The renal effect of replacement fluids in controlled severe hemorrhagic shock: an experimental study

Kontrollü ciddi hemorajik şokta replasman sıvılarının böbreklere etkileri: Deneysel araştırma

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BACKGROUND

This experimental study examined the effects of resuscitation with Ringer's lactate (RL), 6% hydroxyethyl starch (130/0.4-HES), and the combination of RL and HES on renal function in hemorrhagic shock (HS).

METHODS

Twenty-four male New Zealand white rabbits weighing 2198-3435 g were divided at random into four groups. HS was constituted by maintaining the mean arterial blood pressure at 30 mmHg and blood lactate at >4 mM/L. Subsequently, Group 1 (control) was not resuscitated, while the study rabbits' resuscitation was initiated with RL (Group 2), HES (Group 3), or the combination of RL and HES (Group 4).

RESULTS

In all groups, the serum creatinine and blood urea nitrogen (BUN) levels were observed to be within normal limits, while the lactate dehydrogenase and α-1 microglobulin levels statistically significantly increased when time points were compared with beginning values ($p<0.05$). Furthermore, cystatin-C levels were observed to be increased after the HS ($p<0.05$), but returned to the normal level after resuscitation in all the study groups. Interleukin (IL)-6 and tumor necrosis factor (TNF)-α levels were increased in all the rabbits after HS ($p<0.05$), and there were no significant differences among the study groups after resuscitation ($p>0.05$). There were no differences in the histological imaging between the groups ($p>0.05$).

CONCLUSION

The 6% HES (130/0.4) did not have any harmful effects on the kidney when it was used alone or in combination with crystalloid for resuscitation of HS in rabbits.

Key Words: Hemorrhagic shock; hydroxyethyl starch; Ringer's lactate; renal injury.

AMAÇ

Bu deneysel araştırmada, hemorajik şokta Ringer laktat (RL), %6'lık hidroksietil nişasta (130/0,4-HES) ve RL-HES kombinasyonu ile resüsitasyonun böbrekler üzerine etkileri araştırıldı.

GEREÇ VE YÖNTEM

Çalışmada ağırlıkları 2198-3435 g olan 24 adet erkek Yeni Zelanda beyaz tavşanı rastgele dört gruba ayrıldı. Hemorajik şok, ortalama arter basıncı 30 mmHg ve kan laktatı >4 mM/L olacak şekilde oluşturuldu. Grup 1'de sıvı replasmanı uygulanmaz iken, Grup 2'de RL, Grup 3'te HES ve Grup 4'te de RL+HES ile sıvı replasmanı ile şok düzeltildi.

BULGULAR

Bütün gruplarda, serum kreatinin ve kan üre azotu düzeyleri hemorajik şoktan ve sıvı resüsitasyonundan sonra normal sınırlarda kaldı. Laktat dehidrogenaz ve α-1 mikroglobulin düzeyleri şoktan ve sıvı resüsitasyonundan sonra anlamlı olarak arttı ($p<0,05$). Ancak, gruplar arasında bir fark yoktu ($p>0,05$). Sistatin-C düzeyleri şoktan sonra artmasına karşın ($p<0,05$) sıvı replasmanı ile normal değerlere geri geldi. IL-6 ve TNF-α düzeyleri şoktan sonra bütün deneklerde arttı ($p<0,05$). Ancak, sıvı replasmanından sonra gruplar arasında bir fark gözlenmedi ($p>0,05$). Böbreklerin histopatolojik incelemelerinde gruplar arasında bir fark bulunmadı ($p>0,05$).

SONUÇ

%6'lık HES (130/0,4), gerek tek başına gerekse RL ile beraber hemorajik şokun tedavisinde kullanıldığında tavşan böbrekleri üzerine zararlı bir etkisi olmadı.

Anahtar Sözcükler: Hemorajik şok; hidroksietil starch; Ringer laktat; böbrek hasarı.

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Allogeneic blood resuscitation is the major treatment modality in hemorrhagic shock. Plasma expanders, including crystalloids and colloids, may be used when blood cannot be obtained. Fluid resuscitation after traumatic hemorrhage has been historically instituted as soon as possible after the injury in an attempt to normalize blood pressure, heart rate, urine output, and mental status, which are the traditional end-points of resuscitation. Fluid resuscitation is an integral, mandatory component in the management of patients in shock from traumatic hemorrhage. However, the classic theory of instituting resuscitative fluids early after the injury is now being disputed. Adequacy of resuscitation is no longer judged by the presence of normal vital signs, but by the achievement of normalization of organ-specific and tissue-specific measured values. The most important point is to recognize the presence of shock after traumatic hemorrhage and to resuscitate the patient with the appropriate fluid, in the appropriate amount, and at the appropriate time.[1]

To avoid prolonged periods of shock and tissue hypoperfusion in cases in which blood loss exceeds 40% of the circulating intravascular volume (class IV hemorrhage or hemorrhagic shock), rapid fluid administration has been recommended to quickly restore the hemodynamic stability.[2] A major goal of fluid administration is to restore and maintain adequate perfusion and function of vital organs, such as the kidney. However, the issue of the type and quantity of fluid to be administered for resuscitation after hemorrhagic shock is controversial. It has been observed that early colloid infusion might result in prompt recovery of the tissue perfusion, when compared with the crystalloid.[3]

The lack of acceptance of hydroxyethyl starch (HES) for volume replacement therapy is most likely due to the reports of abnormal coagulation and negative effects of HES on renal function.[4] The use of colloids may induce acute renal failure by raising the plasma colloid osmotic pressure.[5] The dehydrated patient who receives considerable amounts of hyperoncotic colloids without additional crystalloids is especially at risk of developing hyperoncotic acute renal failure. Concerns about the adverse effects of HES on renal function were first raised by Legendre et al.,[6] who reported an association between organ donors' exposure to HES and osmotic nephrosis-like lesions in the transplant recipients.

