Araştırma

THE EFFECTS OF RESUSCITATION FLUIDS ON HEMODYNAMIC PARAMETERS AND BLOOD BIOCHEMISTRY OF RATS BLED TO HYPOVOLEMIA

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SUMMARY

Purpose: Hemorrhagic shock is a rare but serious complication which may lead to hemodynamic instability, decrease in oxygen delivery and decreased tissue perfusion, cellular hypoxia, organ damage and death. Current study was designed to study the efficiency of whole blood, low molecular weight starch solution (LWS) and hypertonic saline with dextrane (HSD) on hemodynamics parameters, coagulation and tissue perfusion of rats bled to hypovolemia.

Methods: Experiments were performed with Sprague Dawley rats (n=7 per group), weighing 200–250 g. The rats were bled through left iliac artery for 20 min in three successive steps until the mean arterial pressure fell to and stabilized at approximately 20 mmHg. Then animals in each group was treated either with HSD (4 ml/kg; i.v.) or LWS solution (4 ml/kg; i.v.) or stored whole blood transfusion (2 ml/100 g; i.v.) for 30 minutes.

Results: In rats treated either with whole blood or HSD, the mean arterial pressure values were found to be higher than the rats treated with LWS solution. There was no significant difference in heart rate values among all three groups. LWS solution treatment adversely affected coagulation ($p<0.01$) whereas no effect was recorded with HSD and whole blood treatments. The partial pressure of blood gases and oxygen saturation did not show any significant difference in all three groups. Base excess has not been found to be different in terms of pre- and post-treatment values. Metabolic acidosis was observed in HSD treatment group. Lactate was detected to be statistically lower in rats treated with whole blood ($p=0.03$).

Conclusion: Use of combination of small volume of HSD and whole blood as fluid resuscitation may exert a favorable extended profile of hemodynamic effects, better hemostasis and tissue perfusion in hemorrhagic shock.

Key words: coagulation, asid-base disorders, mean arterial pressure, whole blood, hydroxyethyl starch , hypertonic saline

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Kanama ile Hipovolemi Oluşturulan Sıçanlarda Resüsitasyon Sıvılarının Hemodinamik Parametreler ve Kan Biyokimyasi Üzerine Etkileri

Amaç: Hemorajik şok nadir rastlanılan ancak hemodinamik bozukluk, oksijen taşınımında ve doku perfüzyonunda azalma, hücresel hipoksi, organ hasarı ve ölüme neden olan ciddi bir komplikasyondur. Bu çalışma, kanama ile hipovolemi oluşturulan sıçanlarda tam kan, düşük moleküler ağırlıklı nişasta solüsyonu ve dekstranlı hipertonik saline solüsyonlarının hemodinamik parametreler, koagülasyon ve doku perfüzyonu üzerine olan etkinliklerini araştırmak amacıyla tasarlanmıştır.

Metodlar: Deneylerde 200–250 g Sprague Dawley sıçanlar (her grupta n=7) kullanılmıştır. Sıçanlar, sol iliak arterden 20 dakikalık üç birbirini takip eden basamakla kanama yapılarak ortalama kan basıncı yaklaşık 20 mmHg da stabilize edilene kadar düşürülmüştür. Gruplardaki sıçanlara sonrasında dekstranlı hipertonik saline solüsyon (4 ml/kg; i.v.), düşük moleküler ağırlıklı nişasta solüsyonu (4 ml/kg; i.v.) ya da saklanmış tam kan transfüzyonu (2 ml/100 g; i.v.) 30 dakika süresince uygulanmıştır.

Bulgular: Tam kan ya da dekstranlı hipertonik saline solüsyonu uygulanan sıçanlardaki ortalama kan basıncı düşük moleküler ağırlıklı nişasta solüsyonu uygulanan sıçanların ortalama kan basıncından yüksek bulunmuştur. Gruplar arasında kalp hızı değerleri arasında anlamlı bir farklılık görülmedi. Düşük moleküler ağırlıklı nişasta solüsyonu koagülasyonu olumsuz yönde etkilerken ($p<0.01$), diğerlerinde böyle bir etki görülmedi. Kan gazlarını parsiyel basıncı ve oksijen saturasyonu gruplar arasında farklılık göstermedi. Baz fazlalılığı tedavi öncesi ve sonrasında farklı bulunmadı. Dekstranlı hipertonik saline solüsyonu grubunda metabolik asidoz görüldü. Tam kan uygulanan sıçanlarda laktat düzeyi anlamlı olarak düşük bulundu ($p=0.03$).

Sonuç: Dekstranlı hipertonik saline solüsyonu ve tam kan kombinasyonu daha olumlu geniş profilli hemodinamik etkiler sunmakta, hemorajik şokta daha iyi hemostaz ve daha iyi doku perfüzyonu sağlamaktadır.

