The antioxidative and antiinflammatory effects of hypoxemic resuscitation with HES 130/0.4 and modified gelatin solution on acute hemorrhagic shock in rabbits

Akut hemorajik şok oluşturulan tavşanlarda HES 130/0.4 ve modifiye jelatin solüsyonları ile yapılan hipoksemik resüsitasyonun antioksidatif ve antiinflamatuvar etkileri

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Background: The aim of this study was to investigate antioxidative effects and antiinflammatory properties of colloid fluids such as HES 130/0.4 or modified gelatin solution in rabbits during resuscitation of acute hemorrhagic shock under hypoxemic conditions.

Methods: Twenty-one New Zealand Wistar albino rabbits were randomly allocated to one of the three experimental groups: HES 130/0.4 solution (n=7, Voluven®, group HES), gelatine solution (n=7, Gelafusine®, group GEL) and control (n=7, sodium chloride 0.9%, Saline group). Hemorrhagic shock was induced by controlled blood withdrawal (40% of total estimated blood volume) from the left carotid artery over 30 minute under hypoxemic conditions. The period of hypovolemia was maintained for 10 minute All resuscitation solutions, except saline solution (3 volumes of saline/1 volume of blood loss for saline), were infused as withdrawn blood volume via the jugular vein over 30 minute after the hypovolemic period under hypoxemic conditions. Rabbits were breathing spontaneously via tracheotomy in room air during hemorrhage and fluid resuscitation periods (hypoxemic resuscitation). Malondialdehyde (MDA), glutathione (GSH), interleukin (IL)-6, IL-10 and tumor necrosis factor alpha (TNF)-α concentrations were measured before hemorrhagic shock and after fluid resuscitation in all blood samples.

Results: The values of the MDA, TNF-α, IL-6 levels were the highest, whereas IL-10 and GSH levels were the lowest in the Saline group compared to the other groups. The MDA, TNF-α, IL-6 levels were lower, but the IL-10 and GSH levels were higher in group HES compared to group GEL ($p<0.05$).

Conclusion: Hypoxemic resuscitation with HES 130/0.4 solution (Voluven®) for hemorrhagic shock decreases oxidative stress (MDA) and proinflammatory mediators (TNF-α, IL-6), but increases antioxidative (GSH) and antiinflammatory markers (IL-10) when compared to modified gelatine solution (Gelafusine®). Further studies must be carried out with experimental models and different solutions to reach an ultimate conclusion.

Key words: Fluid resuscitation; hemorrhagic shock; hypoxemia; organ damage; rabbit.

Amaç: Bu çalışmada tavşanlarda hipoksemik koşullarda gerçekleştirilen akut hemorajik şok modelinde HES 130/0.4 veya modifiye jelatin solüsyonu gibi kolloid solüsyonlarının antioksidatif ve antiinflamatuvar etkileri araştırıldı.

Çalışma planı: Yirmi bir adet Wistar albino cinsi Yeni Zelanda tavşanı rastlantısal olarak üç gruba ayrıldı; HES 130/0.4 solüsyonu (n=7 Voluven®, HES grubu), Jelatin solüsyonu (n=7 Gelafusine®, GEL grubu) ve kontrol grubu (n=7 %0.9 sodyum klorür). Hemorajik şok modeli, hipoksemik koşullar altında sol karotis arterinden 30 dakikada tahmin edilen kan hacminin %40'ını çekecek şekilde uygulandı. Hipovolemi dönemi 10 dakika süresince sağlandı. Hipovelemik dönem sonunda çekilen kan hacmi kan resüsitasyon sıvısı sodyum klorür hariç (sodyum klorür solüsyonu 1 birim hacim kaybı için 3 birim olacak şekilde) jugüler ven yoluyla 30 dakika süresince verildi. Tavşanlar hemoraji ve sıvı resüsitasyonu sırasında trakeostomi ile oda havasında spontan solunum yapmaktaydı (hipoksemik resusitasyon). Malondialdehit (MDA), glutatyon (GSH), interlökin (IL)-6, IL-10, tümör nekroz faktör alfa (TNF)-α konsantrasyonları hemorajik şok öncesi ve sıvı resüsitasyonu sonrasında tüm kan örneklerinde ölçüldü.

Bulgular: Kontrol grubunda diğer gruplara göre MDA, TNF-α, IL-6 değerleri en yüksek, buna karşılık GSH ve IL-10 değerleri en düşüktü. Grup HES'de grup GEL'e göre MDA, TNF-α ve IL-6 değerleri daha düşük fakat IL-10 ve GSH düzeyleri ise daha yüksekti ($p<0.05$).