Recently, it has been reported that HES therapy was associated with higher rates of acute renal failure and renal-replacement therapy than was Ringer's lactate (RL).[7] On the other hand, Godet et al.[8] suggested there were no drug-related adverse effects of HES 130/0.4 on renal function in patients with decreased renal function undergoing elective abdominal aortic surgery.

Our objective was to evaluate whether the type of fluid administered after hemorrhagic shock was associated with renal failure. Therefore, we examined the effects of resuscitation with RL, 6% HES with a molecular weight of 130 kDa and molar substitution of 0.4 (HES 130/0.4), and the combination of HES 130/0.4 with RL on renal function in a rabbit model of near-fatal hemorrhagic shock.

MATERIALS AND METHODS

Animal Preparation

This study design was approved by the Institutional Animal Investigations Ethics Committee of Atatürk Training and Research Hospital. Twenty-four male New Zealand white rabbits weighing 2198-3435 g were included in this study. Before the experiment, the rabbits were acclimated for a minimum of 72 hours (h), and carefully checked for preexisting diseases. The daily food ration was not withdrawn until the procedure. All the procedures were performed between 16:00 and 24:00 h. On the day of the experiment, anesthesia was induced with 30 $mg.kg^{-1}$ intramuscular (i.m.) ketamine (Ketasol 10%, Richter Pharma AG, Wels, Austria) and 10 $mg.kg^{-1}$ i.m. xylazine (Alfazyne 2%, Alfasan International BV, Woerden, Netherlands). After cannulation into the right-ear marginal veins of the animals, anesthesia was maintained intravenously with 10 $mg.kg^{-1}.h^{-1}$ ketamine. The left-ear marginal artery was cannulated for mean arterial blood pressure (MAP) measurements (Petas KMA 800, Professional Electronic Industry and Tic. AS, Turkey). The rabbits were placed in the lateral decubitus position and were warmed to maintain a constant body temperature. The trachea was not intubated, and the animal breathed room air spontaneously. The right-ear marginal artery was cannulated for withdrawing blood to produce hemorrhagic shock and to obtain blood samples. A urinary catheter was inserted for urinary analyses.

Hemorrhagic Shock

Hemorrhagic shock was induced by drawing blood at a rate of 1.5 ml kg^{-1} min^{-1} from the right-ear marginal artery. Controlled hemorrhagic shock

(CHS) was constituted by maintaining the MAP at 30 mmHg and blood lactate at >4 mM/L. This blood volume corresponded to more than 50% of total blood volume. The CHS was allowed to last for 30 minutes (min). After this period, before each experiment, the rabbits were randomized to one of the four groups. In Group 1, the rabbits (control, n=6) were not resuscitated; in Group 2 (n=6), resuscitation of the study rabbits was initiated with RL (three times the lost blood volume); in Group 3 (n=6), HES (equivalent to the volume of blood lost) was used to initiate resuscitation; and in Group 4 (n=6), a combination of HES (half of the lost blood volume) with RL (1.5 times the lost blood volume) was used. The study personnel involved in the treatment of animals were blinded to the group assignments. After the fluids were administered in fixed volumes, we continued to administer the fluids until the hemodynamic targets were reached. The fluid resuscitation period was 30 min. The goal of the fluid resuscitation was to maintain MAP above 50 mmHg. The blood pressures of all animals were equally maintained in the study and control groups during the resuscitation and afterwards. The animals that died and those in which target MAP and serum lactate levels were not maintained were excluded from the study.

Blood samples were collected from all the rabbits before (baseline) and 30 min after the hemorrhagic shock. In the study rabbits, blood samples were also collected at the end of the resuscitation and 2 h later. Urine samples were collected from all the rabbits before the hemorrhagic shock, and from the study groups (Group 2, 3, and 4) 2 h after the resuscitation of hemorrhagic shock. We were unable to collect any urine samples from the control group animals after the CHS, because urine flow ceased during near-fatal hemorrhagic shock.

Glomerular function was determined by blood urea nitrogen (BUN), serum creatinine, and cystatin-C, while tubular functions were determined by α-1 microglobulin and urine N-acetyl-β-D-glucosaminidase (NAG). The cellular damage was ascertained with serum lactate dehydrogenase (LDH), and interleukin (IL)-6 and tumor necrosis factor (TNF)-α were measured to determine whether the treatment fluids for resuscitation had any pro-inflammatory effects.

Blood Gases

Arterial pH, PO_2, PCO_2, and lactate were measured with a blood-gas analyzer (Model stat profile M, Nova Biomedical, Waltham, USA) using 0.5 ml sample of heparinized blood. We measured the blood gases after the constituted shock.