Anahtar kelimeler: Koagülasyon, asit-baz bozuklukları, ortalama kan basıncı, tam kan, nişasta solüsyonu, hipertonik salin

INTRODUCTION

Hemorrhagic shock is inadequate tissue perfusion and inadequate removal of cellular waste products causing failure of oxidative metabolism that can involve decreases in oxygen delivery and usage[1]. The type of fluid, the dose and the infusion rate are critical issues while treating hemorrhagic shock that define the therapeutic endpoints[2]. The efficacy of fluid resuscitation after injury can be assessed by many criteria including recovery of cardiovascular instability, restoration of organ perfusion and duration of hemodynamic effects[3]. Crystalloid solutions and blood transfusion are the mainstays of pre-hospital and in-hospital treatment of hemorrhagic shock where in the pre-hospital setting, four types of fluid are recommended: crystalloid solutions, colloid solutions, hypertonic saline and oxygen-carrying blood substitutes[4,5].

The concept of resuscitation with the use of a small volume (4 to 6 mL/kg) of very hypertonic (7.5%) sodium chloride solution has been described in experimental and clinical conditions involving hypovolemia. Small volume hypertonic saline infusions rapidly increase cardiovascular and metabolic functions by producing plasma volume expansion through the displacement of intracellular and interstitial fluid to the vascular compartment[6]. The use of hypertonic saline (6%) with dextran-70 (HSD), which is hypertonic and hyperoncotic, is a new approach for intravascular volume replacement since it prolongs volume expansion through an endogenous fluid redistribution[6]. Colloids considerably reduce total fluid requirement, prolong volume expansion and obtain hemodilution with increased microvascular perfusion[7].

It has been reviewed that whole fresh blood is preferred to resuscitate the patients with acidosis, hypothermia or coagulopathy[5]. Massive blood transfusion is also recommended in hemody-

namic resuscitation without reaching a particular hemoglobin or hematocrit target level[8].

In this current work, we aimed to investigate the effects of low molecular weight starch solution (LWS), hypertonic saline with dextrane (HSD) or transfusion on systemic hemodynamic recovery, coagulation, acid-base status and arterial blood gas parameters in rats bled to hypovolemia.

MATERIALS AND METHODS

Animals

Experiments were performed with male albino Sprague Dawley rats, weighing 200–250 g. An approval of Institutional Animal Care and Use Committee was taken before the experiments. The animals were kept in a temperature-controlled room with 12-h light and dark cycle and fed with standard animal food and water ad libitum.

Experiments were performed under urethane (1.2 g/kg, i.p.) anesthesia. Normal body temperature was maintained by continuous monitoring via a rectal thermometer and a heating pad during the experiments.

Direct measurement of blood pressure

Bilateral iliac arteries were catheterized with a PE-10 catheter attached to PE-50 polyethylene tubing, one for direct measurement of blood pressure and the other for bleeding. Arterial blood pressure was recorded on a polygraph (Grass Model 7, USA) via a pressure transducer (Grass). The heart rate was monitored via a tachograph (Grass Model 7P44D, USA). The left iliac vein was also catheterized for intravenous administration of drug solutions. All catheters were filled with 1% heparin–saline solution. Heparin-saline solutions were discarded before collection of blood samples.

Experimental protocol

Upon completion of catheterisation the rats were connected to polygraph and blood pressure and heart rate was monitored for 10 min. Then, the rats were bled through left iliac artery for 20 min in three subsequent steps until the mean arterial pressure fell to and stabilized at approximately 20 mm Hg. A total of 1.99±0.1 ml blood/100 g was withdrawn that is approximately equivalent to 50% of the total blood volume in rats[9].

Either hypertonic saline with dextrane (4 ml/kg; i.v.) or low molecular weight starch solution (4 ml/kg; i.v.) was injected or stored whole blood transfusion (2 ml/100 g; i.v.) was performed after the stabilization of mean arterial pressure (MAP) at around 20 mm Hg. The blood withdrawn for inducing hypovolemia was used for biochemical assays or collected in citrated tubes (containing 1 mg citrate) for transfusion. The rats were monitored continuously 30 min and final blood samples (3 ml) were taken before sacrification.

The coagulation tests were measured with MDA®, microbial analyzing system (Trinity Biotech, USA) and partial pressures of gases in blood are measured by Radiometer model ABL825 (Radiometer Medical APS, Copenhagen).

Drugs

Low molecular weight starch solution (LWS solution; %6 HydroxyEthylStarch 130/0.4 in 0.9% sodium chloride solution, Voluven®, Fresenius Kabi, Canada) and hypertonic saline with dextrane (HSD; 7.5% hypertonic saline + 6% dextrane70, RescueFlow®, BioPhausia AB, Sweeden) were used in the study. The blood withdrawn for inducing hypovolemia was stored and used for transfusion.

