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Influence of Acute Serious Haemorrhagic Shock on Erythrocyte Glutathione and Sodium – Potassium Contents

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Summary: The aim of the present study is to describe erythrocyte glutathione level and sodium – potassium contents at acute serious haemorrhage. The experiment was performed on adult male 15 Sprague-Dawley rats. Haemorrhage was performed by withdrawing a total volume of 2.1 ml of blood / 100 g body weight over a period of 10 min. Blood was collected twice; before haemorrhage and 1 hour after haemorrhage. Haemorrhage caused approximately 20 % decrease in hematocrit, red blood cells count, amount of haemoglobin and about 60 % decrease in mean arterial pressure but 46 % increase in heart rate. Interestingly, erythrocyte glutathione value does not change in pre- and post-haemorrhage. Moreover, haemorrhage increased plasma and erythrocyte sodium values but decreased plasma and erythrocyte potassium values. In conclusion, due to hemorrhagic shock, increased production of reactive oxygen species is a feature of haemorrhagic shock and decreased erythrocytes by haemorrhage can respond to oxidative stress by upregulating antioxidant defence in terms of increased production of glutathione. Moreover, the observed high sodium concentrations associated with a potassium decrease in red blood cell can be defect in cellular function due to severe hemorrhagic shock.

Key Words: Haemorrhage, Shock, Erythrocyte, Glutathione, Sodium and Potassium.

Alyuvar Glutatyon ve Sodyum – Potasyum Seviyelerine Ciddi Akut Hemorajik Şokun Etkisi

Özet: Çalışmanın amacı akut ciddi kan kaybında alyuvar glutatyon ve alyuvar sodium – potasyum seviyesini belirlemektir. Çalışmada 15 adet Sprague Dawley ırkı erkek yetişkin sıçan kullanılmıştır. Kanatma işlemi 10 dakikalık bir periyotta 100 g vücut ağırlığı başına 2.1 ml kan alınarak gerçekleştirildi. Kan örnekleri kanatmadan önce ve kanatmadan 1 saat sonra olmak üzere iki kez toplandı. Kanatma, hematokrit, alyuvar sayısı ve hemoglobin miktarında yaklaşık % 20, arterial kan basıncında yaklaşık % 60 ve kalp atım sayısında yaklaşık olarak % 46 artmaya neden oldu. İlginç olarak alyuvar glutatyon seviyesi, kanama öncesi ve kanama sonrasında değişiklik göstermedi. Ayrıca, kanama plazma ve alyuvar sodyum seviyelerini arttırırken plazma ve alyuvar potasyum seviyelerini düşürdü. Sonuçta, hemorajik şoktan dolayı reaktif oksijen türlerinin üretimi artabilir ve bu da azalan alyuvarlar glutatyon üretimini artırarak antioksidan savunmayı düzenlemeye ve oksidatif strese cevap vermeye çalışıyor olabilir. Ayrıca, ciddi kan kaybından kaynaklanan alyuvarlarda düşük potasyum seviyesi ile birlikte yüksek sodyum konsantrasyonu hücresel fonksiyonlarda defekte neden olabilir.

Anahtar Kelimeler: Kan Kaybı, Şok, Alyuvar, Glutatyon, Sodyum ve Potasyum.

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Introduction

Hemorrhagic shock is one of the most important and life threatening hypotensive situations. During hemorrhagic shock, a lot of alterations occur in body. In hemorrhagic shock, the observed response was a sustained arteriolar constriction and a decrease in flow and in tissue oxygen tension[1]. After haemorrhage, organism has high oxygen demand. Moreover, trauma and hemorrhagic shock are known to cause changes in red blood cell deformability and resting shape[2]. Moreover, acute serious hemorrhage is characterized by overproduction of oxygen free radicals[3,4].

Organisms have antioxidant defence system to protect biological integrity against free oxygen radical injury[5]. Glutathione is an important antioxidant enzyme which takes part in cellular defence mechanism against oxygen cytotoxin[6,7]. Glutathione (GSH) is a mitochondrial enzyme which catalyzes hydrogen peroxide and lipid peroxides reduction, and is the most effective antioxidant against oxidant stress in erythrocytes[5].

It is of major importance that erythrocytes containing GSH and GSH activity show deviation from normal level according to various illness or circumstances. Decrease of GSH activity causes increase of hydrogen peroxide activity and cell injury. In the lack of GSH, both erythrocyte membrane and organelles of erythrocyte are subject to oxidant effect[8]. Erythrocyte membrane contains almost the same rate lipid and protein. The lack of enzyme affects the fraction of erythrocyte membrane and also causes alteration in membrane fluidity[9,10].