Sonuç: Hemorajik şok için hipoksemik koşullarda HES 130/0.4 (Voluven®) ile yapılan sıvı resüsitasyonunda modifiye jelatin solüsyonuna (Gelafusine®) göre oksidatif stres (MDA) ve proinflamatuvar belirteçler (TNF-α ve IL-6) seviyeleri azalırken, antioksidatif (GSH) ve antiinflamatuvar belirteçler (IL-10) düzeylerinde ise artış olduğu saptandı. Gelecekteki çalışmalarda farklı deneysel modeller ve farklı solüsyonlar kullanılarak daha kesin ve mükemmel sonuçlara ulaşılabilinecektir.

Anahtar sözcükler: Sıvı resüsitasyonu; hemorajik şok; hipoksemi; organ hasarı; tavşan.

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Critical decrease in blood pressure often is associated with a state of shock - a condition in which tissue perfusion is not capable of sustaining aerobic metabolism.[1] In the hypovolemic patient, adequate volume restoration is essential to prevent irreversible shock and to avoid subsequent development of organ failure, or even multiple organ dysfunction syndrome (MODS).[2] Hemorrhagic shock may also lead to haemodynamic instability, decreased tissue perfusion, cellular hypoxia, organ damage, and death.[2,3]

The definition of the ideal volume replacement strategy still remains one of the major problems in shock. The choice between colloid and crystalloid solutions continues to generate controversy.[4] Colloid solutions can be blood products (human albumin solution, plasma protein fraction) or synthetic (modified gelatines, dextrans, hydroxyethyl starch [HES] preparations).[5] There are different types of colloids and these may have different effects. Although the review of trials did not find enough evidence to be sure that any particular colloid is safer than any other, newer colloids have been modified to limit effects on the coagulation system, and they may be used to modulate the inflammatory response, which could prove to be particularly useful in the management of critically ill patients.[6-9]

Reperfusion following a period of ischemia leads to stimulation of the inflammatory response of the host. It was shown that the sera of rabbits sampled during the period of resuscitation from hemorrhagic shock could stimulate monocytes to produce of pro-inflammatory [tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)] and antiinflammatory (IL-10) cytokines.[8]

It was also suggested that theories of pathogenesis implicate oxygen free radical formation following reperfusion as a mainstay for the production of pro-inflammatory mediators.[5,9] The recent application of the concept to generalized ischemia, namely to the entirely ischemic body secondary to hemorrhagic shock, afforded attenuation of oxidative and inflammatory response and better hemodynamic restoration.[8,10]

Although hypoxemic resuscitation with blood products has been well documented in previous studies[9,11] the effect of colloid solutions on hypoxemic resuscitation has not been investigated in hemorrhagic shock model to the best of our knowledge. The aim of this study was to investigate the antioxidative and antiinflammatory properties of colloid fluids [HES 130/0.4 (Voluven®)] and modified gelatine solution (Gelofusine®) in rabbits during resuscitation of acute hemorrhagic shock under hypoxemic conditions.

MATERIALS AND METHODS

Animal preparation

Twenty-one New Zealand Wistar albino rabbits ranging from 2500 to 3500 g with a mean weight of 3000 g were divided in three groups consisting of seven rabbits each. All animals received humane care according to guidelines that complied with the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals formulated by the National Academy of Sciences. This study was approved by Adnan Menderes University Veterinary Faculty Ethical Committee.

Anaesthetic techniques

Intramuscular ketamine and xylazine (35 mg/kg and 5 mg/kg, respectively) were used for anaesthetic induction. An intravenous line was placed via the right ear marginal vein and pulse-oximetry monitoring was performed for all. Rabbits were breathing spontaneously by tracheotomy in room air during hemorrhage and fluid resuscitation period (hypoxemic resuscitation).

Polyethylene catheters were inserted in the right atrium through the right jugular vein for fluid infusion and venous blood sampling. The other catheters were inserted in the left carotid artery to allow blood withdrawal during hemorrhagic shock and for arterial blood sampling. Arterial blood pressure was measuring continuously by the right femoral artery.

Haemodynamic monitoring

Invasive arterial pressure and saturation were monitored on the Petas KMA® 800 (Turkey) monitor with the help of an Abbott transducer. Arterial tension value was recorded continuously during the hemorrhagic shock and fluid resuscitation. We also observed oxygen saturation and heart rate via pulse-oximetry monitoring.

Resuscitation fluids

Gelofusine® 4% (Gelofusine, B. Braun, Switzerland): A modified fluid gelatine (22.6-25 kDa) was used as the study solution. Gelofusine® is a sterile, apyrogenic, isotonic and iso-oncotic volume expansion solution.