Data analysis

The results were expressed as “mean ± standart error of mean (sem)”. Mean arterial pressure was calculated as “1/3 pulse pressure+diastolic blood pressure”. Analysis of variance for repeated measures (two-way) and Bonferroni post test were used to analyse the effect of solutions on mean arterial blood pressure or heart rate. Paired Student’s t-test was used to compare pre- and post-bleeding biochemical values. One-way analysis of variance followed by Dunnet's post test was used for comparing the post-treatment biochemical values in different groups receiving different treatments. The level of statistical significance was accepted as $P<0.05$.

RESULTS

The effect of whole blood, HSD and LWS treatments on MAP has been found to produce a significant time course difference ($p<0.0001$; $F=35.75$, $df=6$) as shown in Fig.1 where the treatment blocks were found to be different ($p=0.0007$; $F=11.24$, $df=2$) and a significant interaction was detected ($p<0.0001$; $F=6.32$, $df=1$). In rats treated either with whole blood or HSD, the MAP values were found to be higher than the rats treated with LWS. Neither of the solutions displayed any quick restoration of MAP values within 10 min like whole blood. There was no significant difference in heart rate values among all three groups (Fig. 2).

When the blood chemistry was investigated, the groups did not produce a significant difference in terms of pre-treatment values of three treatment approaches. LWS treatment prolonged international normalized ratio (INR; $p=0.0062$) and LWS treatment was found to be different HSD and whole blood treatments in terms of coagulation ($p<0.01$; Table1).

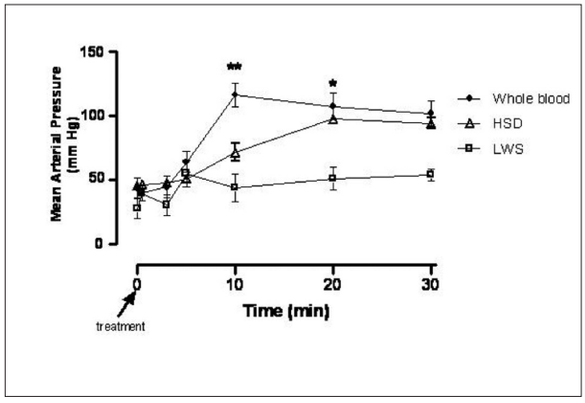
Base excess has not been found to be different in terms of pre- and post-treatment values or treatment groups (Table.1). According to the pH values listed in Table.1, we observed metabolic acidosis in HSD treatment group ($p=0.0292$). Lactate was detected to be lower in rats treated with whole blood ($p=0.03$). Whole blood increased hemoglobin values when compared to HSD ($p<0.01$) and LWS ($p<0.05$).

The comparison of partial pressure of blood gases and oxygen saturation did not produce any significant difference both in the pre-treatment and post-treatment values in three resuscitation fluids (Table 2).

Hemoglobin values increased from 14.44 ± 0.59 to 15.99 ± 0.76 in rats treated with whole blood ($p= 0.0014$) but decreased in HSD or LWS treated rats ($p=0.0734$ and $p=0.0095$, respectively).

DISCUSSION

In hemorrhagic shock, the main management strategies are the control of bleeding and the



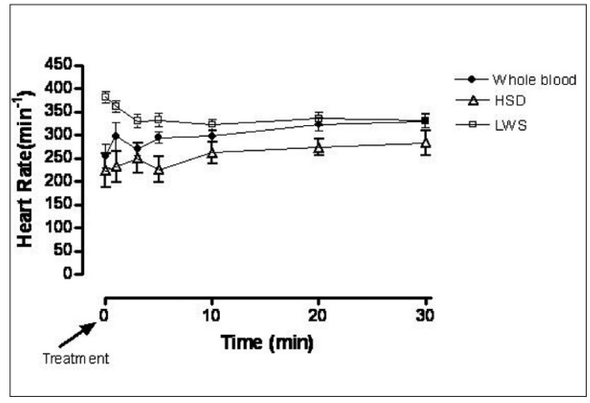


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Fig. 1 The effect of whole blood, hypertonic saline-dextrane 70 (HSD) and low molecular weight starch solution (LSW) on mean arterial pressure in rats bled to hemorrhagia (n=7 per group). Treatments were given at time 0.

*, $p < 0.05$ (Bonferroni post-test, compared to LSW).

