EFFECTS OF HYPERTONIC SALINE, HAES AND DYMETHYLSULPHOXYDE ON FREE OXYGEN RADICALS IN HAEMORRHAGIC SHOCK

HEMORAJİK ŞOKTA OKSİJEN SERBEST RADİKALLERİ ÜZERİNE HİPERTONİK SALİN, HAES VE DİMETİLSÜLFOKSİT'İN ETKİLERİ

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ÖZET

Amaç: Bu çalışmada amaç hemorajik şok sırasında kullanılan resusitasyon sıvılarının ve bunlara antioksidan eklenmesinin doku iskemisi üzerine etkilerini araştırmaktır.

Gereç ve yöntem: Kırk adet Yeni Zelanda tipi tavşan K (kontrol), I (hipertonik salin), H (HAES) ve D (dimetilsulfoksit-DMSO) olarak dört gruba ayrıldılar. Tavşanlar karotid arter yolu ile kanatıldılar. Otuz dk. şoktan sonra K grubuna sıvı resusitasyonu yapılmazken, I grubuna kanama miktarının 1.5 katı olacak şekilde %7.2'lik hipertonik salin, H grubuna kanama volümüne eşit volümde HAES %10 verildi. D grubuna ise HAES %10 ile birlikte antioksidan olarak DMSO eklendi. Sıvıların ve DMSO'nun etkisini değerlendirmek için kan, karaciğer ve ince barsak TBARS (thiobarbütürik asit reaktif maddeleri) ve laktat düzeyleri çalışıldı.

Bulgular: Grupların doku ve plazma TBARS ve laktat seviyeleri birbiri ile karşılaştırılınca istatistiksel olarak anlamlı bir fark olmadığı görüldü.

Sonuç: Replasman sıvılarının ve bunlara antioksidan ajan eklemenin hemorajik şokta gelişen doku iskemisini azaltmada birbirlerine üstünlüğü yoktur.

Anahtar kelimeler: Hemorajik şok, hipertonik salin, oksijen serbest radikalleri, dimetilsülfoksit antioksidanlar

ABSTRACT

Background: The aim of this study was to investigate the effects of antioxidant and resuscitation fluids which were used during haemorrhagic shock on tissue ischemia.

Methods: Forty New Zealand type rabbits were divided into four groups as C (control), I (hypertonic saline), H (HAES) and D (Dymethylsulphdxide-DMSO). Haemorrhagic shock was induced by bleeding from carotid artery. Thirty minutes after shock, Group C was not resuscitated while Group I was resuscitated with Hypertonic saline 7.2, Group H with 10 % HAES and Group D with HAES 10 % and DMSO. Thiobarbituric acid reactive substances (TBARS) and lactate levels in blood, liver and small bowel samples were measured.

Results: There were no significant differences among the groups tissue and plasma TBARS and lactate levels.

Conclusion: Resuscitation fluids and addition of antioxidants to the resuscitation fluids do not have any superiorities over each other to prevent tissue ischemic insult in haemorrhagic shock.

Key words: Haemorrhagic shock, hypertonic saline, oxygen free radicals, dimethylsulphoxide, antioxidants

INTRODUCTION

Blood flow toward the body is re-regulated as a result of haemorrhagic shock. The organs, such as heart and brain have their own auto-regulation systems for blood flow. In skin tissue, splenic regions and kidney, blood flow is regulated by sympathetic tonus. In such regions, blood flow is reduced and directed to more vital organs in shock.[1,2] During haemorrhagic shock, ischemia becomes apparent in all tissues and organs.[3] Anaerobic metabolism is stimulated during ischemic state and may cause the activation of some lytic enzymes, by increasing lactic acid level. Production of free oxygen radicals and lipid peroxidation products occurs via this way.[4] It has been reported that the antioxidant agent DMSO has scavenging effects of hydroxyl radicals, which is the most disturbing oxygen radical for the tissue.[5] Breaking down this chain by removing or preventing the cell ischemia as rapidly and efficiently as possible constitutes the basis of efficient treatment.

The aim of this study was to compare the efficiencies of hypertonic saline 7.2%, hydroxyethyl starch (HAES 10%) and HAES %10 plus DMSO as an antioxidant in eliminating tissue ischemia in an experimentally induced haemorrhagic shock model.