Voluven® (Fresenius-Kabi, Sweden): Low molecular weight HES with low degree of substitution (mean molecular weight 130±20 kDa, degree of substitution 0.4) was formulated at 6% (weight/vol).

0.9% sodium chloride (NaCl): Saline solution contained 0.9 g/dL–1 NaCl. (Polifleks 0.9% isotonic sodium chloride, Eczacıbaşı-Baxter, Turkey).

The animals were randomly allocated to one of the three experimental groups: Voluven® (n=7, HES group),

Gelofusine® (n=7, GEL group) and control (n=7, Saline group).

Animal model of hemorrhagic shock

After a stabilization phase of 30 minute, defined as the time point of completion of instrumentation and stabilization of all variables, hemorrhagic shock was induced by controlled blood withdrawal (40% of total estimated blood volume) from left carotid artery over 30 min, according to the estimated blood volume Formula.[11,12]

$$V\ (mL)=(\text{body weight (g)}\times 6.5/100)\times 0.5.$$

Hypovolaemia was maintained for 10 minutes. A volume of resuscitation fluids, identical to the volume of blood withdrawn, was injected via the jugular vein. Saline solution was infused at 3 volumes of saline for each volume of blood loss. For the all solutions, the time of infusion was 30 minutes. Rabbits were breathing spontaneously by tracheotomy in room air during hemorrhage and fluid resuscitation period (hypoxemic resuscitation). Hemorrhagic shock model was summarized in Figure 1.

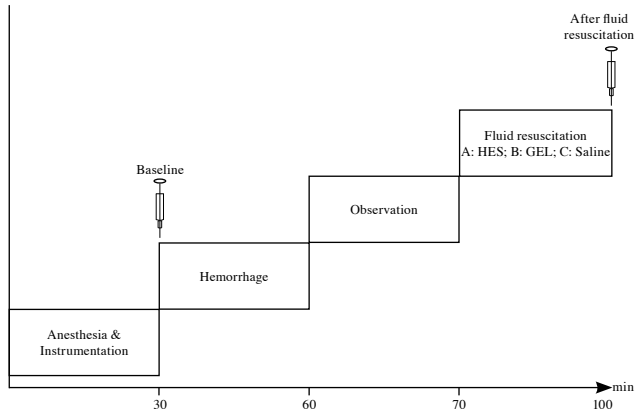

Fig. 1. Hemorrhagic shock model was summarized. Blood samples were collected before hemorrhage (baseline) and after fluid resuscitation.

Biochemical results

TNF-α, IL-6, and IL-10 determination

For rabbit serum TNF-α determination, human TNF-α ELİSA kit was used (Bender MedSystems Cat No: BMS223/4, BMS223/4 TEN, Vienna, Austria, Europe).

For rabbit serum IL-10 determination, human IL-10 ELISA kit was used (Bender MedSystems Cat No: BMS215/2, Vienna, Austria, Europe).

For rabbit serum IL-6 determination human IL-6 ELISA kit was used (Bender MedSystems Cat No: BMS213/2, BMS213/2 TEN, Vienna, Austria, Europe).

The principles of tests are the same: These are enzyme-linked immunosorbent assays for the quantitative detection of IL-6, TNF-α and IL-10. The anti-human antibodies are adsorbed on the microwells. TNF-α, IL-6 and IL-10 are presented in the sample or standard binds to antibodies adsorbed to the microwells. Biotin conjugated monoclonal anti IL-10, IL-6 and TNF-α antibodies are added and bind to IL-6, TNF-α, and IL-10 captured by the first antibody. Following incubation, unbound biotin conjugated anti IL-6, TNF-α, and IL-10 is removed during a wash step. Streptavidin HRP is added antibodies are removed during wash step. A colour product is formed, the reaction is terminated by addiction of acid and absorbance is measured at 450 nm. The results are calculated by standard curve and expressed as pg/ml.[13]

Malondialdehyde determination

Serum malondialdehyde concentration was measured as an indirect marker of oxidative stress in terms of thiobarbituric acid reactive substances, spectrophotometrically.[14] Serum samples (0.125 ml) were mixed with 20% trichloroacetic acid (1.25 ml) and 0.67% thiobarbituric acid (0.5 ml). Mixture was then boiled at 95 °C for 30 minute, immediately followed by cooling on ice. Reaction mixture was then vortexed, following the addition of n-Butanol (2 ml). All vials were then centrifuged at 3000 rpm for 10 minute. Absorbance of the

Table 1. Time course of changes in mean arterial pressure, heart rate and oxygen saturation

