

THE EFFECTS OF CEFTRIAZONE, TEICoplanin AND MEROPENEM ON THE HEMORHEOLOGICAL PARAMETERS

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ABSTRACT

Objective: Microcirculatory blood flow is altered in sepsis. Changes in hemorheological parameters may contribute to the alterations in microcirculatory blood flow. This study was conducted to observe the effects of ceftriazone, teicoplanin and meropenem, commonly used antimicrobial agents in sepsis, on hemorheological properties.

Material and Method: A total of 40 adult Wistar albino rats divided in 4 groups were included. Group 1 received ceftriazone (50 mg/kg/day), group 2 received meropenem (100 mg/kg/day), group 3 received teicoplanin (10 mg/kg/day) and group 4 received normal saline solution intraperitoneally for 10 days. On the 11th day hematological parameters (erythrocyte, leukocyte and platelet counts, hemoglobin, hematocrit and mean corpuscular volume)

and hemorheological parameters (blood and plasma viscosity, red blood cell, polymorphonuclear cell and mononuclear cell deformability) were analyzed.

Results: Erythrocyte rigidity was increased in the ceftriazone group and mononuclear cell rigidity was increased in the meropenem group ($p<0.05$). Ceftriazone resulted in an increase in plasma and blood viscosity.

Conclusion: We conclude that some antimicrobial agents may affect rigidity and viscosity. The effects of antibacterial drugs on hemorheological parameters may be distinguishing especially when the maintenance of microcirculation is important.

Key Words: Ceftriazone, teicoplanin, meropenem, hemorheology, rigidity, viscosity. *Nobel Med 2012; 8(2): 107-110*

SEFTRİAKSON, TEİKOPLANİN VE MEROPENEMİN HEMOREOLOJİK PARAMETRELERE ETKİSİ

ÖZET

Amaç: Sepsiste mikrosirkulatuar kan akımı bozulur. Hemoreolojik parametrelerdeki değişiklikler mikrosirkulatuar kan akımının bozulmasına katkıda bulunur. Bu çalışma sepsiste sık kullanılan antibiyotikler olan seftriakson, teikoplanin ve meropenem hemoreolojik parametreler üzerine etkisini görmek amacıyla planlanmıştır.

Materyal ve Metod: Çalışmaya 4 gruba ayrılan toplam 40 erişkin albino Wistar sıçanı alındı. 1. gruba seftriakson (50 mg/kg/gün), 2. gruba meropenem (100 mg/kg/gün), 3. gruba teikoplanin (10 mg/kg/gün) ve 4. gruba normal salin solüsyonu 10 gün boyunca intraperitoneal olarak verildi. 11. günde he-

matolojik (eritrosit, lökosit ve trombosit sayısı, hemoglobin, hematokrit ve ortalama eritrosit hacmi) ve hemoreolojik (kan ve plazma viskozitesi, eritrosit, lökosit ve mononükleer hücre deformabilitesi) parametreler incelendi.

Bulgular: Seftriakson grubunda eritrosit rijiditesi, meropenem grubunda ise mononükleer hücre rijiditesi artmıştı ($p<0,05$). Seftriakson plazma ve kan viskozitesinde artışa yol açtı.

Sonuç: Bazı antibiyotiklerin rijidite ve viskoziteyi etkilediği belirlenmiştir. Mikrosirkulasyonun devamı önemli olduğunda antibiyotiklerin hemoreolojik parametreler üzerine bu etkisi tedavi seçiminde belirleyici olabilir.

Anahtar Kelimeler: Seftriakson, teikoplanin, meropenem, hemoreoloji, rijidite, viskozite. **Nobel Med 2012; 8(2): 107-110**

INTRODUCTION

The microcirculation is a dynamic process involving microvasculature, the endothelium and the rheological factors. Red blood cell (RBC) deformability and blood viscosity are essential factors to maintain the blood flow in terminal capillary bed. Deformability is the ability of the cell to reversibly adopt a new shape. The normal state of deformability is essential for RBC to conform its shape and maintain proper blood flow. Increased rigidity of RBCs, which is inversely proportional with RBC deformability, alter the flow rate of blood in the microvascular bed.¹⁻⁴

Sepsis is associated with microcirculatory disturbances.⁵ Decreased RBC deformability and increased viscosity have been extensively investigated in patients with sepsis.⁶⁻¹⁰ White blood cell (WBC) deformability is also decreased in sepsis and severe trauma.¹¹⁻¹⁴ Alterations in blood rheology may be responsible for or further aggravate microcirculatory dysfunction.

