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Effects of Different Fluids on Hymodynamic Parameters and Liver Arginase Activities in Hemorrhagic Shock

Farklı Sıvıların Hemorajik Şokta Hemodinamik Parametreler ve Karaciğer Arjinaz Aktivitesi Üzerine Etkileri

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ABSTRACT

AIM: Using a standardized splenic injury model of uncontrolled hemorrhagic shock, we aimed to research the effect of different fluid resuscitation on the liver arginase activity.

MATERIALS AND METHODS: Fifty rats were divided into five groups. In group 1; massive splenic injury was untreated, in group 2 massive injury was treated with 70 ml/kg/hour Ringer Lactate solution, in groups 3,4,5 massive injury was treated with 7.5 ml/kg/hour Hydroxyethyl Starch, Hypertonic Saline and Dextran-40, respectively between 15 and 30 minutes of injury. In all groups, splenectomy was performed at the 30th minute. Hemodynamic monitoring and anesthesia were continued up to 90 minutes. Duration of follow-up was 48 hrs. After 48 hours of survival, liver tissue samples were removed and were kept in -70◦C for the analysis of arginase activities.

RESULTS: Different fluid regimens were found to have no effects on hemodynamic parameters, arteriel blood gases and survival at the early phase of uncontrolled hemorrhagic shock. RL increased liver arginase activity compared with control group ($p<0.02$) and other groups. ($p<0.05$). Unlike Ringer Lactate, other groups had no statistical differences at arginase activities comparing to control group.

CONCLUSIONS: Finally, depending on long term results, Ringer Lactate is more useful fluid choice in hemorrhagic shock.

Key words: Hemorrhagic shock, splenic injury, Ringer lactate, arginase activity

ÖZET

AMAÇ: kontrolsüz hemorajik şokun standart dalak yaralanması modelini kullanarak farklı sıvı resüsitasyonunun karaciğer arjinaz aktivitesi üzerine etkilerini araştırmayı amaçladık.

GEREÇ VE YÖNTEM: 50 adet rat 5 gruba ayrıldı. Grup 1'de massif dalak yaralanması yapıldı, tedavi verilmedi. Grup 2'de massif dalak yaralanması 70 ml/kg/saat Ringer Laktat solüsyonu, Grup 3, 4, 5'te 7.5 ml/kg Hydroxyethyl Starch, Hipertonic Salin ve Dekstran-40 ile, yaralanmanın 15-39. dakikaları arasında tedavi edildi. Tüm gruplarda 30. dakikada splenektomi yapıldı. Hemodinamik monitörizasyon ve anestezi 90. dakikaya kadar sürdürüldü. Takip süresi 48 saatti. 48 saatlik sağkalım sonrası karaciğer doku örnekleri çıkarıldı ve arjinaz aktivitesi analizi için -70◦C'de saklandı.

BULGULAR: Farklı sıvı rejimlerinin hemodinamik parametreler, arter kan gazları ve hemorajik şokun erken dönemlerinde sağkalım üzerine etkileri yoktu. Ringer Laktat, kontrol ve diğer gruplarla karşılaştırıldığında karaciğer arjinaz aktivitesini artırdı. ($p<0.02$; $p<0.05$). Diğer gruplarla kontrol grubu karşılaştırıldığında aralarında arjinaz aktivitesi yönünden istatistiksel farklılık yoktu.

SONUÇ: Sonuç olarak, uzun dönem bulgulara bağlı olarak, Ringer Laktat hemorajik şokta daha yararlı bir seçenektir.

Anahtar Kelimeler: Hemorajik şok, dalak yaralanması, Ringer laktat, arjinaz aktivitesi

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INTRODUCTION

Hemorrhagic shock is one of the major causes of preventable death following trauma [1]. Shock described as generalized cellular hypoxia resulting from imbalance between oxygen consumption (VO2) and oxygen delivery (DO2) due to insufficient tissue perfusion [2]. Successful treatment of hemorrhagic shock is usually accomplished by surgical control of bleeding, restoration of tissue perfusion by replacement of fluid loss. However, in traumatic hemorrhagic shock cases, ideal resuscitation fluid, its applied rate and volume at prehospital and emergency units are controversial [3]. L- arginine (Arg) is classified as semiessential aminoacid [4]. L-arginine is subject to various metabolic fates in its role as precursor for synthesis of nitric oxide (NO), urea, polyamines, proline, glutamate, creatine, agmatine (decarboxylated arginine), and proteins. Its cleavage to L-ornithine and urea, has important role in the urea cycle, is catalyzed by arginase. Urea is excreted via kidney while L-ornithine plays role as precursor of proline, polyamines and glutamate. This experimental study was aimed to evaluate early effects such as hemodynamic parameters, arterial blood gases (ABG) and late effects such as survival, liver arginase activities of different fluid resuscitations in traumatic hemorrhagic shock

