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Third Generation Cephalosporins Altered Human Red Cell Membrane Function *in vitro*: Evidence Observed from Osmotic Fragility Test

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ABSTRACT

Cephalosporins adversely affect the hemopoietic system in terms of inducing immune hemolytic anemia and disturbing the function of blood platelets. In this study, the effect of 3rd generation cephalosporins, namely ceftriaxone, cefotaxime and ceftizoxime, on human red cells was investigated and the study was conducted in Rizgari Teaching Hospital in Hawler, Iraq. The effect of cephalosporins was evaluated by osmotic fragility test using fresh human blood in the presence of cephalosporins in varying concentrations between 25-200 μg mL^{-1}. Although, all tested drugs shifted the osmotic fragility curve to some extent, the concentration of sodium chloride to induce 50% hemolysis (CH_{50}) was higher in the presenceof ceftriaxone, cefotaxime and ceftizoxime at 200 μg mL^{-1} with increments in hemolysis percents of 5.55, 3.55, 3.16% in comparison to controls, respectively. As a result, this study shows that third generation cephalosporins at high concentrations alter directly the red cell membrane function *in vitro*, by shifting the osmotic fragility curve and increasing the levels of CH_{50}.

Key words: Cephalosporins, adverse reaction, hemolysis, fragility

INTRODUCTION

Immune hemolytic anemia is characterized by destruction of red blood cell by antibodies, either I gG or I gM, acting against specific antigens on the erythrocyte membrane. It may be idiopathic or secondary to several causes such as drug-induced hemolytic anemia (Arndt and Garratty, 2005). Cephalosporins, nonsteroidal anti-inflammatory agents, teicoplanin and others who are shown to cause immune hemolytic anemia (Johnson *et al*., 2007).

The increase in red cell fragility was reported for patients with congenital spherocytosis who subjected to oxidative stress conditions such as infections (Saha *et al*., 2011). On the other hand, endogenous substances like bilirubin were reported to cause the normal erythrocytes with more fragile plasma membrane whereas the spherocytes remain unaffected (Roll *et al*., 2005).

Moreover, propolis extract that contained high polyphenolic compounds reduced the erythrocyte membrane fragility of patients with hereditary spherocytosis (Moreira *et al*., 2011). Multiple antioxidant fortifications including vitamin C, vitamin E and carnitine are shown to be effective in overcoming increased red cell instability, osmotic fragility and hemolysis induced by high altitude (Vani *et al*., 2010). Exercise per se induced significant effects on the red cell membrane characterized with considerable increase in the osmotic fragility and decreased deformability of erythrocytes in sedentary humans. These changes accompanied by signs for intravascular hemolysis which prevented by administration of antioxidant vitamins (A, C, E) (Senturk *et al*., 2005). In addition to these, cephalosporins, widely used antibiotics in the management of skin, soft tissue and genitor-tract diseases, were shown to cause adverse immune reactions

(Montannez *et al.*, 2011) and upon administration, 3rd generation cephalosporins cefoxitin, ceftriaxone, ceftizoxime and cefobactam are shown to cause acute hemolytic anemia (Leaf *et al.*, 2010; Imam *et al.*, 2008; Al-Hawsawi *et al.*, 2010; Baek *et al.*, 2009).

Therefore, this study aimed to explore the direct effect of cephalosporin on the human red cell membrane by utilizing osmotic fragility test on fresh blood samples obtained from healhty individuals.

MATERIALS AND METHODS

This study was conducted in the laboratories of Rizgari teaching hospital in Hawler, Iraq during 2011 and approved by the local Scientific Committee of College of Pharmacy, Hawler Medical University. The venous blood samples were obtained from healthy male volunteers (a total number 12 participants) and a verbal consent form was obtained from each individual enrolled in the study. None of them was a smoker or had a history of alcohol intake. The Medical history of volunteers revealed no evidence of familial hereditary hemolytic anemia or previous history of acquired hemolytic anemia, hypertension, diabetes mellitus or renal failure. Venous blood samples were obtained and osmotic fragility test was done in the presence of the solvent or the pharmaceutical preparation of cephalosporins. Four pharmaceutical preparations of each drug, commercially available in vials, namely ceftriaxone, cefotaxime and ceftizoxime, purchased from the local sources to be used in osmotic fragility tests, at final concentrations of 0, 25, 50, 100, 200 μg mL^{-1}. The final concentrations of NaCl used in osmotic fragility test were 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, 0.65, 0.75 and 0.85% (w/v) as percent weight to volume ratio. Osmotic fragility test was performed by the method described in laboratory tests (Tietz, 1995) by using the heparinized whole blood samples mixed with increasing concentration of buffered salt solution (NaCl) followed by incubation at room temperature for 30 min. After incubation with drugs or solvent (vehicle), solutions were centrifuged (1000 rpm for 10 min) and the hemoglobin released from the erythrocytes was measured at 540 nm (The constituents of buffered sodium chloride include; NaCl 90 g; Na_2HPO_4 13.65 g, $NaH_2PO_4.2H_2O$ 34 g dissolved in 1 L distilled water).