	Mean arterial pressure (mmHg)			Heart rate (beat/min.)			Saturation (%)		
Time (min.)	HES group	GEL group	Saline group	HES group	GEL group	Saline group	HES group	GEL group	Saline group
0	78±3.2	75±4.3	76±3.5	256±12	249±19	254±17	99±0.3	99±0.2	99±0.4
30	76±5.1	74±6.2	78±3.2	245±14	250±12	253±18	99±0.4	99±0.3	99±0.3
60	53±4.4	55±5.3	54±4.6	264±17	275±23	278±20	85±0.3	84±0.4	84±0.2
70	44±5.2	41±4.8	43±4.1	276±15	268±14	273±19	82±0.4	81±0.3	82±0.4
100	77±4.5	73±5.1	75±5.3	254±22	251±18	255±21	86±0.5	86±0.2	85±0.3

HES: Hydroxyethyl starch; GEL: Gelofusine; There was no significant difference in all groups for mean arterial pressure, heart rate and oxygen saturation levels during study period.

Table 2. Changes in TNF-alpha, IL-6 and IL-10 levels before hemorrhage and after fluid resuscitation

	TNF-α pg/mL			IL-6 pg/mL			IL-10 pg/mL		
	HES group	GEL group	Saline group	HES group	GEL group	Saline group	HES group	GEL group	Saline group
Basic level	2.2±0.8	2.0±0.9	2.4±0.7	4.1±0.6	3.7±0.7	3.9±0.4	1.9±0.3	2.0±0.6	2.3±0.8
After resuscitation	40.6±0.7†	93.2±3.6	240.1±6.3*	69.1±9.0**	128.8±11.0	312.6±23.3*	162.4±11.7‡	93.8±6.7	48.8±9.4*

HES: Hydroxyethyl starch; GEL: Gelofusine; *p<0.0001 Saline group vs. GEL group and HES group (for TNF-α, IL-6 and IL-10); **p=0.0028 HES group vs. GEL group (for IL-6); ‡p=0.0034 HES group vs. GEL group (for IL-10); †p=0.0019 HES group vs. GEL group (for TNF-α)

supernatant was then measured at 535 nm. Concentration of lipid peroxidation products was calculated as malondialdehyde concentration using the extinction coefficient for malondialdehyde-thiobarbituric acid complex of 1.56x105 mol/cm.

Glutathione (GSH) determination

Blood was collected into tubes containing EDTA as anticoagulant. Reduced GSH level was estimated by monitoring the reduction of DTNB (dithiobis-2-nitrobenzoic acid) forming a yellow colored anion at 412 nm.[15]

Statistical analysis

Statistical analyses were performed using the one-way analysis of variance (ANOVA) with post-hoc Dunnett's multiple comparison tests for comparing means from different treatment groups. Statistical differences of p<0.05 were considered to be significant. Values are means ± standart eror.

RESULTS

There were no significant differences in mean arterial pressure, heart rate and saturation levels during the study period among all three study groups (Table 1). Saturation levels were decreased in all groups after hemorrhagic shock, but there was no significant difference among the groups. There were also no significant differences in basic values of MDA, TNF-α, IL-6, IL-10, and GSH levels of all three groups. The values of the MDA, TNF-α, IL-6 levels were the highest, whereas IL-10 and GSH levels were the lowest in the Saline group compared with the other two study groups after application of resuscitation fluids (p<0.0001; Table 2, 3). The level of MDA, TNF-α, IL-6 levels were lower in group HES compared to group GEL (p<0.001). The values of IL-10 and GSH levels were also higher in group HES compared to group GEL (p<0.001; Table 2, 3).

DISCUSSION

In our study, we found that the values of the MDA, TNF-α, IL-6 levels were the highest, whereas IL-10 and GSH levels were lowest in the Saline group compared with other study groups. The level of MDA, TNF-α, IL-6 levels were lower, but the IL-10 and GSH levels were higher in HES group compared to GEL group.