**, $p < 0.01$ (Bonferroni post-test, compared to LSW group).

Fig. 2 The effect of whole blood, hypertonic saline-dextrane 70 (HSD) and low molecular weight starch solution (LSW) on heart rate in rats bled to hemorrhagia (n=7 per group). Treatments were given at time 0.

replacement of circulating volume[10]. The critical problems of hemorrhagic shock are decreased tissue nutrition and drainage of metabolites into tissues thus producing subsequent hypoxic cellular damage[1]. It was accepted that trauma victims in hypotensive hemorrhage should receive large volumes of fluids as early as possible for increasing cardiac output and oxygen delivery to maintain microvascular perfusion and oxygenation[4]. Correction of the deficit in blood volume with crystalloid volume expanders will generally maintain hemodynamic stability, while transfusion of red cells is used to improve and maintain tissue oxygenation. The main target of blood transfusions in hemorrhagic shock is to restore the capacity of intravascular volume for oxygen transport[11].

The use of hypertonic saline was introduced by Velasco and colleagues in 1980[12]. Using small-volume hypertonic solutions for resuscitation have some advantages in improving microvascular flow, controlling intracranial pressure, stabilizing arterial pressure and cardiac output[4,13]. However, its potent and rapid hemodynamic effects only last for 30 to 60 min. Combining dextran or hydroxyethyl starch (HES) with hypertonic saline increases the duration of its effects up to 3-4 h and expands plasma volume 3-4 times[14,15]. Previously, it was demonstrated that these solution dramatically restore the blood pressure and the cardiac output within 2 min in experimental animals[15,16]. Colloids are often preferred for correcting hypovolemia, improving systemic and microcirculatory hemodynamics[17,18].

In this current study, we demonstrated that the groups receiving whole blood or HSD treatments had more marked elevations in MAP values than the group received LWS solution treatment.

Hemorrhagic coagulopathy is also another major problem that occurs following traumatic injury and underlying mechanism still remains unknown[19]. Intravenous fluids can produce adverse effects on hemostatic mechanisms while stabilizing hemodynamics. Dilutional coagulopathy and secondary clot disruption due to increased blood flow, increased perfusion pressure and decreased blood viscosity are the main reasons for hemorrhagic coagulopathy[7,10]. Dilution of plasma clotting proteins results in prolongation of the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) and this requires the monitorisation of PT, aPTT, and fibrinogen in patients receiving massive blood transfusions[20]. The use of artificial colloids may be associated with untoward effects on coagulation as well. In some studies colloids have been found effective and safe substitutes for blood loss without relevent adverse effects on coagulation, but some showed that it should be avoided especially in patients at risk of increased bleeding[21]. Hydroxylethyl starches (HES) is believed to alter coagulation and platelet function leading to increased bleeding tendency. This effect is caused by physicochemical characteristics of the HES preparation and the electrolyte composition of the solvent[22,23]. Bleeding tendency is also associated with reduction in circulating concentrations of factor VIII and von Willebrand factor (vWF) by the hydroxyethyl starches[24]. The effects on blood coagulation closely depends on physicochemical properties of different types of HES. Whereas some studies have showed lower molar substitution of HES (130/0.4) causes lower perioperative blood loss and has minimal coagulation impairment, other studies suggest that all moleculer substitutes of HES can alter coagulation[24,25].

Fluid resuscitation with hypertonic saline in case of of hemorrhagic shock causes delay in clot formation and greater bleeding results[27]. Hypertonic saline solutions have been investigated in various clinical settings. As a replacement fluid, it is cheaper, free of risk of infection or allergic reactions compared with colloids. In current study, HES statistically prolonged INR when compared to HSD and whole blood ($p<0.05$). Fluid resuscitation with HES caused coagulation impairment.

Large amounts of fluid replacement for hemorragic shock especially associated with untoward changes of acid–base status[25]. pH is usually used as an index for tissue perfusion and it has been verified in a variety of patients and in experimental hemorrhagic shock models[28]. Base ex-

cess is an important marker to identify patients with under-perfused tissues[29]. During hemorrhagic shock, metabolic acidosis is common and hyperlactatemia is the main cause. The most sensitive marker which predicts complications in trauma patients is arterial base deficit and is correlated with arterial lactate concentration. Elevated base deficit and lactate concentrations after shock are related to oxygen transport imbalance at the cellular level[30]. Lactate and base deficit are all more sensitive endpoints of cellular resuscitation[31]. In our study, we demonstrated that lactate decreased significantly in rats treated with whole blood ($p<0.05$). HSD didn't recover metabolic acidosis when compared to LWS and whole blood administration due to its hypernatremic and hypercloremic effects. P02, PCO2 and SPO2 parameters didn't show statistically significant difference among all three group ($p>0.05$).

CONCLUSION

The primary treatment of hemorrhagic shock is control of the source of bleeding as soon as possible and fluid replacement. There are some concerns regarding the best method for resuscitation including the choice of fluid type, its amount, its application time and rate. The results of our study demonstrate that hypertonic saline solution improves hemodynamic status and has beneficial effects on microcirculation in hemorrhagic shock without causing significant side effects. LWS solution may have negative effects on hemostasis. Thus, HSD and whole blood combination is a good approache for fluid resuscitation in massive hemorrhagic shock.

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