Pathological Analyses

The kidneys were removed from the rabbits of the near-fatal hemorrhagic shock model at the end of the experimental period after tying the renal pedicle and were cut in a sagittal section into two halves, which were fixed by immersion in 10% (wt/vol) formaldehyde in phosphate-buffered saline (PBS; 0.01 M; pH 7.4) at room temperature for one day. The kidneys were examined by a pathologist unaware of the specimen's history of replacement fluid exposure. Slides (4 microns) prepared from these blocks were stained by hematoxylin-eosin and examined by Olympus BX50 light microscope. Histological evaluation was based on tubular changes (findings of ischemia), glomerular changes (hemorrhage and congestion), and interstitial changes (polymorphonuclear leukocytes (PMNs) infiltration). For the evaluation of tubular ischemic changes, in every kidney slide, 100 areas were examined and tubular structures observed in every area were scored between 0 and 3 as follows: 0=Normal histology; 1=Tubular cell swelling, loss of brush borders, nuclear loss in 1/3 of tubular structure; 2=Nuclear loss in 1/3–2/3 of tubular structure; and 3=Nuclear loss in >2/3 of tubular structure. Total score was maintained by the sum of every 100 scores, to reach the maximum score of 300.[9] For the evaluation of PMN infiltration in the interstitial tissue, the samples were examined for glomerular hemorrhage and congestion, and the severity of these histomorphological changes was graded subjectively as: 1=minimal, 2=mild, 3=moderate, and 4=marked.[10]

Biochemistry Analyses

Arterial blood samples were withdrawn in tubes with EDTA and centrifuged at 3000 cycles/s for 10 min; serum was aspirated and stored at -80°C. Urine samples were centrifuged at 3000 cycles/s for 5 min, and the supernatant was aspired and stored at -80°C. The biochemistry analyses were examined by a biochemist blind to the study protocol. IL-6 (Biosource, lot-063202/A, CA, USA) and TNF-α (Biosource, lot-064606/A, CA, USA) levels were evaluated using solid-phase Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's instructions. BUN, serum creatinine, and LDH levels were measured using spectrophotometric method (Abbott C 16000). Furthermore, the urine NAG levels were also evaluated using spectrophotometric method (Olympus AU 2700 biochemical

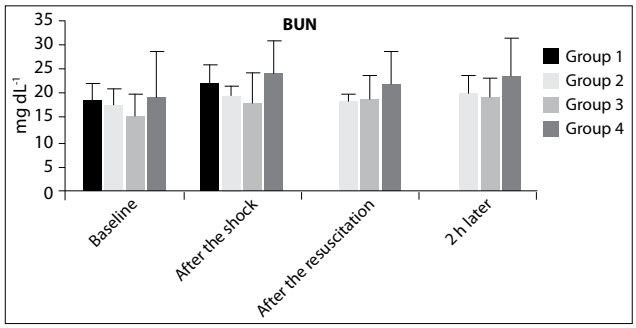

Fig. 1. Blood urea nitrogen levels (mg dL^{-1}) of all the groups when time points are compared with baseline.

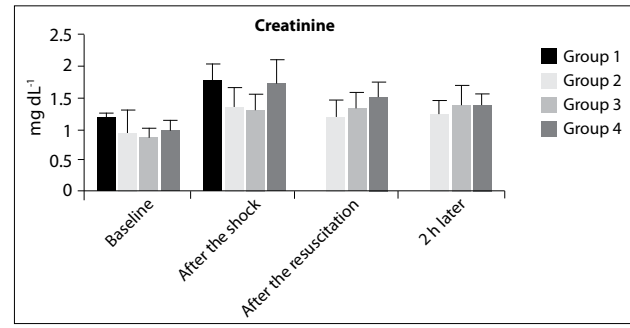

Fig. 2. Serum creatinine levels of all rabbits (mg dL^{-1}).

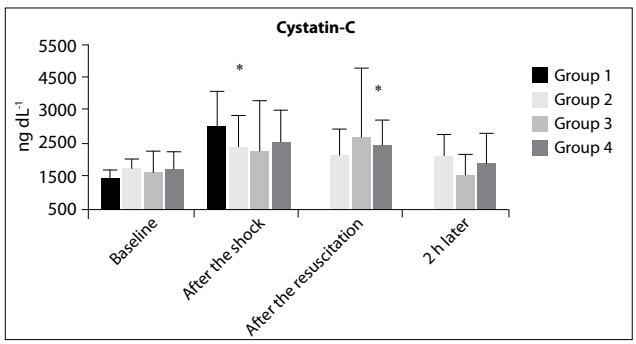

Fig. 3. Cystatin-C levels (ng ml^{-1}) of all the groups when time points are compared with baseline * $p<0.05$.

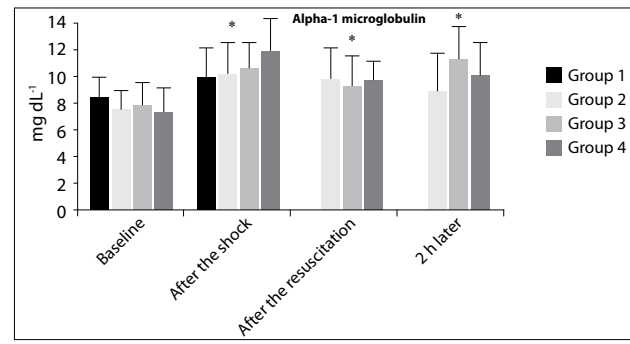

Fig. 4. The α-1 microglobulin levels (mg L^{-1}) when time points are compared with baseline * $p<0.05$.