METHODS

A total of 40 New Zealand type rabbits, weighing between 2000 - 4000 g were used with institutional approval of the Selçuk University Meram Medical Faculty Animal Care and Use Committee. Our institution's guide for the care and use of laboratory animals was strictly followed. The rabbits were divided into four groups, each containing 10 subjects. The groups were named as the control (C) group, hypertonic saline 7.2% (I) group, HAES 10% (H) group and HAES 10% + DMSO (D) group. The subjects were anesthetized by the injection of 25 mg/kg ketamine and 15 mg/kg xylasine dihydrocloride intramuscularly.[6,7] Intra-arterial catheter was inserted into the carotid artery. Each experiment was monitorized to follow-up the mean arterial pressure (MAP), systolic blood pressure, diastolic blood pressure, heart and breathing rate, partial arterial oxygen pressure, respiration and heart rhythms during the experiment. After the initial value of MAP was measured, bleeding was carried out from carotid artery in a manner to reduce MAP to 35 mm/hg level.[8] The rabbits were applied second-degree shock and maintained in shock for a period of 30 minutes. After the shock (30 minutes), 4 ml venous blood sample was taken via internal jugular vein cannulated, for measurement of lactate and TBARS in red blood cells. Following this procedure, resuscitation group I was resuscitated with hypertonic saline 7.2% infusion at a volume equal to 1.5 fold of total blood sample taken, via internal jugular vein. Similarly, H group was resuscitated with HAES 10% and D group was resuscitated with HAES %10 + 20 mg/kg DMSO, both at a volume equal to the total blood sample taken, and in the same route as in group I during 30 minutes period.[9,10] Control group animals were not resuscitated. After the resuscitation period midline laparatomy was performed. Firstly, 4 ml blood sample was taken from vena cava inferior in order to measure lactate and TBARS levels in plasma and red blod cells. Thereafter, tissue samples were taken from the liver and the small intestines into two separate containers, containing isotonic sodium chloride solution, in order to measure lactate and TBARS levels in tissue. The animals were killed with injection of 10%

formaldehyde into the ventricle.

Biochemical Methods

TBARS measurement in plasma: A modification of Drapper-Hadley method was used to measure the plasma TBARS levels.[9,10] 0.5 ml plasma was taken and mixed with 2.5 ml 10% trichlor-acetic acid (TCA). It was incubated in 90°C water bath for 15 min, and than centrifuged for 10 min at 3000rpm. An amount of 2 ml of supernatant was transferred into another tube and 1 ml 0.675% thiobarbituric acid (TBA) was added. This mixed solution was than incubated in 90°C water bath for 15 min. After cooling the tubes, the absorbance of the material was measured using a spectrophotometer at 532 nm.

TBARS measurements in red blood cells: The method of Jain et al.[11] was used for the measurements of TBARS in red blood cells. Blood samples were centrifuged for 10 min at 3000rpm. Equal volume of saline solution was added into the tube after removing the supernatant and centrifuged for 10 min. at 2000/rpm. The washing procedure was repeated three times. Two tubes were taken as sample and control tubes. 200 µl of red blood cells was transferred into sample tube while same amount of distilled water was put into the control tube. Firstly, 800 µl phosphate solution (pH: 7.4) and 25 µl buthylised hydroxytoluen (BHT) and than 500µl30% TCA were added to the tubes. Thereafter, tubes were placed in the refrigerator for two hours and than centrifuged for 15 min at 2000 / rpm. 1 ml of supernatant was transferred to other tubes, and 75µl 0.1M EDTA and 250µl 1% TBA were added and mixed. The final solution was than incubated in 90°C water bath for 15 min. The absorbance of the material was measured using a spectrophotometer at 532 nm.