In addition, previous studies of cellular function have demonstrated alteration of cell homeostasis during haemorrhage[3,11]. It was reported that red cell sodium in shocked animals was significantly higher than in control animals and a small decrease occurred in red cell potassium[12]. In addition, it was also reported that although ratio of sodium and potassium in red blood cell shown no significant difference at 15 minutes after haemorrhage, this ratio elevated at one hour after haemorrhage[3].

Considering all those previous study findings, the present study was designed to determine the erythrocyte glutathione and erythrocyte sodium and potassium contents in hypotension induced by hemorrhagic shock conditions.

Materials and Methods

Animals: Adult, male Sprague-Dawley rats (250-300 g; Experimental Animals Breeding and Research Centre, Uludag University, Medical Faculty, Bursa, Turkey) were used in the present study. Rats were housed under a 12-hour light/dark cycle with free access to food and water. The surgical and experimental protocols were approved by the animal Care and Use Committee of Uludag University and are in accordance with the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals'.

Surgical Procedure: Under ether anaesthesia, the left common carotid artery of rats were canulated with PE50 tubing filled with heparinized saline (100 U/ml). After surgery, the rats were placed in individual cages and allowed to recover from the anaesthesia for 4-5 h. During this period, the rats remained calm and without evidence of pain.

Blood Samples Collection And Haemorrhage Protocol: Before haemorrhage, 2ml of blood samples were collected via arterial catheter from rats and then acute haemorrhage was performed by withdrawing a total volume of 2.1 ml of blood/100g body weight over a period of 10 min. Approximately 35-40 % of the estimated total volume was removed during this period[13]. One hour after haemorrhage, 1 ml of post-haemorrhage blood samples were collected via arterial catheter.

Experimental Protocol: Before and one hour after haemorrhage, the mean arterial pressure (MAP) of rats were measured. For this, arterial catheter was connected to a volumetric pressure transducer (BPT 300) attached to a DA 100C general purpose transducer amplifier (Commat Ltd., Ankara, Turkey). Blood pressure of rats was recorded and analyzed using the MP 100 system and AcqKnowledge software (Biopac System Inc., Goleta, Calif., USA).

The hematocrit (PCV) was determined using a heparinized capillary tube and centrifuging the blood in a micro-hematocrit centrifuge for 6 min at 12000 rpm. Haemoglobin concentration (Hb) was measured by adding 20 µl of well-mixed whole blood to 6 ml of Drabkin's reagent. After 4 min haemoglobin concentration was measured in spectrophotometer (UV-1200 series, Shimadzu Corporation, Japan) set a 540µm[14]. The Red blood cell (RBC) was counted in a haemocy-

tometer Thomas chamber. Sodium (Na) and potassium (K) levels were analyzed by flame photometer[15,16] (Flame Photometer PFP 7, Jenway LTD, England).

Erytrocyte glutathione (GSH) level of blood samples were determined with spectrophotometer (UV-1200 series, Shimadzu Corporation, Japan) according to previously defined procedure[17].

Data And Statistical Analysis: Data are presented as mean ±SEM and % change. Student's *t* test was used to test the significance of differences between before and after haemorrhage values.

Results

Before haemorrhage, MAP, heart rate, PCV, RBC, Hb, erythrocyte GSH, E_{Na} and E_K were 113 ± 2 mm Hg, 276 ± 18 beat/min, 47.0 ± 0.6 %, 11.8 ± 0.5 x 10^6/mm^3, 11.7 ± 0.3 g/100ml, 83.16 ± 5.6 mg/100ml erythrocyte, 42.8 ± 0.9 mEq/l, and 65.9 ± 1.4 mEq/l respectively. After acute haemorrhage, mean arterial pressure, heart rate, PCV, RBC, Hb, erythrocyte GSH, E_{Na} and E_K were 46 ± 2 mm Hg, 403 ± 24 beat/min, 38.4 ± 1.0 % ($p<0.05$), 9.4 ± 0.3 x 10^6/mm^3 ($p<0.05$), 9.6 ± 0.2 g/100ml ($p<0.05$), 82.08 ± 5.9 mg/100ml erythrocyte ($p>0.05$), 71.9 ± 2.1 mEq/l ($p<0.05$), and 61.4 ± 1.8 mEq/l ($p<0.05$) respectively.

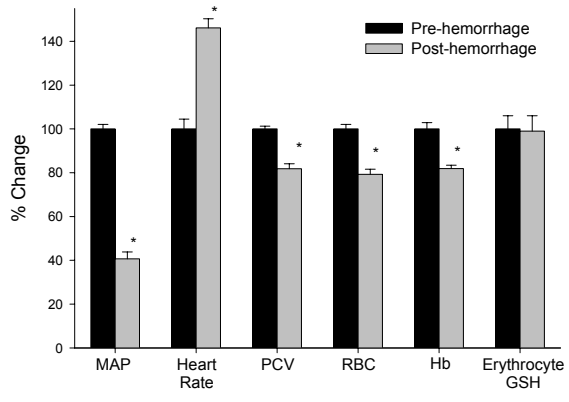

Figure 1:
Effect of acute serious haemorrhage on % changes of mean arterial pressure, heart rate, PCV, RBC, Hb and erythrocyte GSH

*, $p < 0.05$ significantly different from the value of pre-haemorrhage. MAP; mean arterial pressure, PCV; hematocrit level, RBC; red blood cell counts, Hb; haemoglobin value, GSH; glutathione level.