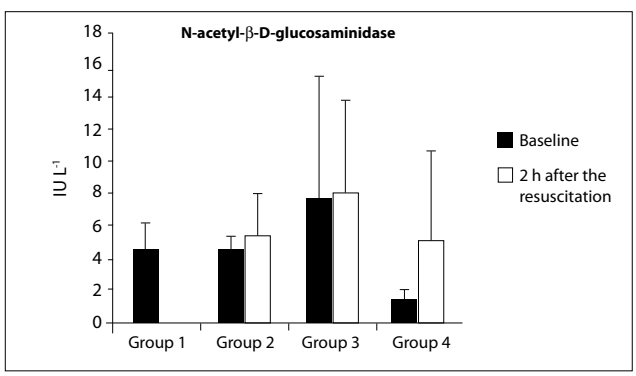

Fig. 5. Urine N-acetyl-β-D-glucosaminidase level (IU L^{-1}) of all the groups.

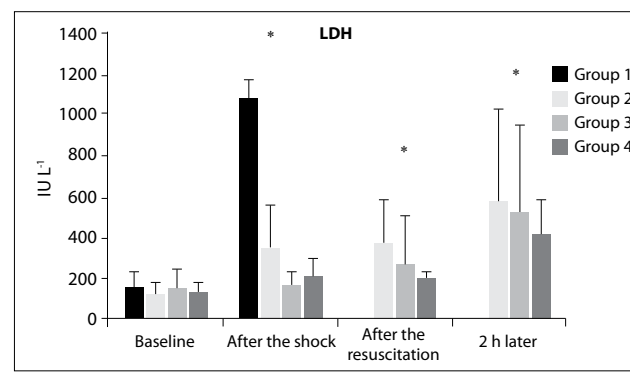

Fig. 6. Serum lactate dehydrogenase levels (U L^{-1}) of the groups when time points are compared with baseline * $p<0.05$.

analyzer). Plasma cystatin-C (Biovendor Cystatin C ELISA kit) and α-1 microglobulin (Immune diagnostic α1-microglobulin ELISA kit) levels were evaluated using solid-phase ELISA according to the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as median (mean ± SD in figures). The differences between groups for the quantitative data were evaluated using Kruskal-Wallis and Mann-Whitney U tests. In each group, Wilcoxon and Friedman tests were used for comparing the quantitative data. Qualitative data (renal interstitial and glomerular pathology of the rabbits) were tested by the χ^2-test. A value of $p<0.05$ was considered as the significance level.

RESULTS

Glomerular Function

Glomerular function was determined by BUN, serum creatinine, and cystatin-C. In all groups (control and study), the BUN (median values of group 1 18.5 - 21.5; group 2 16.5 - 19.5 - 18.5 - 20; group 3 15.5 - 18 - 19.5 - 20; group 4 16.5 - 25.5 19.5 - 22.5 mg kg^{-1})

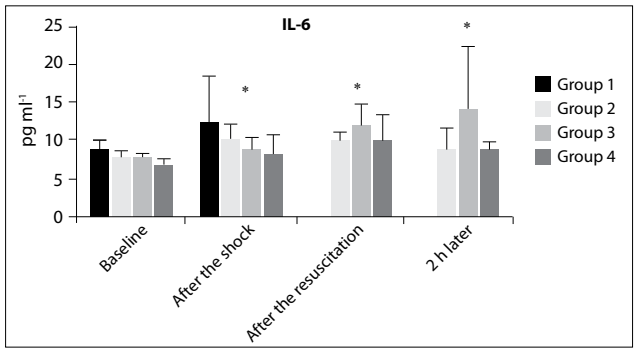

Fig. 7. IL-6 levels (pg ml^{-1}) of all groups, * p<0.05 compared to beginning.

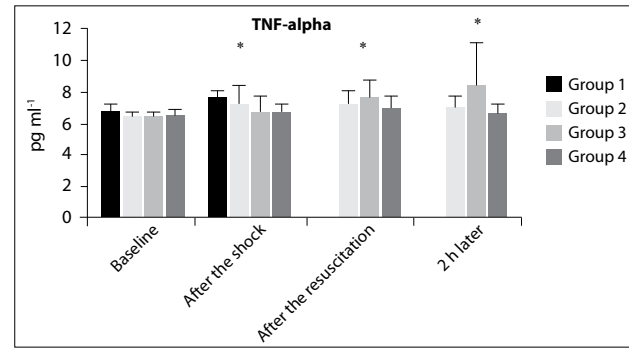

Fig. 8. TNF-α levels (pg ml^{-1}) of all groups, * p<0.05 compared to beginning.

and serum creatinine levels (median values of group 1 1.17 - 1.76; group 2 0.8 - 1.2 - 1.18 - 1.3; group 3 0.8 - 1.3 - 1.4 - 1.5; group 4 0.9 - 1.6 - 1.5 - 1.4 mg kg^{-1}) were observed to be in normal limits, when the time points were compared to baseline (Figs. 1, 2). Cystatin-C levels of all the groups were observed to be increased after CHS (median values of group 1 from 1378 to 2474; group 2 from 1588 to 2189; group 3 from 1469 to 1808; group 4 from 1905 to 2881 ng ml^{-1}) (p<0.05), but returned to the normal level after resuscitation in all study groups (median values of group 2 1947 - 1775; group 3 1992 - 1261; group 4 2664 - 1762 ng ml^{-1}) (Fig. 3). There were no statistically significant differences between the groups regarding BUN, creatinine and cystatin C (p>0.05).