After a research in the Pubmed database, we could not find any article addressing the rheological effects of antibacterial drugs. Thus, this study was conducted to investigate the effects of ceftriaxone, teicoplanin and meropenem, the antimicrobial agents commonly used in sepsis, on hemorheological parameters.

MATERIAL and METHOD

A total of 40 adult Wistar albino rats were included. They were divided in 4 groups: Group 1 received

ceftriaxone (50 mg/kg/day), group 2 received meropenem (100 mg/kg/day), group 3 received teicoplanin (10 mg/kg/day), and group 4 received normal saline solution intraperitoneally for 10 days. On the 11th day all of the rats were anaesthetized with pentothal sodium and blood samples were obtained by left heart puncture.

Hematological parameters [RBC, WBC and platelet counts, hemoglobin, hematocrit (Hct) and mean corpuscular volume (MCV)] were determined by an automatic counter (TechniconH2). The following hemorheological parameters were analyzed: blood and plasma viscosity, erythrocyte, polymorphonuclear (PMN) and mononuclear cell (MNC) rigidity.

Blood and plasma viscosity were determined in blood samples containing EDTA by Wells-Brookfield LUT cony-plate rotator viscosimeter (MA O20 2072 Engineering Laboratories, Stoughton, USA). The procedure was carried out at a shear rate of 60 rpm. Deformability was determined in blood samples containing EDTA by microfiltration technique in terms of pressure versus cell rigidity. RBC, PMN and MNC suspensions were prepared.

RBC suspension: Blood was filtered through cotton wool to remove leukocytes and platelets. The RBCs were then washed three times in buffer solution and resuspended in HEPES buffer at a hematocrit of 5% by adding HEPES buffer (137 mmol of NaCl, 4 mmol of KCl, 1.8 mmol of CaCl₂, 0.7 mmol of MgSO₄.7H₂O, 0.2 mmol of Na₂HPO₄.2H₂O, 8.4 mmol of Hepes-Merck).¹⁵→

PMN suspensions: After centrifugation of whole blood on Ficoll gradient, the cell pellet was subjected to osmotic lysis of RBCs. PMNs were centrifuged and resuspended in Ringer solution.¹⁵

MN suspensions: MN's were isolated from whole blood by Ficoll gradient centrifugation at 700 g for 20 minutes. The MNs were washed and resuspended in Ringer solution.¹⁵

Filtration: The cell suspensions were pumped at a constant rate (6.05 ml/min) through polycarbonate filters (Nucleopore Corp. Pleasanton, CA) with nominal pore sizes of 3 µm for RBCs and 5 µm for WBCs and MNs at room temperature (20-23°C). The filtration pressure was measured on the upstream side of the filter with a pressure transducer (Gould, Model TMP400, P231D) connected to an amplifier and recorded on a polygraph (Nikon Kohden RM 6000). Then the data obtained were saved by an analog-digital converter. Prior to the filtration of a cell suspension, buffer solution alone was filtered to obtain the filtration pressure (P0) for suspending medium. Cell rigidity (k) was calculated by using the filtration pressures before and after the start of pumping.¹⁵⁻¹⁸

All of the chemicals were supplied by Merck Chicago and they were of an analytic grade.

The experiments were conducted according to the applicable local and international guidelines and regulations about the ethical use and care of laboratory animals.

Statistical analysis was conducted by using "SPSS for Windows Release 13.0 Software (SPSS Inc, 92 Chiago, IL). We used Kruskal Wallis test to compare the groups. Results were expressed as means ± standard deviation (SD). Differences were considered significant at p<0.05. Post-hoc tests were performed by NCS 2000 software. A two-tailed Student Newman Keuls test was used for multiple comparisons with significance at p<0.05.

RESULTS

We observed an increase in erythrocyte rigidity in the ceftriaxone group compared to the teicoplanin group although it did not differ in groups 2 and 4. There was not a statistically significant difference in PMN rigidity between groups, however a significant difference was observed in MN rigidity. Meropenem resulted in an increased MN rigidity compared to ceftriaxone. Ceftriaxone was associated with an increase in both plasma and blood viscosities compared to both teicoplanin and meropenem.

