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Experimental Hemorrhagic Shock Protocol in Swine Models: The Effects of 21-Aminosteroid on the Small Intestine

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ABSTRACT

Background: The protective potential of lazaroids has been reported in previous studies on ischemia/reperfusion and induced hemorrhagic shock protocols.
Objectives: The present study is the first experimental protocol on the effects of the antioxidant factor U-74389G on the small intestine of swine models in a hemorrhagic shock protocol and resuscitation with 3 different types of fluids.
Methods: The study included 49 Landrace breed swine that were divided into groups of 7 each. Hemorrhage was provoked 45 minutes after starting the surgical protocol (0 minutes), followed by resuscitation starting 30 minutes after haemorrhage ceased by using 3 different fluids. Three groups (Group A, resuscitation using blood; Group B, resuscitation with Ringer's lactate solution; and Group C, resuscitation with hypertonic saline solution) underwent resuscitation with fluid alone, and another 3 groups (named A', B,' and C') were administered lazaroid U-74389G in addition to fluid. Control Group S underwent all the surgical procedures without hemorrhagic shock. Vital signs, complete blood count, and biochemical markers were analyzed, and tissue samples of the small intestine were collected from all animals. Further, malondialdehyde, tumor necrosis factor-α, and levels of inflammation in the tissue sample were measured.
Results: In Group S-A-A' and Group S-C-C', the analysis did not show statistically significant differences in the percentage changes of histopathology, malondialdehyde, and tumor necrosis factor-α through time. In Group S-B-B', the malondialdehyde levels in the small intestine were reduced in both the B and B' groups, without lazaroid (Group B) ($P = 0.038$) and lazaroid (Group B') ($P = 0.011$), compared with Group S (control), but the group without lazaroid (Group B) had greater reduction in malondialdehyde levels than the group treated with lazaroid (Group B'). With regard to the biochemistry results, 24% reduction was observed for alkaline phosphatase ($P = 0.022$) in Group A' treated with lazaroid compared with that in the untreated group. Lastly, for the complete blood count parameters, a 14% reduction in white blood cells was observed in Group B', which was treated with lazaroid in all phases ($P = 0.015$) (absolute value = 6.23) compared with Group B (absolute value = 13.74).
Conclusions: Despite few initial findings of this study suggesting that administration of lazaroid U-74389G may have some potential in attenuation of the effects of hemorrhagic shock in the small intestine of swine models, no differences remained after correction for multiple comparisons was made. Therefore, further research is required to investigate this result thoroughly.

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Introduction

In 1862, Samuel Gross described shock as the "rude unhinging" of the cycle of life. Adequate oxygen delivery and metabolism are essential for the maintenance of cellular energy stores. Failure of adequate oxygen delivery to and use by tissues during shock can lead to organ dysfunction and death.[1]

Hemorrhagic shock is an ischemic insult against an entire organism, and the restoration of the intravascular volume with fluid administration may cause a subsequent systematic ischemia-reperfusion injury.[2,3] Ischemia-reperfusion injury following major trauma can induce some inflammatory sequelae.[4,5] The multiorgan failure caused by hemorrhagic shock may lead to morbidity and mortality after severe trauma-related injury.[6,7] The inflammatory response to hemorrhagic shock causes the expression of cytokines[8] and the accumulation of neutrophils[9] in a variety of tissues.[10]

Hemorrhagic shock induces bacterial translocation in the small intestine[11] and increases mucosal permeability, membrane disintegration, and lipid peroxidation due to the action of oxygen free radicals targeting the cell membranes of the small intestine. The result of this increased mucosal permeability is the activation and adhesion of polymorphonuclear neutrophils and the release of proinflammatory factors.[12,13]

Numerous methods of resuscitation have been reported to manage hemorrhagic shock, such as blood volume replacement (ie, blood transfusion)[14] and fluid replacement with Ringer's lactate (RL)[15] or normal saline (NS) 7.5%.[16] However, replacing the current blood volume and fluid seems insufficient, and pharmacologic support is often needed.[17] The 21-aminosteroids, or lazaroids, belong to the group of lipid peroxidation inhibitors and their potential therapeutic effect has been widely studied. Lazaroids are a family of glucocorticoids without adverse side effects, and they have been found to scavenge lipid peroxyl and phenoxyl radicals, to inhibit iron-dependent lipid peroxidation, and to protect against peroxynitrite-induced cell toxicity.[18]

The present experimental protocol aimed to determine the effect of lazaroids on the small intestine postresuscitation with 3 different types of fluids in a hemorrhagic shock model. The study also investigated the effects of lazaroids on other organs (ie, liver, brain, heart, and stomach); however, these results will be presented separately in the future. The current protocol is the first experimental model of hemorrhagic shock in swine models that investigated the effect of the 21-aminosteroid U-74389G on the small intestine.