Tubular Function

Tubular function was evaluated by plasma α-1 microglobulin and urine NAG. The α-1 microglobulin levels were observed to be statistically increased when the time points were compared to baseline (median values of group 1 from 8.2 to 10; group 2 from 7.1 to 9.6 - 9.8 - 8.9; group 3 from 7.9 to 10.7 - 10 - 11.6; group 4 from 6.8 to 11.7 - 10 - 10.8 mg L^{-1}) (p<0.05) (Fig. 4). However, the increase in the urine NAG levels was statistically insignificant in the study groups (median values of group 2 from 2.5 to 2.7; group 3 from 2.2 to 2.9; group 4 from 2.5 to 2.9) (p>0.05) (Fig. 5). Moreover, the plasma α-1 microglobulin and urine NAG levels were similar among the groups (p>0.05).

Cellular Damage

Cellular damage was determined by serum LDH. The level of LDH was found to be statistically significantly increased when the time points were compared with baseline (median values of group 1 from 148 to 967; group 2 from 134 to 289 - 285 - 381; group 3 from 131 to 171 - 161 - 373; group 4 from 140 to 225 - 213 - 340 U L^{-1}) (p<0.05); however, no statistically significant differences were observed between the groups (p>0.05) (Fig. 6).

Pro-Inflammatory Effect

Interleukin-6 and TNF-α were measured to deter-

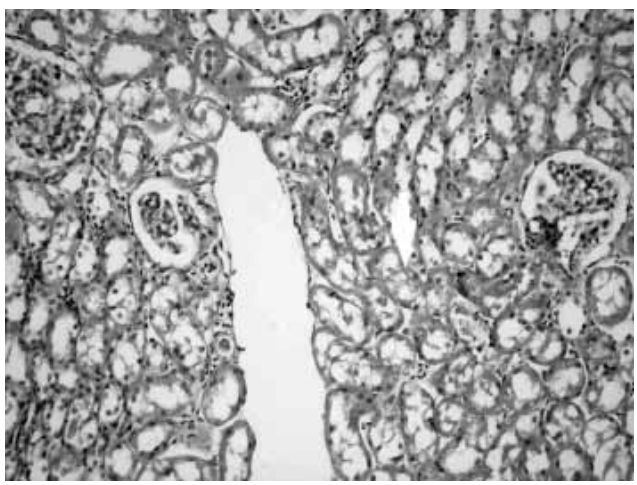

Fig. 9. The areas of acute tubular necrosis (H-E x 20).

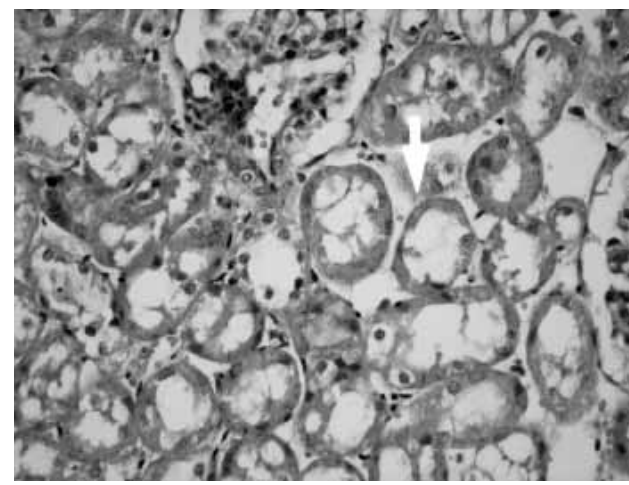

Fig. 10. Acute tubular necrosis (H-E x 20).

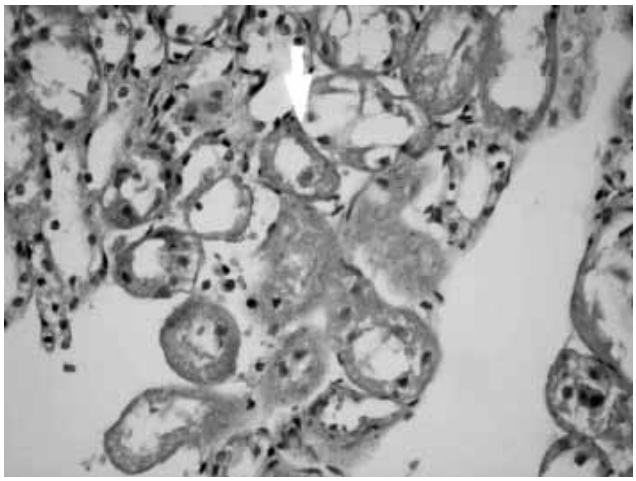

Fig. 11. Acute tubular necrosis (H-E x 40).

mine whether the treatment fluids for resuscitation had any pro-inflammatory effects. The increase in the levels of IL-6 (median values of group 1 from 8.9 to 10.3; group 2 from 8 to 9.3 - 10 -10; group 3 from 7.1 to 8.4 - 10.4 - 10.7; group 4 from 7 to 8.3 - 9.1 - 8.8 pg ml^{-1}) and TNF-α (median values of group 1 from 6.6 to 7.5; group 2 from 6.4 to 7 - 7.1 - 7.7; group 3 from 6.2 to 6.4 - 7.4 - 7.9; group 4 from 6.3 to 6.8 - 6.8 - 6.9 pg ml^{-1}) was statistically significant in all rabbits after CHS ($p<0.05$) (Figs. 7, 8). There were no significant differences between the study groups after resuscitation ($p>0.05$).