MATERIALS AND METHODS

General Preparation:

After the approval of our study by ethical committee of Ondokuz Mayıs University, School of Medicine (TCAM-01/34), adult male Sprague-Dawley rats (n=50) were fasted overnight before the experiment, but allowed water ad libitum. The rats were anesthetized by pentobarbital (50 mg/kg i.p) after measurement of body weights (250-350 gr). They were allowed to breathe room air spontaneously. The animals were placed supine and body temperature was monitored with a rectal temperature probe (Thermistor 8402-20, Cole-Parmer Instrument Company, Niles, IL, USA). Both left femoral artery and femoral vein were isolated by cut-down under sterile conditions then cannulated (22G,Vasofix, Braun Melsungen, Germany). The femoral artery was used to measure mean arterial pressure (MAP) and for ABG 2 sampling. An arterial catheter containing a calibrated pressure transducer (Deltran II, Disposable Pressure Transducer System, Utah Medical Products, Midvale, UT, USA) was connected directly to a monitor (Kontron Instruments Minimon 7131, ENGLAND). Another catheter (20 GA, Venflon Cannula, SWEDEN) was advanced into the inferior vena cava in left femoral vein for blood withdrawal and it was also used for the administration of drugs. An infusion pump was introduced into this vein as described by Guven et al [5]. Blood collected in this system was used for hemogram and determination of biochemical parameters.

Uncontrolled Hemorrhagic Shock Protocol:

The experimental hemorrhagic shock was induced by creation of a massive splenic injury. Shortly, after anesthesia and cannulation, a midline laparotomy was performed. An orthodontic wire that passed inside of epidural tuohy needle was turned around spleen Grade-4 splenic injury in animals was started by pulling cerclage wire and the splenic parenchyma was sharply and completely transected transversely. At the same time (accepted as baseline, t0), a 2 ml/100g controlled bleeding was started from venous catheter. At 15th minute controlled bleeding was stopped. The cut edges of the spleen were allowed to bleed freely into the peritoneal cavity, and the laparotomy incision was closed with a running suture. After 15 minutes, animals were randomized into five groups according to received fluid regimen treatment. The experiment consisted of four phases (P); prehospital (P1) (0-15 min), emergency unit (P2) (15-30 min), operative (P3) (30-90 min), intensive care unit (P4) (90min-48hrs). During P1, rats did not have any fluid resuscitation. In P2, different fluid regimens; Hydroxyethyl Starch (HES), hypertonic saline (HS) and Dextran-40 from 7.5 ml/kg/hr, ringer lactate (RL) from 70 ml/kg/hr were administered. In P3, bleeding was controlled by splenectomy and all groups took standart fluid regimens RL 70ml/kg/hr until the end of 90 minutes. This period mimicks “operation room” period. In P4, mean arteriel pressure (MAP), rectal temperature and breath rate were observed within 10 minute intervals. Blood samples were drawn at t0,15, 30 and 90.th minutes for ABG analysis and at 48th hour for complete blood count (CBC) and biochemical tests. At the end of 90 minute, all catheters were removed, vessels were tied. At the end of 48 hours, animals were sacrified. Blood samples were analysed for ABG by blood gas analyzer (CHIRON BGA 248, East Walpole, MA,USA) and for biochemical tests by autoanalyser (HITACHI-917, Roche, Germany). Removed liver tissue samples were immediately stored at -80 ^{o}C for later assays of arginase.

Methods of Tissue Arginase Analysis:

We homogenized tissues weighing 40-50 mg with pestle in liquid nitrogen. After addition of 35 mmol/L TRIS-HCl and 1mmol/L MnCl2 (pH 7.5) buffer, tissues were further homogenized by a sonicator. Homogenate was santrifuged at 18.000 g +4 ^{o}C degrees for 10 minutes. We determined arginase enzyme activity by a spectrophotometric method as described by Kocna et al [6] with UV-160A SHIMADZU spectrophotometer at 515 nm. Tissue protein levels were measured by Lowry [7]. Arginase activity was expressed as unite/mg protein.