Hemolysis was expressed as a percentage and 100% hemolysis was determined from the absorbance of the distilled water (0% NaCl). The NaCl concentration that induce hemolysis in 20% (CH_{20}), 50% (CH_{50}) and 80% (CH_{80}) were calculated from the percentage of hemolysis in buffered salt solutions at various concentrations.

Statistical analysis: The osmotic fragility curve was constructed with Microsoft Excel and the regression equation of the best line and the standard error of coefficient factor were calculated to determine CH_{20}, CH_{50} and CH_{80}. The standard experimental error (or standard deviation) for each data point was calculated by using the same program.

RESULTS

Figure 1 shows that ceftizoxime at 200 μg mL^{-1} shifts the osmotic fragility test to the right while at low concentration (25 μg mL^{-1}) shifts slightly the curve towards the left side. Cefotaxime shifts the curve of osmotic fragility towards the right side (i.e., hemolysis of red cells at high sodium chloride concentration compared with control) at all concentrations used in the study (Fig. 2). The effect of ceftriaxone on the human red cell fragility is more obvious than cefotaxime and ceftozoxime. Ceftriaxone shifts the osmotic fragility curve towards the right side at all tested concentrations (Fig. 3). Table 1 shows that the concentration of sodium chloride that induced

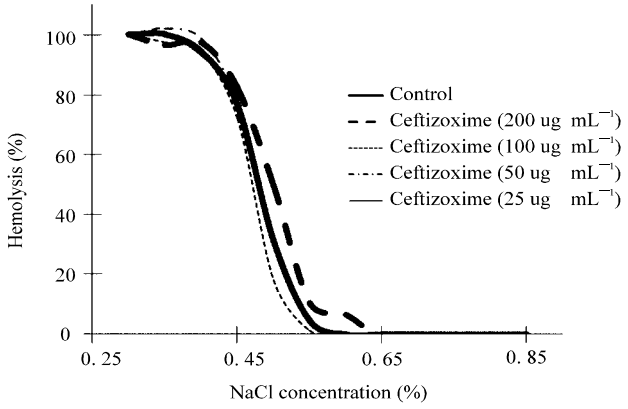

Fig. 1: Effect of ceftizoxime on the osmotic fragility of human red cells

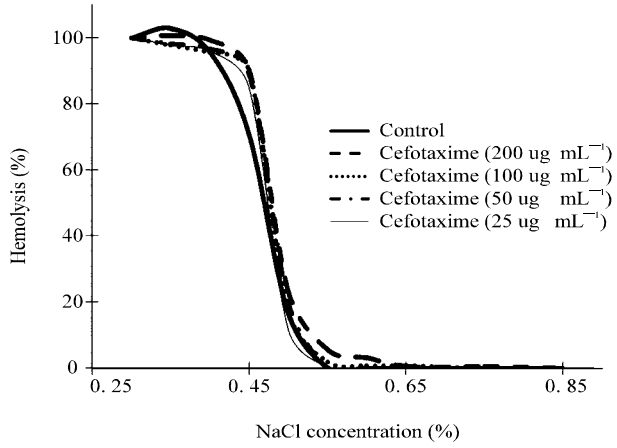

Fig. 2: Effect of cefotaxime on the osmotic fragility of human red cells

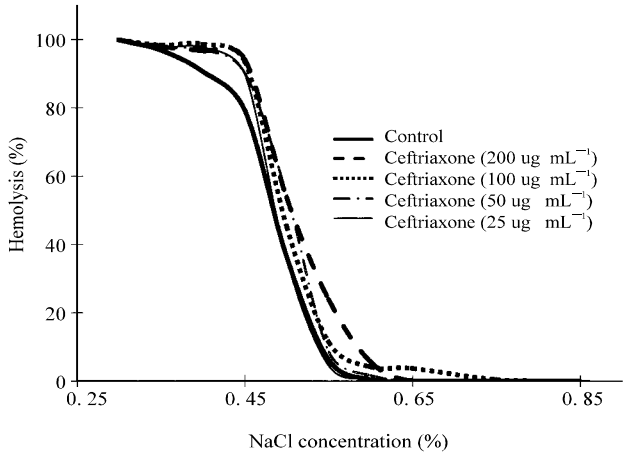

Fig. 3: Effect of ceftriaxone on the osmotic fragility of human red cells

Table 1: The concentration of NaCl (%) at which cephalosporins induced corresponding percent of hemolysis in human red cells (the mean experimental errors should be given for each data point that includes the results of 12 samples, such as 0.6298±0.0025)

Drug (μg mL^{-1})	CH_{20}	CH_{50}	CH_{80}	Standard error of correlation factor
Control	0.6298	0.4998	0.3698	0.115
Ceftizoxime (200 μg mL^{-1})	0.6445	0.5156	0.3866	0.128
Ceftizoxime (100 μg mL^{-1})	0.6212	0.4882	0.3552	0.14
Ceftizoxime (50 μg mL^{-1})	0.6398	0.5025	0.3742	0.14
Ceftizoxime (25 μg mL^{-1})	0.6301	0.5002	0.3703	0.147
Control	0.6211	0.4901	0.3592	0.131