Table: Comparison of rheological parameters in the study groups

Parameter	Group 1	Group 2	Group 3	Group 4	p
RBC rigidity (k)	0.0534±0.005	0.0479±0.17	0.0435±0.007	0.0476±0.006	< 0.05
PMN rigidity (k)	0.1935±0.045	0.235±0.081	0.1762±0.018	0.1779±0.025	> 0.05
MN rigidity (k)	0.198±0.035	0.248±0.033	0.2274±0.012	0.217±0.025	< 0.05
Blood viscosity	5.53±1.05	3.84±0.54	3.72 ± 0.06	4.23±0.77	0.001
Plasma viscosity	2.13±0.19	1.35±0.28	1.38±0.13	1.78±0.38	< 0.0001
WBC (x10 ⁹ /mm ³)	4.27±0.3	5.98±1.02	3.6±0.1	4.6±0.2	< 0.0001
RBC (x10 ⁶ /mm ³)	7.8±2.2	5.9±2.2	4.6±1.2	6.3±1.8	< 0.05
Hgb (g/dl)	11.1±0.9	12.1±1.8	9.9±1.2	15.2±1.1	< 0.0001
Hct (%)	37.7±3.2	38.3±3.2	42.1±2.7	48±2.3	< 0.0001
MCV (fl)	52.5±3.8	56.2±3.9	54.3±3.2	56.9±6.3	> 0.05
Platelet (x10 ⁹ /mm ³)	732±1.6	721±18.8	166±0.9	213±2.1	< 0.0001

RBC: Red blood cell, PMN: polymorphonuclear cell, MN: mononuclear cell, WBC: white blood cell, Hgb: hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume

WBC count was increased in the meropenem group. This increase was significant when compared with the other three groups. RBC, WBC and platelet counts and hemoglobin was decreased in the teicoplanin group. Meropenem caused a significant increase in WBC compared to ceftriaxone and teicoplanin. Ceftriaxone increased RBC compared to teicoplanin. The decrease in hemoglobin was also observed in the ceftriaxone group compared to the control group. Hct was decreased in both group 1 and 2 compared to the group 4. Thrombocytosis was observed in both ceftriaxone and meropenem groups. The increase in platelet group was significant in these groups compared to the meropenem and control groups. The results are summarized in Table.

DISCUSSION

The decreased RBC deformability and viscosity may unfavorably contribute to the microcirculatory blood flow.^{2,3} The rheology of WBCs has also significant implications in their flow through the microcirculation.¹⁹ Although much work has been done on the rheological properties of RBCs and leucocytes, there is little information available on the monocytes. Evans et al. demonstrated distinct sub-populations of monocytes with differing rheological properties.²⁰

Blood rheology in sepsis has been studied to a great extent. Alterations in rheological parameters such as viscosity and disturbances of RBC and WBC rheology have a negative impact on microcirculation in patients with sepsis.⁷ RBC deformability is accepted to be an important determinant of blood flow resistance. Thus decreased deformability of RBCs contributes to impaired tissue perfusion encountered in sepsis.⁸ →

Voerman et al. noted that they observed a decrease in blood viscosity and RBC deformability.⁹ RBC deformability has also been studied and found as a marker for prognosis and monitoring of severity.¹⁰ Yodice et al. reported decreased neutrophil deformability in patients with sepsis.¹³ Drost et al. hypothesized that PMN rigidity might lead to sequestration of those cells in the capillaries and impairment of microvascular perfusion in sepsis.¹¹ Skoutelis et al. noted that neutrophils from septic shock patients were significantly more rigid than neutrophils from sepsis patients. Increased neutrophil rigidity is found to reduce neutrophil flow causing release of oxygen radicals and contributing to ischemia and tissue injury.²¹ Nishino et al. investigated the serial changes in leukocyte deformability in sepsis. They found that deformability and hemorheological properties changed rapidly and dynamically in relation with the clinical course.¹⁴

We intended to bring up the hemorheological effects of antibacterial agents. Since sepsis itself negatively affects deformability, we should avoid using drugs further aggravating it. Thus we chose

three antibacterial drugs commonly used in sepsis and septic shock. We found that ceftriaxone increased RBC rigidity in rats. Blood and plasma viscosities were also increased in this group. If the same effect is observed in human studies with adequate number of subjects, especially in the condition of sepsis, then the preference of antibacterial drugs will be revised.

We observed the highest RBC and platelet counts and the lowest Hct and MCV in the ceftriaxone group. High RBC and platelet counts are threats for blood flow in capillary bed. In a study by Sordia et al. the RBC deformability index was found to be decreased by 71% and the systemic Hct lowered by 31% in an experimental rat model of endotoxic shock as compared to the same parameters in the control group. They stated that the decreased Hct favoured the blood flow in the capillary networks, but on the other hand it reduced the oxygen supply to the tissues.²²

We conclude that some antimicrobial agents may affect hemorheological parameters. These effects may be distinguishing when prescribing in conditions with altered microcirculation.