Histopathological Evaluation

In all groups, we found variable severity and diffusion tubular necrosis (Figs. 9-11). Numerous vacuoles and loss of the brush border were observed in the proximal tubules, which were more prominent in the control group than in Groups 2, 3, and 4; however, there were no statistically significant differences among the groups ($p>0.05$) (Fig. 12). Cortical fields of the kidneys had PMNs focally (Figs. 13, 14). Furthermore, inflammatory infiltration generally had mononuclear cells, and in various points, leukocyte infiltration was also observed. When the severities of these histomorphological changes were graded subjectively among the groups, no statistically significant differences were obtained (Table 1) ($p>0.05$). Glomerular congestion and hemorrhage were observed with variable severity in all the rabbits (Figs. 15, 16), but were statistically insignificant ($p>0.05$) (Table 1).

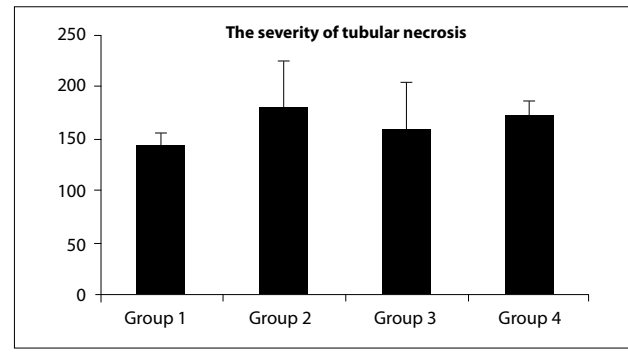

Fig. 12. Tubular ischemic disturbances.

DISCUSSION

Renal hypoperfusion, which occurs in hemorrhagic shock, creates an environment in which cellular injury and organ dysfunction can occur during the episode of shock. The characteristic ultrastructural features of hemorrhagic shock on the kidney appear to be severe tubular degeneration and mild to moderate changes in the glomeruli. In this study, we observed that hemorrhagic shock induced numerous vacuoles and loss of the brush border in the proximal

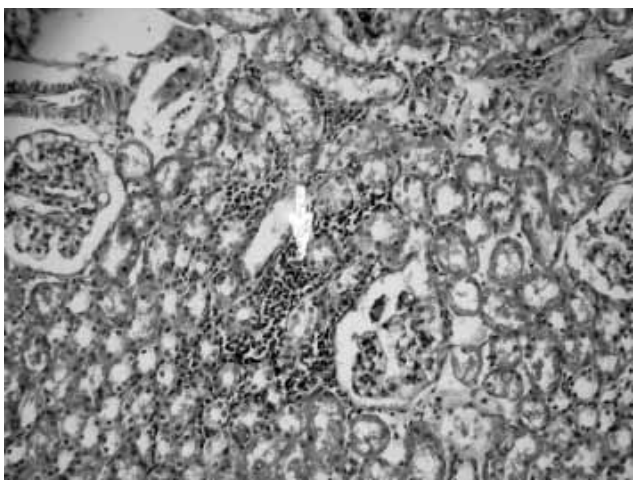

Fig. 13. Interstitial inflammatory infiltration (H-E x 20).

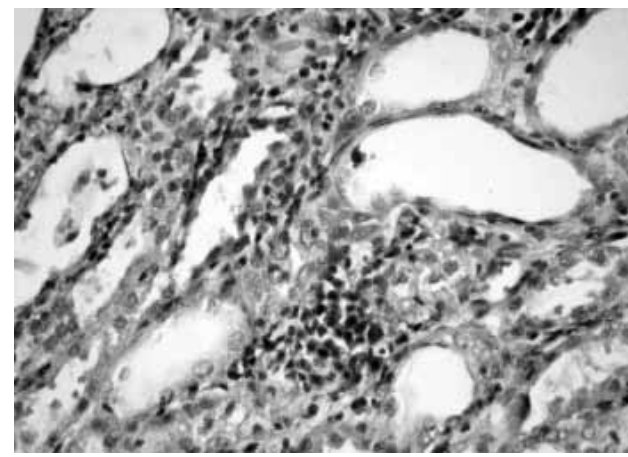

Fig. 14. Mixed type (polymorph and mononuclear cells) inflammatory infiltration (H-E x 40).