Methods

The experimental protocol of hemorrhagic shock in swine models was conducted in our Experimental Research Centre. A total of 49 laboratory animals, divided into groups of 7 each, were used. They were Landrace breed swine with a mean (SD) weight of 30 (2) kg. The animals were fed with standard laboratory food and kept adequately hydrated with free access to water. Twelve hours before the operative procedures, the animals were deprived of solid food, but their access to water was not limited. The experimental protocol was acute (all animals were euthanized immediately after the operative procedure and resuscitation, firmly applying all domestic and international laws for treating animals in experimental model protocols). Each animal was placed in the supine position on the operating table, which was covered by a heated mattress and blanket to maintain the core temperature of each animal at 39°C (±0.5°C).

This experiment was performed by a single qualified, experienced veterinarian.

Preanesthesia

Midazolam 0.5 mg/kg animal body weight was used (Dormicum 50 mg/10 mL → ~4 mL).

In addition, ketamine 15 mg/kg animal body weight and atropine 0.045 mg/kg animal body weight (1 mg/1 mL→ ~1 amp) was administered 10 minutes before intubation.

Introduction

The animals were intubated with a 6- or 7-mm endotracheal tube. They were infused with a solution of propofol 3 mg/kg (1% 10 mg/mL → 2–6 mL), fentanyl 0.012 mg/kg (0.05 mg/mL → 6 mL) and cisatracurium besylate 0.5 mg/kg (2 mg/mL → 6 mL) by bolus intravenously.

Maintenance

Initial ventilator settings: Fraction of inspired oxygen (FiO2) 40%, 20 breaths per minute.

Propofol 1% (6–8 mg/kg/h), fentanyl (2 mg = 4 amp in 500 mL NS), and cisatracurium besylate (200 mg = 10 amp Nimbex in 500 mL NS) were administered by infusion 60 to 80 mL/h intravenously.

Within 10 minutes, FiO_2 increased up to 60%, with 14 breaths per minute and inspiration volume of 15 mL/kg. Then, 750 mg intravenous cefuroxime was administered. The settings of the ventilator were continuously changed with the goal of maintaining the partial pressure of carbon dioxide in the arterial blood in the 35 to 45 mm Hg range; FiO_2 was kept as low as possible while still maintaining oxygen saturation of 98%. The ventilator was a Siare Alpha Delta Lung Ventilator, Italy (SN: MJ0097MM). An Irma Blood Gas Analyzer, Italy (SN: PIN406700) was used to measure blood gas.

Surgical protocol

The laboratory animals, sedated under general anesthesia and mechanically ventilated, underwent an open surgical dissection to initially expose the left common carotid artery in the left cervical area and then the left external jugular vein. The left common carotid artery was catheterized with a 20G probe for continuous monitoring of the arterial pressure and sampling for blood gas analysis. The left external jugular vein was catheterised with a probe of larger diameter (7Fr) for the administration of fluids. Subsequently, through an open surgical technique, the right external jugular vein in the right cervical area was prepared and catheterized with a 7.5Fr pulmonary artery probe.

Subsequently, a central abdominal incision (laparotomy) and splenectomy were performed. The spleen was weighed, and RL solution was rapidly delivered intravenously in a volume equal to 3 times the weight of the removed spleen. Subsequently, the inferior vena cava was catheterized through direct venepuncture with a 14G catheter and fixed in place with a 5-0 nylon suture. An extension line was placed, exiting the abdomen without any tension. The extension was fixed at the lower point of the abdominal surgical wound to control the hemorrhage after closure of the midline laparotomy. Finally, a urinary catheter (Foley 16Fr) was placed in the urinary bladder through open cystotomy and was connected to a urinary bag collector by exiting the abdomen through the lower part of the laparotomy surgical wound. Subsequently, a quick closure of the laparotomy wound was performed.

At the end of the surgical procedure and after a stabilization period of 30 minutes, the mean arterial pressure (MAP) was recorded, and this was considered the reference MAP (ie, baseline) for the animal. Subsequently, the phase of provoked hemorrhage occurred. Blood was withdrawn from the inferior vena cava, which was catheterized as mentioned previously to achieve a mean (SD)