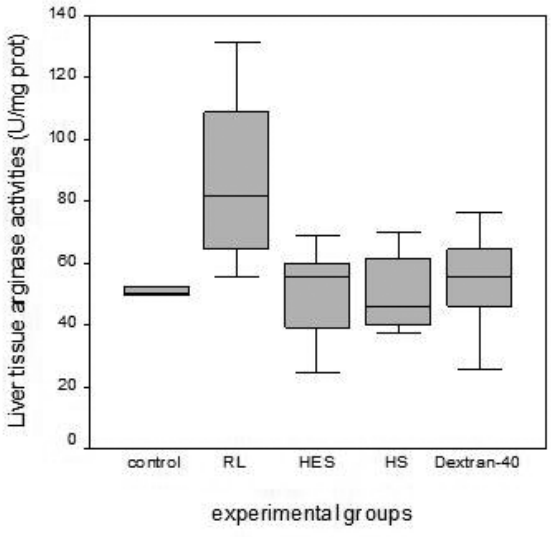
Statistics:

For statistical evaluation, we used the software package SPSS 15.0 and probability value of less than 0.05 was accepted as statistically significant. One way anova test was used to compare all groups. Subgroup analysis were performed with Tukey’s HSD test for arginase level. For other parameters, variance analysis for dependent and repeated groups was used. Survival rates compared with Kaplan-Meier survival analysis.

Table I: *Liver tissue arginase activities (U/mg prot)(mean±SEM)*

	mean±SD
Control	50.59±10.26
RL	86.99±29.01*
HES	50.64±16.18
HS	50.90±13.23
Dextran -40	53.76±15.66
P value: control and RL	*p<0.009*
HES and RL	*p<0.009*
HS and RL	*p<0.006*
Dextran 40 and RL	*p<0.007*

Figure 1*. Liver tissue arginase activities in experimental groups*



RESULTS

Five rats from control group, three rats from RL, and two rats from each other groups died during 48 hours after hemorrhagic shock. In this experimental model, resuscitation with different iv fluids at the early phase of uncontrolled hemorrhagic shock after massive splenic injury did not improve the survival until 48 hours. ABG, CBC, MAP, rectal temperature and breath rate, biochemistry parameters such as electrolyte (Na, Cl), liver and kidney functions (AST, ALT, urea, creatinine) showed no significant differences among fluid regimen groups.

Liver arginase activity levels were shown on Table 1. Liver arginase activity in RL group was statistically higher than other regimens and control group *(Figure 1)*.

DISCUSSION

Timely and appropriate fluid resuscitation may mean the difference between survival and death. Controversy surrounds the best method of fluid resuscitation for optimum recovery [8]. Fluid resuscitation in travmatic hemorragic shock management is essential. In various clinical trails it was shown that early fluid resuscitation and surgical intervention decreased acut renal failure insidance and increased survival rate [9]. Bolus 2 liters cristalloid infusion followed by blood transfusion according to responce is routine treatment protocol in hemorrhagic shock patients [10]. In this protocol, suggested by American Association for the Surgery of Trauma, the mechanism of trauma, the place of bleeding, its amount and rate were not considered. In our study, massive splenic injury was performed as uncontrolled bleeding model. We can assume that this bleeding model is not as severe as an aort or arterial injury.

In this study, we determined liver arginase activities in different fluid resuscitation groups. There are two known isoforms of arginase. Arginase I is located in the cytosol and strongly expressed in liver, whereas arginase II is confined to the mitochondrial matrix and mainly expressed in kidney. The role of arginase I in the liver is well-defined, where it catalyzes the deamination of L-arginine to produce ornithine and urea. Ornithine, the downstream product of arginase activity, is known to be further metabolized into polyamines that are involved in tissue repair and growth, as well as proline, the precursor of collagen formation [11,12]. In our experiment, liver arginase activity in RL group was significantly higher than control and other groups. This shows a better ammonia detoxification in RL group where L-arginine is converted to ornithine and urea by arginase enzyme[11]. Besides, ornithine is precursor of proline and poliamines used in cell proliferation and wound healing [13]. Because of higher liver arginase activity in RL group, we believe above mechanisms occur predominantly in this group. Ornithine supports liver regeneration. Since liver is major organ of metabolism, its protection is important for body functions.

Cytokines stimulate arginase expression and activity in trauma. Similarly, catecholamines stimulate arginase activity in trauma. So, arginase activity in trauma is more dominant than nitric oxide synthase (NOS) activity without any exterior effect [14,15,16]. Low NO production might show a restricted bleeding by vasoconstriction.

Recent study indicates that resuscitation with RL leads to greater hypercoagulability and less blood loss than resuscitation with NS in uncontrolled hemorrhagic shock [16]. Another recent study suggest that the timing of fluid resuscitation and the type of fluid used to treat hemorrhagic shock contribute to the inflammatory response as well as cell death.

Cellular destruction and a pro-inflammatory response follow hemorrhagic shock. Early resuscitation with isotonic crystalloid fluids decreases these responses [17]. In conclusion, we consider that late effects of different fluid resuscitation as well as early effects are very important. Although ABG, CBC, MAP, vital signs and biochemistry parameters showed no significant differences in all groups, RL increases liver arginase activity with compared to other groups. Finally, depending on long term results, RL is more useful fluid choice in hemorrhagic shock.

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