Ischemia and reperfusion participate in oxidative stress and systemic inflammatory response syndrome (SIRS) arising during post-ischemic resuscitation.[16-18] The pro-inflammatory cytokines (such as TNF-α and IL-6) in SIRS may account for many of the presenting signs of shock, such as respiratory failure, capillary leak, shunting, redistribution, depressed myocardial function, oxygen uncoupling, and cellular ischemia.[19-22] Antiinflammatory mediators such as IL-10, with their ability to depress cytokines and suppress effect or functions of immune cells, have been suggested to play a major role in counter regulation of the early inflammatory response.[23] Some studies indicate beneficial effects of early systemic IL-10 release following injury and shock.[23-26]

The proper fluid resuscitation of hemorrhagic shock has been a long-standing subject for debate.[27-30] In shock models, crystalloid administration is associated with higher concentrations of pro-inflammatory cytokines and subsequently higher expression of adhesion molecules compared to HES solution.[27] Similarly, we also found that, the values of the TNF-α, IL-6 levels were

Table 3. Changes in malondialdehyde and glutathione levels before hemorrhage and after fluid resuscitation

	Malondialdehyde levels (nmol/mL)			Glutathione (μmol/g Hb)		
	HES group	GEL group	Saline group	HES group	GEL group	Saline group
Basic level	1.3±0.7	1.1±0.4	1.3±0.8	32.3±5.6	31.7±4.7	32.5±7.2
After resuscitation	2.3±0.7	2.5±1.1	4.2±1.1*	39.4±4.3*	32.5±6.6	30.7±4.4

HES: Hydroxyethyl starch; GEL: Gelofusine; *p<0.001 HES group vs. HES group and Saline group (for malondialdehyde and glutathione).

the highest, whereas IL-10 levels were lowest in the Saline group compared with HES and GEL groups after application of resuscitation fluids in our study. Schmand et al.[31] investigated the effects of an HES solution and Ringer lactate (as crystalloid solution) on cell-mediated immunity after trauma-hemorrhagic shock and noted the HES group had improved peritoneal macrophage function and lower circulating IL-6 concentrations compared to the Ringer lactate group. In animals undergoing severe hemorrhage, plasma levels of pro-inflammatory cytokines (i.e, IL-6) were not negatively affected by HES.[31-33] The plasma expander HES also has an antiinflammatory and protective effects against reperfusion injury.[30] Lee et al.[34] investigated the effects of different resuscitation fluids (such as lactated Ringer solution; 4% hydroxyethyl starch solution, and 4% modified fluid gelatin) on the production of pro-inflammatory and antiinflammatory cytokines in an animal model of hemorrhagic shock in rats. They found that resuscitation with gelatine may be associated with cytokine production favouring a pro-inflammatory response. They also suggested that the marked elevation of IL-6 observed in the gelatin-treated animals may play a role in the relatively high frequency of anaphylactoid reaction in clinical use of gelatin. We observed that in fact, pro-inflammatory cytokines were lower in HES group when compared to GEL group.

Gelatin polypeptides are derived from bovine collagen, whereas HES solutions are modified natural polymers of amylopectin. The animal peptide nature of gelatin may render an enhanced immunogenicity compared to HES.[34] Gelatin has also been reported as the colloid that is most likely to induce anaphylactoid reactions.[10] We thought that the effects of gelatin on the release of IL-6 cannot be attributed solely to the reinforcement of shock specific effects; it can be affected by anaphylactoid reaction as well.

Oxygen radicals are highly toxic metabolites causing lipid peroxidation and injury of cell membranes, which in turn, lead to capillary leakage and induction of adhesion molecules in shock. This process may activate hypoxia-stimulated macrophages in the liver, causing synthesis of pro-inflammatory mediators such as TNF-α and interleukin in shock.[35] Lipid peroxidation is believed to be an important cause of oxidative damage to cellular membranes, and eventually, cell death. MDA is a good indicator of oxidative injury and an end product of lipid peroxidation. Glutathione is also crucial in the cellular defence against oxidative insult.[35] Some studies suggest that the adverse sequences after fluid resuscitation are the induction of the inflammatory response and the generation of reactive oxygen radicals.[36,37] Tsai et al.[35] suggested that HES solution is an appropriate resuscitation fluid for restoring hemorrhagic hypotension without inducing the adverse effect of oxidative stress.

On the other hand, Douzinas et al.[10] suggested that hypoxemic resuscitation from hemorrhagic shock was more efficient than normoxemic in restoring the blood pressure and exerted a protective effect by inhibiting the oxidative stress and the excessive inflammatory responses observed during normoxemic resuscitation of the experimental models. Our study was distinct from the Douzinas's study; we used different colloid solution (HES and gelatine) instead of blood transfusion. We also found that MDA levels (as oxidative stress marker) was lower but GSH levels (as antioxidative marker) were higher in HES group compared with GEL and Saline groups in hypoxemic conditions. In conclusion, our study demonstrates that resuscitation of hemorrhagic shock with different fluids was associated with different oxidative stress and cytokine responses. We suggest that by hypoxemic resuscitation with HES solution, the balance of pro- and antiinflammatory cytokines was shifted in favour of antiinflammatory response, and the antioxidative effect was also attenuated when compared with gelatin and isotonic saline.

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