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Superiority of acetate compared with lactate in a rodent model of severe hemorrhagic shock

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ABSTRACT

Background: Recently, we have shown that the use of lactated Ringer's (LR) solution is inferior to pure Ringer's solution (RS) in treatment of severe hemorrhagic shock in rats. The present study was performed to evaluate whether this is a specific effect of lactate or also applies to another metabolizable anion, namely acetate.

Material and methods: We subjected male Wistar rats to hemorrhagic shock by dropping the mean arterial blood pressure to 25–30 mm Hg for 60 min, resuscitated with acetated Ringer's (AR) solution, LR solution, RS, or normal saline (NS) within 30 min, and further observed the animals for 180 min.

Results: Administration of AR solution prolonged median survival to 115 min compared with 50 min for resuscitation with LR solution or 85 and 90 min for NS and RS, respectively. Resuscitation with AR solution and LR solution clearly improved metabolic acidosis compared with NS and RS but tissue injury, indicated by plasma enzyme activities, was most pronounced in the LR solution group, medium in the NS and RS groups, and least in the AR solution group.

Conclusions: In severe hemorrhagic shock, resuscitation with both RS and NS is superior to administration of LR solution but initial outcome is even further improved if AR solution is used. Mere amelioration of the acid–base status by AR solution may explain its superior role compared with RS and NS but cannot be responsible for its superiority compared with LR solution. Here, direct injury by lactate has to be discussed.

1. Introduction

Hemorrhagic shock is one of the major causes of death in the setting of trauma [1]. Severe loss of blood leads to tissue hypoxia, and death may result either from exsanguination or from the consequential multiorgan failure [2]. To protect the patient against these fatal consequences, it is necessary to replace the lost volume, stabilize circulation, and ensure tissue perfusion [3]. Currently, in the initial preclinical treatment of hemorrhagic shock crystalloid solutions are used for volume replacement. The American College of Surgeons recommends the use of normal saline (NS) or lactated Ringer's solution (hereafter named LR) in preclinical treatment dependent on the concomitant trauma [4]. This recommendation is controversially discussed and not obligatory. Several experimental studies give reason to doubt the infallibility of LR in the resuscitation of hemorrhagic shock [5,6]. Others, however, showed markedly improved

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hemodynamics, acid–base balance, and less organ injury after resuscitation with LR [7]. Therefore, the S3 guideline of the German Society for Trauma Surgery (Deutsche Gesellschaft für Unfallchirurgie) recommends the infusion of acetated Ringer's solution (hereafter named AR) or Ringer's malate before infusion of LR or NS [8].

Metabolizable anions, such as acetate and lactate, have been included in saline solutions to avoid hyperchloremic (dilutional) acidosis [9]. In the presence of molecular oxygen (O_2), they are degraded to CO_2 and H_2O or consumed in anaplerotic pathways, such as gluconeogenesis. In both cases, one H^+ is consumed (or one HCO_3^- is formed) for each carboxylate group (COO^-) metabolized. The location (organ, cell) of their metabolism may vary. Lactate is almost exclusively metabolized by the liver, whereas acetate is degraded more ubiquitously, among others, to a large extent in the muscle, but only to a negligible extent in the liver [10].

In marked contrast to the anticipated beneficial role of metabolizable anions, we recently demonstrated in a rat model of severe hemorrhagic shock (shock induction within 30 min to a mean arterial blood pressure [MAP] of 25–30 mm Hg, duration of shock 60 min) that LR used as a volume replacement solution shortens survival and increases organ injury in comparison with pure Ringer's solution (RS) [11]. To find out whether this unexpected negative result is confined to lactate or may also apply to other metabolizable anions, we here compare the effects of resuscitation with LR, AR (acetate as a metabolizable anion alternative to lactate), RS, or NS on survival, acid–base status, and tissue injury using the same model of severe hemorrhagic shock as depicted previously. The comparison of AR and LR with both RS and NS was chosen to ascertain whether the additional presence of potassium and calcium (in RS compared with NS; Table 1) affects successful resuscitation.