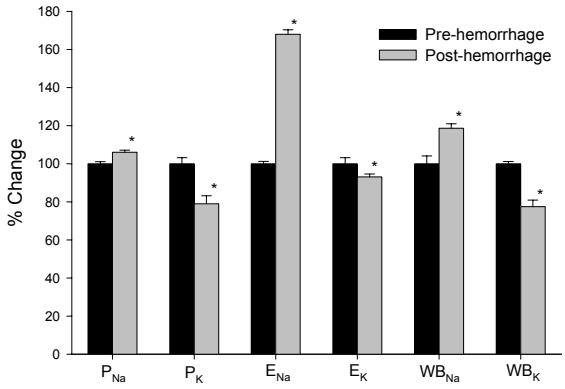

Figure 2:
Effect of acute serious haemorrhage on % changes of sodium-potassium contents of plasma, erythrocyte and whole blood.

*, $p < 0.05$ significantly different from the value of pre-haemorrhage. P_{Na}; plasma sodium value, P_K; plasma potassium value, E_{Na}; erythrocyte sodium value, E_K; erythrocyte potassium value, WB_{Na}; whole blood sodium value, WB_K; whole blood potassium value

Discussion

In the present study, erythrocyte GSH level and erythrocyte sodium and potassium level were investigated in acute serious hemorrhagic shock. Circulatory shock, including hemorrhagic shock, is a severe condition for mammals. Hemorrhagic shock is characterized by typical alterations in clinical and laboratory test results. In agreement with other studies[13,18], both clinically decreased mean arterial pressure and increased heart rate and haematologically decreased hematocrit level, red blood cell count and haemoglobin level were observed in the present study.

It was reported that hemorrhagic shock[4,19], cardiovascular disease[20] and hypoxemia[21] are characterized by overproduction of oxygen free radicals. Erythrocyte GSH has a central role in defence against oxidative damage. GSH is an important antioxidant enzyme which takes part in cellular defence mechanism against oxygen cytotoxin[6,7]. Besides, GSH is a mitochondrial enzyme which catalyzes hydrogen peroxide and lipid peroxides reduction, and is the most effective antioxidant against oxidant stress in erythrocytes[5].

In the present study, pre- and post- haemorrhage erythrocyte GSH values were found out as almost the same surprisingly. Pre- and post-haemorrhage erythrocyte GSH levels were 83.16 ± 5.6, 82.08 ± 5.9 mg/100ml erythrocyte respec-

tively. Erythrocyte GSH values in acute myocardial infarction patients were found higher than in normal healthy subjects. This increase was explained with the erythrocyte response to oxidative stress that acute myocardial infarction caused[20]. After haemorrhage, vital organs should be oxygenated to survive. Therefore, decreasing RBC count and Hb can be subject to more oxygen bind to meet oxygen need of vital organs per minute. This can cause oxidative injury. This oxidative stress could also cause an increase in erythrocyte GSH values but because of the decrease in normal erythrocyte count depending on haemorrhage, GSH level increase in the rest of the erythrocyte might have been established as no change in pre- and post-haemorrhage erythrocyte GSH level. In relation to this, in moderate haemorrhage, perhaps erythrocyte GSH level will be higher than the level before haemorrhage.

In the present study, levels of P_{Na}, E_{Na} and WB_{Na} after haemorrhage were higher than levels of those before haemorrhage. After haemorrhage, intracellular fluid passes plasma to compensate volume loss[22]. Therefore, P_{Na} and WB_{Na} levels could increase but P_K and WB_K levels could decrease relatively. Erythrocyte Na-K balance is saved by energy dependent active transport system located in erythrocyte membrane. Moreover, it was reported that haemorrhage decreased erythrocyte ATPase activity in dogs[3] and also resulted in diminution in erythrocyte membrane permeability[11]. This situation may cause an alteration in erythrocyte element contents

According to the results obtained from the study, having almost the same pre- and post haemorrhage erythrocyte GSH levels might be considered as a compensator response in order to minimize the oxidative injury that will occur after acute serious haemorrhage. Moreover, the observed high sodium concentrations associated with a potassium decrease in red blood cell appeared to be only one manifestation of generalized defect in cellular function and composition during severe hemorrhagic shock.

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