TBARS measurements in tissues: The method of Uchiama-Mihara[12] was used for the measurements of TBARS in tissue samples. For the measurements of TBARS in liver and small intestine, tissues were homogenized using a Microsom homogenizer (Biobak New York-USA) at 12 rms and 156 mM potassium chloride (KCL) in order to prepare 10% homogenate. 0.3 ml of KCL was added to each 0.3 gr tissue. 2 ml of homogenate was taken and mixed with 2 ml of 8% cold $HCLO_4$ (perclorate). It was centrifuged for 10 min at 3000 rpm. A certain amount of supernatant was transferred to another tube in order to measure lactate. 3 ml of 1% phosphoric acid was placed in to a tube, and 1 ml

0.675% TBA and 10% tissue homogenate were added and mixed. The solution was than incubated in 90°C water bath for 45 min, and after cooling 4 ml n-buthanol was added. The absorbance of the material was measured using a spectrophotometer at 532 nm against n-buthanol.

Tissue and plasma lactate measurements: These measurements were studied by calorimetric test using a Technicon RA-XT (Technicon Ltd. Swords Co./Dublin-Ireland) analyzer.

Statistical Methods

In statistical evaluations, for the values having parametric test condition, ANOVA one way variance analysis was used. If there was a difference between the groups, Tukey HSD test was used as a Post Hoc test for those parameters. The values were presented as mean ± standard deviation in figure.[1-5]

Kruskal-Wallis variance analysis was applied for the values that do not provide parametric test condition. In significant values ($p < 0.05$) Bonferroni correction and Mann Whitney U tests were applied. $P \leq 0.05$ was considered as statistically significant.

### RESULTS

There were a statistically significant decrease in mean plasma TBARS values after shock (30th min) compared to the 60th min values in all groups including the control ($p < 0.01$). Comparison of plasma TBARS levels among the groups following the shock (30th min) and after replacement (60th min) did not indicate any statistically significant difference among the groups. (The mean values of plasma TBARS were shown in figure 1).
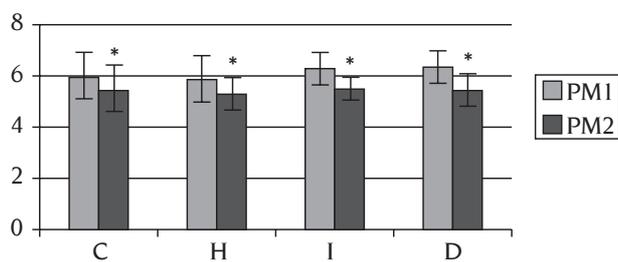
Figure 1. The plasma TBARS levels of the groups.
PM1: Plasma TBARS levels after shock (30 min);
PM2: Plasma TBARS levels after replacement (60 min).
(60 min)*shows significant difference at $p < 0.05$.

The mean values for liver and small intestine TBARS were given in figure 2. There was not any
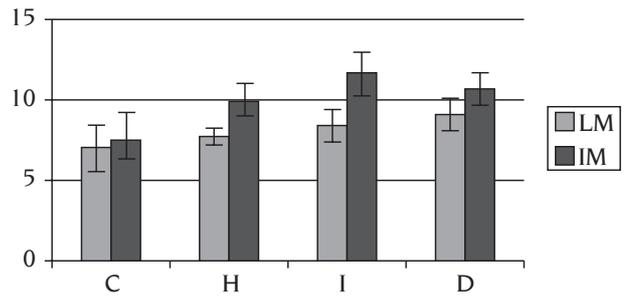
Figure 2. The liver and small intestine TBARS levels of the groups.
LM: TBARS levels of the liver; **IM:** TBARS levels of the small intestine

significant difference among the groups with respect to liver TBARS levels. Similarly, small intestine TBARS levels among the groups did not indicate any significant difference either.

Mean red blood cell TBARS levels in the groups were shown in figure 3. When red blood cell TBARS levels of the groups were compared after-shock (60th min), no significant difference was observed.
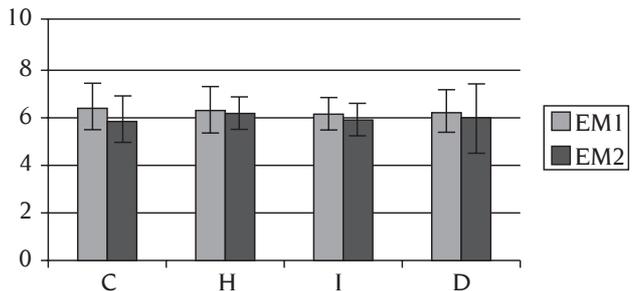
Figure 3. The red blood cell TBARS levels of the groups.
EM1: Red blood cell TBARS levels after shock (30 min);
EM2: Red blood cell TBARS levels after replacement (60 min)

Mean plasma lactate levels at 60th min. (PL2) was significantly higher than 30th min. (PL1) level in control group ($p < 0.05$). But no statistically significant difference was found in the other groups. Comparison of group C with other groups with respect to average plasma lactate levels following the shock (30th min) and after the replacement (60th min) did not indicate any statistically significant difference (Figure 4).