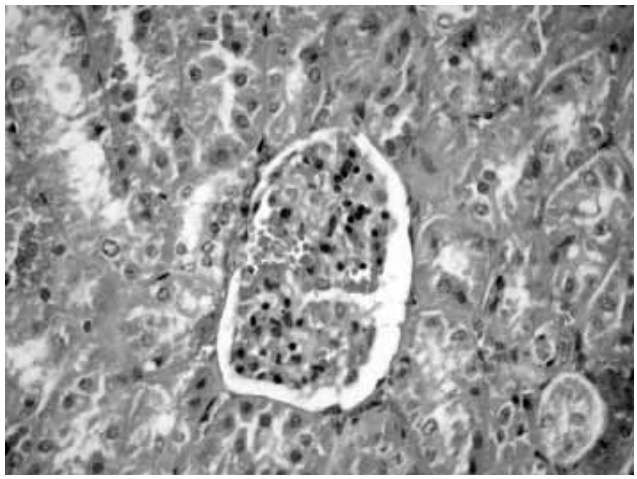

Fig. 15. Glomerular congestion (H-E x 40).

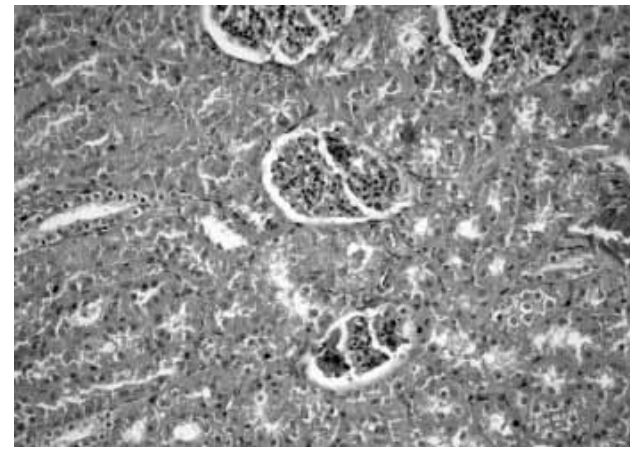

Fig. 16. Glomerular congestion and vascular hemorrhage (H-E x 20).

tubules in rabbits that underwent near-fatal hemorrhagic shock. However, we did not observe any additional harmful effect of resuscitation of hemorrhagic shock with RL, HES, or RL + HES on the renal function in rabbits. We presume that the high levels of IL-6 and TNF-α observed after the shock might signify the systemic inflammatory response caused by the hemorrhagic shock. Furthermore, the resuscitation of CHS with RL, HES, or RL + HES did not produce any additional harmful effects on the levels of IL-6 and TNF-α.

The concerns about HES contributing to acute kidney injury[11-13] may again lead to the predominant use of crystalloid for fluid resuscitation.[14] However, all HES solutions are not identical, and they widely differ with respect to their physicochemical characteristics (concentration, mean molecular weight [M_w], degree of substitution [DS], and C_2/C_6-substitution ratio). These differences have important consequences on the adverse effects, such as alterations in the coagulation process and on the kidney function. Conflicting results about the effects of different HES solutions on renal function may also be due to the varying clinical protocols, selection of patients, and different criteria for volume replenishment. Theoretical and documented hazards are associated with each kind of volume replenishment therapy. There appears to be no reason to ban the modern HES preparations with a low or medium M_w (e.g., 70, 130, or 200 kD) and a low DS (0.4 or 0.5) in patients without pre-existing kidney dysfunction. In patients with known renal dysfunction (e.g., plasma creatinine level of >3 mg/dl), all HES preparations should be used cautiously and other volume replenishment regimens (e.g., gelatins) should be considered, since no convincing data are available yet for the latest generation of HES (M_w=130; DS=0.4).[4] Furthermore, an adverse impact on renal function has been observed in patients receiving HES with molecular weights of 70, 130, 200, 450, and 670, substitutions of 0.4, 0.5, 0.62, 0.7, and 0.75, and C_2/C_6 ratios of 4, 4.6, 5, and 9.[15] Thus, the adverse renal effects of HES have been reported over the entire spectrum of available molecular weights, substitutions, and C_2/C_6 ratios. There is presently no proof that new starches are devoid of the same renal effects.

Sakr et al.[16] investigated the effects of HES administration on renal function in patients included in a large European database of 3147 critically ill patients. They observed that HES did not have a sys-

Table 1. Severity of the histomorphological changes graded subjectively (grade 1-4)

	Inflammatory infiltration				Glomerular congestion			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Group 1	2	2	2	0	0	4	2	0
Group 2	1	2	3	0	2	4	0	0
Group 3	1	3	2	0	1	5	0	0
Group 4	3	2	1	0	0	4	2	0

In all groups, the number of the rabbits with the respective degree of severity of histomorphological changes is presented.

tematic adverse effect on renal function. A moderate increase in the renal component of the Sequential Organ Failure Assessment (SOFA) score occurred regardless of the type of fluid administered during the intensive care unit (ICU) stay. However, in a multivariable analysis, none of the colloids used were found to be associated independently with an increased risk of subsequent need for renal replacement therapy in the ICU.

However, Winkelmayer et al.[17] retrospectively studied 238 patients who underwent coronary artery bypass grafting surgery. The use of high molecular weight HES (670/0.75) was independently associated with a modest reduction in the glomerular filtration rate (GFR) on postoperative days 3 and 5. Similarly, Boldt et al.[18] found kidney-specific proteins in patients who were receiving 6% HES (130/0.4) or gelatin. However, none of the patients developed acute renal failure.