2. Material and methods

2.1. Chemicals and materials

RS and LR (containing only L-lactate) were from Fresenius Kabi (Bad Homburg, Germany), AR and NS were purchased from B. Braun (Melsungen, Germany), ketamine 10% was from Ceva (Düsseldorf, Germany), lidocaine (Xylocaine 1%) from AstraZeneca (Wedel, Germany), and acid citrate dextrose A solution from Baxter (Deerfield, IL). Portex catheters (inner diameter: 0.58 mm, outer diameter: 0.96 mm; Smiths Medical International, Hythe, UK) and medical oxygen (Air Liquide, Düsseldorf, Germany) were obtained from the vendors listed.

Table 1 – Composition of NS, RS, LR, and AR.

Ions	NS	RS	LR	AR
Na^+	154 mM	147.2 mM	131 mM	130 mM
Cl^-	154 mM	155 mM	112 mM	112 mM
K^+		4 mM	5.36 mM	5.4 mM
Ca^{2+}		2.25 mM	1.84 mM	0.9 mM
Mg^{2+}				1.0 mM
Lac^-			28.3 mM	
Ac^-				27 mM

Lac^- indicates lactate and Ac^- indicates acetate.

2.2. Animals

Male Wistar rats (400–450 g) were obtained from the central animal unit of the Essen University Hospital. Animals were kept under standardized conditions of temperature (22°±°1°C), humidity (55°±°5%), and 12-h/12-h light–dark cycles. They were fed *ad libitum* (Ssniff-Spezialdiäten, Soest, Germany) with free access to water and not fasted before the experiments. All animals received human care according to the standards of Annex III of the directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes [12]. The experimental protocol has been approved by the North Rhine-Westphalia State Office for Nature, Environment and Consumer Protection (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen), Germany, based on the local animal protection act.

2.3. Anesthesia, analgesia, and surgical procedures

Anesthesia, analgesia, catheter insertions, shock induction, resuscitation schedule, blood sampling, and organ resection were basically performed as described previously [11,13], with slight modifications. Rats were anesthetized with isoflurane (2% in 100% medical O_2 at 4 L/min for induction of anesthesia, 1%–1.5% at 1 L/min throughout the experiment) through face masks connected to a vaporizer (Isoflurane Vet. med. Vapor; Dräger, Lübeck, Germany) and received ketamine (50 mg/kg, subcutaneously) into the right chest wall for analgesia. Lidocaine (5 mg/kg, subcutaneously) was administered before a skin-deep incision along the left groin. Subsequently, a Portex catheter was placed within the femoral artery and the femoral vein. Each catheter was fixed with surgical suture. To avoid occurrence of pain, half of the initial dose of ketamine was injected if needed.

2.4. Induction of hemorrhagic shock and resuscitation regimen

After insertion of the catheters, animals were allowed to adapt for 20 min before hemorrhagic shock was induced by removing 2 mL blood every 3 min through the femoral artery catheter using a 2-mL syringe (Terumo, Leuven, Belgium). The first syringe was prefilled with 0.2 mL of acid citrate dextrose A solution. The syringe with citrated blood was stored at 37°C. Blood withdrawal was continued until the MAP dropped to 25–30 mm Hg; this typically took about 20 min. During the following 10 min, the MAP was fine-tuned by sampling of smaller blood volumes (0.5–1 mL). For the next 60 min, the MAP remained between 25 and 30 mm Hg, typically without the need of any further intervention. In some individual cases, small amounts (0.1–0.5-mL aliquots) of citrated blood had to be administered or additional small blood samples (0.5–1-mL aliquots) to be withdrawn, to keep the MAP within the desired range. After the shock phase, study group–specific resuscitation fluids were infused into the femoral vein within 30 min