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REFERENCES

- Chien S. The Microcirculatory Society Eugene M. Landis Award lecture. Role of blood cells in microcirculatory regulation. *Microvasc Res* 1985; 29: 129-151.
- Pries AR, Secomb TW. Rheology of the microcirculation. *Clin Hemorheol Microcirc* 2003; 29: 143-148.
- Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. *Semin Thromb Hemost* 2003; 29: 435-450.
- Reggiori G, Occhipinti G, De Gasperi A, Vincent JL, Piagnerelli M. Early alterations of red blood cell rheology in critically ill patients. *Crit Care Med* 2009; 37: 3041-3046.
- Hinshaw LB. Sepsis/septic shock: participation of the microcirculation. *Crit Care Med* 1996; 24: 1072-1078.
- Astiz ME, DeGent GE, Lin RY, Rackow EC. Microvascular function and rheologic changes in hyperdynamic sepsis. *Crit Care Med* 1995; 23: 265-271.
- Piagnerelli M, Boudjeltia KZ, Vanhaeverbeek M, Vincent JL. Red blood cell rheology in sepsis. *Intensive Care Med* 2003; 29: 1052-1061.
- Baskurt OK, Belmont D, Meiselman HJ. Red blood cell deformability in sepsis. *Am J Respir Crit Care Med* 1998; 157: 421-427.
- Voerman HJ, Fonk T, Thijs LG. Changes in hemorheology in patients with sepsis or septic shock. *Circ Shock* 1989; 29: 219-227.
- Moutzouri AG, Skoutelis AT, Gogos CA, Missirlis YF, Athanassiou GM. Red blood cell deformability in patients with sepsis: A marker for prognosis and monitoring of severity. *Clin Hemorheol Microcirc* 2007; 36: 291-299.
- Drost EM, Kassabian G, Meiselman HJ, Belmont D, Fisher TC. Increased rigidity and priming of polymorphonuclear leukocytes in sepsis. *Am J Respir Crit Care Med* 1999; 159: 1696-1702.
- Kirschenbaum LA, Aziz M, Astiz ME, Saha DC, Rackow EC. Influence of rheologic changes and platelet-neutrophil interactions on cell filtration in sepsis. *Am J Respir Crit Care Med* 2000; 161: 1602-1607.
- Yodice PC, Astiz ME, Kurian BM, Lin RY, Rackow EC. Neutrophil rheologic changes in septic shock. *Am J Respir Crit Care Med* 1997; 155: 38-42.
- Nishino MM, Tanaka H, Ogura H, et al. Serial changes in leukocyte deformability and whole blood rheology in patients with sepsis or trauma. *J Trauma* 2005; 59: 1425-1431.
- Betticher DC, Keller H, Maly FE, Reinhart WH. The effect of endotoxin and tumour necrosis factor on erythrocyte and leukocyte deformability in vitro. *Br J Haematol* 1993; 83: 130-137.
- Reinhart WH, Usami S, Schmalzer EA, Lee MM, Chien S. Evaluation of red blood cell filterability test: influences of pore size, hematocrit level, and flow rate. *J Lab Clin Med* 1984; 104: 501-516.
- Chien S, Schmalzer EA, Lee MM, Impelluso T, Skalak R. Role of white blood cells in filtration of blood cell suspensions. *Biorheology* 1983; 20: 11-27.
- Skalak R, Impelluso T, Schmalzer EA, Chien S. Theoretical modeling of filtration of blood cell suspensions. *Biorheology* 1983; 20: 41-56.
- Chien S, Sung KL, Schmid-Schönbein GW, et al. Rheology of leukocytes. *Ann N Y Acad Sci* 1987; 516: 333-347.
- Evans SA, Adams R, Rainger GE. Monocytes are a rheologically heterogeneous population of cells. *Clin Hemorheol Microcirc* 2001; 25: 63-73.
- Skoutelis AT, Kaleridis V, Athanassiou GM, et al. Neutrophil deformability in patients with sepsis, septic shock, and adult respiratory distress syndrome. *Crit Care Med* 2000; 28: 2355-2359.
- Sordia T, Tatarishvili J, Mchedlishvili G. Hemorheological disorders in the microcirculation during septic shock in rats. *Clin Hemorheol Microcirc* 2006; 35: 223-226.