On the contrary, there are studies that showed no harmful effects of 6% HES on renal function. In a recent study, Mahmood et al.[19] suggested that volume expansion with both types of HES (200/0.62 and 130/0.4) during abdominal aortic aneurysm surgery improved renal function and reduced renal injury when compared with gelatin. No adverse effects on kidney function were observed even with large repetitive doses of HES (130/0.4), when used according to the product information.[20,21] Furthermore, in patients undergoing major spine surgery with hypotensive anesthesia and normovolemic hemodilution for blood conservation, HES (130/0.4) showed better preservation of renal function than a crystalloid-based fluid replacement regimen.[22] In addition, Boldt et al.[23] showed that the HES preparation with low molecular weight and a low molar substitution given in cardiac surgery patients with preoperative compromised kidney function did not negatively influence kidney integrity, compared with a human albumin-based volume replacement strategy. Similarly, the use of HES (200/0.5) in cardiac surgery did not seem to be associated with a clinically significant deterioration of postoperative renal function.[24] In another study, volume therapy with 6% HES (130/0.4) was observed to be associated with less marked changes in the kidney function and endothelial inflammatory response than 4% gelatin in cardiac-surgery patients aged >80 years.[25]

The pathological changes occurring after an ischemic renal insult in part are related to the length of the ischemic interval. After 25 minutes of shock, proximal tubular cells undergo progressive injury, with loss of the brush border, formation of cytoplasmic blebs, necrosis of epithelial cells, and abundant intratubular casts.[26] We chose 30 minutes of shock, as it was a reasonable approximation of clinically relevant ischemic periods.

Serum cystatin-C does not depend on muscle mass, gender, or age and is not affected by inflammation, fever, or extrinsic substances; it probably is also not affected by the malignant processes.[27] A meta-analysis found cystatin-C to be superior to serum creatinine as a marker of kidney function.[28] Beta-NAG is a specific proximal tubular lysosomal enzyme. It has a large molecular weight (>130 kDa) that prevents glomerular filtration, and it is neither absorbed nor secreted by the tubules.[29] Thus, any detection of NAG in the urine reflects tubular cell damage. These characteristics make NAG a sensitive and early marker of renal tubular damage.[30] Therefore, when evaluating renal function, we used plasma creatinine, BUN, and cystatin-C to evaluate glomerular functions and plasma α-1 microglobulin and urine NAG values to evaluate the tubular functions.

The RL solution is the most widely available and frequently used balanced salt solution for fluid resuscitation in hemorrhagic shock. It has been shown that aggressive crystalloid resuscitation was followed by increased cytokine activation, including IL-1, IL-6, and TNF.[31] The administration of crystalloid (RL) or colloid (Hextend) after 30 minutes of hemorrhagic shock induces significant local upregulation of IL-6 and granulocyte colony-stimulating factor, and further upregulation of TNF-α gene transcription. However, the increase in the proinflammatory cytokine mRNA does not occur in the absence of fluid resuscitation.[32] However, in our study, the increasing values of IL-6 and TNF-α owing to CHS did not change after fluid resuscitation.

Considering the GFR as an index of renal performance after fluid replacement following hemorrhagic shock, the low-volume resuscitation fluids, hypertonic saline (HS), the addition of 6% dextran-70 to HS, RL, and HES are equally effective in restoring renal function in a two-hour period.[33] Therefore, we evaluated the effects of fluid resuscitation after near-fatal shock, immediately as well as two hours after resuscitation.

The precise mechanism by which HES may alter kidney function and whether the amount of adminis-

tered HES is of importance when considering kidney dysfunction are still unknown. In the study by Schortgen et al.[13] on septic patients and those in septic shock, acute renal failure developed, even though the volume of administered HES (median dose, 31 ml/kg) was less than the maximal dose recommended by the manufacturer (33 ml/kg). Boldt and Priebe[34] in their review emphasized the need for caution while using HES in dehydrated patients with high serum creatinine levels (>2-3 mg/dl). On the contrary, HES was used in patients who showed mild to severe renal dysfunction. After 500 ml of HES (130/0.4), kidney function was not affected, indicating no negative effects of this new preparation with respect to kidney function.[35]

Cheung et al.[36] demonstrated that the microvascular and systemic functions returned to pre-hemorrhagic shock levels with 6% HES and crystalloid resuscitation in dogs. They suggested that volume replenishment played an important role in pre-hospital route treatment for hemorrhagic shock. In the experimental model of metabolically controlled near-fatal hemorrhagic shock, Ferreira et al.[37] demonstrated that HES (130/0.4) is the best fluid for immediate and early intravascular volume replacement, because even a small volume restored CO with no significant increase in MAP (hypotensive resuscitation), as well as recovered the tissue perfusion, as demonstrated by the metabolic variables.

In the present study, the near-fatal shock generated moderate renal dysfunction and structural defect. Renal glomerular functions improved with the use of 6% HES (130/0.4), RL, and the combination of RL + HES (increased cystatin-C values owing to shock regressed to pre-shock levels after fluid resuscitation). In terms of tubular functions, although the increased plasma α-1 microglobulin levels did not regress to the pre-shock levels after fluid resuscitation, urine NAG levels were similar to the pre-shock levels. Our hemorrhagic shock model in laboratory animals generated moderate renal dysfunction and structural defect, and 6% HES (130/0.4) did not have a harmful effect on the kidney, either when used alone or in combination with RL for resuscitation of CHS in rabbits.

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