Effects of three fluid resuscitation methods on apoptosis of visceral organs in rats with hemorrhagic shock

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Received June 2, 2005; revision accepted July 17, 2005

Abstract: Objective: To observe the effects of three fluid resuscitation methods on apoptosis of visceral organs in rats with hemorrhagic shock. Methods: A model of rat with severe hemorrhagic shock and active bleeding was established in 32 SD (Sprague-Dawley) rats. The rats were randomly divided into control group, no fluid resuscitation group (NF group), controlled fluid resuscitation group (NS40 group) and rapid large scale fluid resuscitation group (NSS80 group). Each group contained 8 rats. The curative effects were compared. At the same time, the apoptosis in liver, kidney, lung and small intestinal mucosa of survivors after hemorrhage and resuscitation was detected by light microscopy in HE (hematoxylin and eosin) stained tissue sections, flow cytometry and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL). Results: The survival rate of early fluid resuscitation (14/16) was markedly higher than that of NF group (3/8). There was some apoptosis in liver, kidney, lung and small intestinal mucosa of all survivors. Compared with NF and NS40 groups, the apoptosis of liver, kidney and small intestinal mucosa of NS80 group was obviously increased. Conclusions: Among three fluid resuscitation methods, controlled fluid resuscitation can obviously improve the early survival rate and the apoptosis of liver, kidney and small intestinal mucosa in rats with severe and uncontrolled hemorrhagic shock, and may benefit improvement of prognosis.

Key words: Shock, Hemorrhagic, Resuscitation, Apoptosis

INTRODUCTION

Hemorrhagic shock is a common clinic emergency case. Successful treatment includes surgical control of hemorrhage and restoration of tissue perfusion. Current guidelines for presurgical treatment of patients with hemorrhagic shock recommend rapid volume resuscitation to normal blood pressure as quickly as possible. The practice is controversial because aggressive restoration of intravascular volume and rapid increasing of blood pressure before securing hemostasis may exacerbate hemorrhage and worsen outcome.

Controlled resuscitation allows prehospital treatment to work with compensatory mechanisms. The concept is to restore some intravascular fluid while taking into consideration hemostatic mechanisms. Such a scheme would balance the seemingly mutually exclusive processes of tissue perfusion and hemostasis.

Our study used a model of rat with severe hemorrhagic shock and active bleeding to test the effects of different resuscitation methods on apoptosis of visceral organs.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Sir Run Run Shaw Hospital, School of
Medicine, Zhejiang University. Forty-two male Sprague-Dawley (SD) rats, weighing 270–460 g, were obtained from the Medical Institute of Zhejiang Province, China. They had unlimited access to food and water before the experiments. After being weighed, the rats were anesthetized with pentobarbital (40 mg/kg intraperitoneally) and were placed in a supine position on a warming pad (25 °C).

After applying povidone-iodine solution, the right carotid artery was isolated and cannulated with polyethylene catheter through a neck incision. The arterial catheter was used for blood withdrawal and was connected to a pressure transducer and computerized physiograph system for continuous hemodynamic monitoring. In the same way, the left femoral vein was cannulated for fluid infusion. Blood losses of the procedure were measured by mopping all blood from the incision with preweighed gauze sponges, which were then reweighed. A transformation formula of 1 g = 0.9 ml of blood was used. Only animals whose blood losses were lower than 0.2 ml during the above-mentioned procedure and were spontaneously breathing 10 min after the procedure were included in the study. Among the test rats, thirty-two fulfilled the inclusion criteria.

The model of rat with severe hemorrhagic shock and active bleeding was established (Capone et al., 1995). Under light anesthesia, the injury began (time = 0) with blood withdrawal through the carotid arterial cannula for four times (at a rate of 1 ml per 100 g per 5 min in the first two times, 0.5 ml per 100 g per 5 min in the late two times). The shed blood was collected in glass syringes with heparin and reinfused during resuscitation. At 30 min, uncontrolled hemorrhagic shock was added to the volume-controlled shock by amputation of the tail at 75 percent of its length, measured from the tip. The bleeding tail was immediately directed into a container (with heparin) and the amount of shed blood was measured. This phase was called “prehospital phase” and continued for 60 more minutes. During this period, the rats were early resuscitated by infusing normal saline (NS). Fluids were administered via the femoral vein with an infusion pump at the rate of 2 ml/(kg·min). The pump was turned on and off to maintain the MAP (mean arterial pressure) goal.

At 90 min, a phase simulating hospital treatment (hospital phase) began. Hemostasis was achieved by tail wound closure. Simultaneously, resuscitation began with infusion of blood and normal saline solution. The “hospital phase” lasted 60 min and the end points were Hct (hematocrit) of 30 percent and MAP of 80 mmHg.

Thirty-two rats were randomly divided into four groups of eight rats each with the sequence of the experiments randomized in blocks of four (one from each group): group 1 (control group), neither fluid resuscitation nor hemostasis in the “prehospital” or “hospital phases” (no treatment); group 2 (no fluid resuscitation group, NF group), no fluid resuscitation in the “prehospital phase” (no field fluid resuscitation); group 3 (controlled fluid resuscitation group, NS40 group), NS infusion during the “prehospital phase” to reach and maintain MAP at 40 mmHg, beginning immediately after the tail cut (field fluid resuscitation to MAP 40 mmHg); and group 4 (rapid large scale fluid resuscitation group, NS80 group), NS infusion during the “prehospital phase” beginning immediately after the tail cut, to reach and sustain MAP of 80 mmHg (field fluid resuscitation to MAP 80 mmHg). Groups 2, 3, and 4 had a “hospital phase” with the same end points: control of bleeding, fluid resuscitation with blood and NS to Hct of 30 percent and MAP of 80 mmHg.

Blood samples (0.3 ml/sample) were taken separately from rats for complete blood count at 0, 120 and 150 min, and blood samples (0.2 ml/sample) were collected separately for determining serum lactate levels at 0, 30, 60 and 90 min.

Rats who lived for 150 min were regarded as survivors. The surviving rats were immediately sacrificed after resuscitation and hemostasis. The liver, kidneys, lungs and small intestine were taken out quickly and flushed with 0.01 mol/L cold phosphate buffer solution (pH 7.4).

The left part of liver, kidneys, lungs and part of the small intestine were fixed with 10% buffered formaldehyde for routine pathological examination and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL). All histological specimens were examined by a pathologist who was blind to the animals’ resuscitation protocol. The TUNEL detection kit (in situ Cell Detection Kit, AP) was purchased from American Promega Corporation. The detection procedure was mainly done according to the instructions provided by the corporation. Under high power