There were no significant differences between the groups liver and small intestinal tissue lactate levels, and also no significant differences were observed in groups' levels (Figure 5).
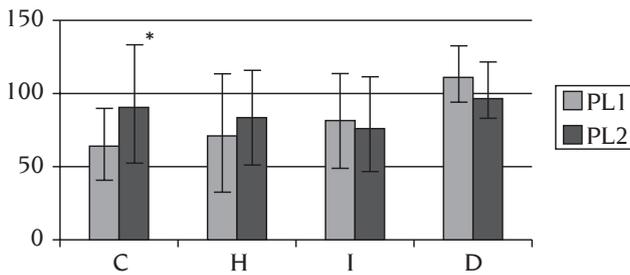
Figure 4. The plasma lactate levels of the groups.
PL1: Plasma lactate levels after shock (30 min);
PL2: Plasma lactate levels after replacement
(60 min)*shows significant difference at p<0.05.
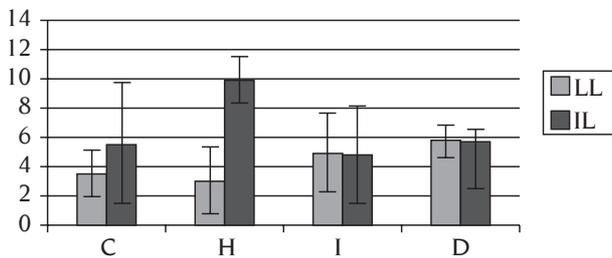
Figure 5. The liver and small intestine lactate levels of the groups.
LL: Liver lactate levels; ***IL:*** Small intestine lactate levels

DISCUSSION

Tissue hypoperfusion following the shock has an inhibiting effect on the energy sources of the organism. Oxygenation disruption in the tissue impairs the use of high phosphated compounds. Reduction of such energy sources as adenosine tri-phosphate (ATP) or insufficient utilization of energy sources of the cell lead the organism towards to anaerobic metabolism. As a consequence of initialization of anaerobic metabolism, free oxygen radicals are produced in the tissue. These formed free oxygen radicals can cause microvascular damage and this situation will deepen hypoxia and accelerates anaerobic metabolism.[3,13] It is possible to reduce tissue ischemia that is formed after haemorrhagic shock by either blood or fluid replacement. Some authors report that a - tochopherol, allopurinol, superoxide dismutase, vitamin-E analogues, DMSO and similar antioxidants used in experimental hypovolemic shock treatment along with replacement fluids, prevent serious tissue damage.[14-22] Therefore, we used DMSO as an antioxidant along with replacement fluids in our study for the haemorrhagic shock treatment.

In this study, a reduction of tissue and plasma TBARS levels of group D was much more than group I and H. This finding was expected because of the additional antioxidant. We also expected no difference or increase in the control group TBARS levels after replacement treatment. A decrease was observed in the plasma TBARS levels after replacement (60th min). This reduction was highest in group D (14%) and lowest in group C. In addition, the reduction in group D plasma TBARS levels was higher than in group I, which had only replacement fluid.

Bauer et al.[15] compared the effects of the fluids and hydroxyethyl starch + desferroxamin used for replacement on tissue ischemia and microcirculation in their haemorrhagic shock model. They found that thiobarbutiric acid reagents decreased significantly in the group which hydroxyethyl starch %6+ desferroxamin were administered after replacement (60th min), compared to the group which only replacement fluid administered. Similarly, Murthy et al.[6] reported that plasma TBARS level in the groups which were administered pre-ischemic vitamin-E analogues and 5 aminosalicylic acid were lower than the control group in an experimental hypovolemic shock model.

In this study, in-group plasma TBARS levels following fluid replacement at 60th min. were found significantly lower than plasma TBARS levels of all groups following the shock at 30th min. in all groups. Most significant reduction in TBARS level was seen in hypertonic saline (I) group and HAES 10% + DMSO (D) group. This finding seems consistent with the findings of Murthy et al.[6] This was attributed to a compensation mechanism that was activated as a result of ischemia. These mechanisms play a role in protecting tissues from the damage caused by ischemia, however, if these mechanisms work longer it may increase the tissue damage. This may explain the decrease observed in TBARS levels of the control group.

Our hypothesis was that the tissue damage related to ischemia might probably be less in group D compared to the other groups because of the added antioxidant. Tissue TBARS levels of group I and H were also expected to be lower compared to the control group. However, the results showed that there was no significant difference between the groups related with liver and small intestine TBARS levels. In some studies the antioxidant agents were applied to subjects before the shock.[16] In our study, DMSO was given to the subjects after shock and that might be the

explanation for different results obtained in the present and the previous studies.

Besides that, as it was reported in a previous study performed by Itoh et al.[16], the addition of DMSO as an antioxidant to the replacement fluids in hypovolemic shock model has a preventing effect from ischemia. Itoh et al.[16], used allopurinol, superoxide dismutase (SOD) and DMSO as antioxidants along with the replacement fluid in their experimental hypovolemic shock model. They reported that, the group with allopurinol and SOD was protected from ischemic damage but this effect was not visible in DMSO group. Although, there was a change in the mean values, the addition of DMSO to the replacement fluids did not significantly affect the results in the present study which means that DMSO did not indicate any protection against ischemic damage. Similarly, Jiang Huai et al.[20] found that there was not any significant difference between intestine TBARS levels of the control group and the rats kept in ischemic situation and underwent reperfusion for a period of 60 min. This finding is consistent with our results.

Although no statistically significant difference was found between the red blood cell TBARS levels measured during ischemia and after fluid replacement, a slight degree of reduction was observed in TBARS levels of group C, group I and group D after replacement (3.9%, 3.9% and 7.4% respectively). We actually expected a reduction in red bloo cell TBARS levels after replacement (60th min) with the reduction being maximum in DMSO group. However, similar effects have not been observed in the tissue level, which might be explained by the lower rate of transport of the antioxidant agent to the tissues or an inadequate waiting period after replacement.

Before the study was performed, it was presumed that the plasma lactate level may increase during the shock, and will than decrease after replacement. Although plasma lactate levels we measured during the ischemia and after the replacement did not show any statistically significant difference apart from the control group,

lactate levels increased in groups C and H (39% and 19% respectively) whereas in group I and D decreased (6% and 14% respectively). This finding indicates that hypertonic saline may be more effective compared to HAES 10% in haemorrhagic shock treatment. Additionally, the results shows that the addition of an antioxidant along with the replacement fluid may provide a protective effect for the tissues against the ischemia developed after haemorrhagic shock. On the other hand, the increase in the lactate level of the control group may indicate that the compensatory mechanisms in the organism may be adequate to a certain level. Similarly to our findings, Kapoor et al.[21] reported that plasma lactate level in all groups during the shock increased progressively. They stated that reduction was observed in the group that SOD and catalase were given as antioxidants along with fluid replacement was more than those observed in the group that only replacement fluid applied.

Deb et al.[22] compared HAES %6, lactated ringer solution and plasma replacement that are used in haemorrhagic shock treatment, with respect to controlling ischemic damage in the tissue. They reported that cellular ischemia and damage was the least in the control group with no replacement performed and in the plasma administered group. In accordance with this study, no significant difference was found between the groups related with tissue TBARS levels following fluid resuscitation (60th min) in the present study. This may bring the question of whether it is accurate to perform the treatment while the compensatory mechanisms of the organism are active.[23] While the organism is in a stable position with its own defense mechanisms during the haemorrhagic shock, some aggressive attempts may disturb this balance.

In conclusion, HAES 10% and hypertonic saline 7.2% does not have any superiority over each other in the protection of the tissue from oxidative stress. It was also observed that the use of antioxidants along with the replacement fluids has no beneficial effects.

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