Cefotaxime (200 μg mL^{-1})	0.635	0.5075	0.3799	0.128
Cefotaxime (100 μg mL^{-1})	0.6281	0.4984	0.3682	0.14
Cefotaxime (50 μg mL^{-1})	0.6277	0.4989	0.3701	0.14
Cefotaxime (25 μg mL^{-1})	0.6231	0.4926	0.362	0.147
Control	0.6323	0.5007	0.3691	0.107
Ceftriaxone (200 μg mL^{-1})	0.6549	0.5285	0.402	0.087
Ceftriaxone (100 μg mL^{-1})	0.6487	0.5216	0.3945	0.139
Ceftriaxone (50 μg mL^{-1})	0.6454	0.5187	0.3921	0.105
Ceftriaxone (25 μg mL^{-1})	0.6364	0.5089	0.3814	0.119

The results calculated from the best fit line of corresponding osmotic fragility, CH_{20}, CH_{50}, CH_{80}: The concentration of NaCl that produced hemolysis in 20, 50 and 80% of human red cells

hemolysis in 20, 50 and 80% in presence or absence of cephalosporins. These percents were chosen because they represent the straight line of sigmoid shape of fragility test. The significance of these values was to explore the effective concentration of the cephalosporins to induce hemolysis in 50% of red cells and to show whether the hemolysis was concentration-dependent. The results clearly demonstrated that the hemolysis did not depend on the concentration of cephalosporin. Table 1 shows that the concentration of sodium chloride to induced 50% hemolysis is higher in presence of ceftriaxone, cefotaxime and ceftizoxime (at 200 μg mL^{-1}) than corresponding control and this account an increment in hemolysis percents of 5.55, 3.55 , 3.16%, respectively. At the concentration of 25 μg mL^{-1}, the increments in CH50 are 1.64, 0.5 and 0.08% for ceftriaxone, cefotaxime and ceftizoxime, respectively (Table 1).

DISCUSSION

The results show that 3rd generation cephalosporins disturb the function of red cell membrane by shifting the osmotic fragility test in a mechanism not related to the antigen-antibody complex.

Recent reports show that new generation cephalosporins induced immune hemolytic anemia (Salama, 2009) and ceftriaxone induced red cell antibody in about 12.5% and caused red cell hremolysis in 3.1% (Quillen *et al*., 2008). Further study showed that ceftriaxone-dependent antibody also bound to glycoprotein receptors of blood platelet (GPIIb/IIIa) subunits and induced thrombocytopenia (Grossjohann *et al*., 2004). There is evidence that cephalosporins inhibit the activity of the glutathione enzymes that involved in the antioxidant system (Sukoyan *et al*., 2005). Cephalosporins inhibit ATP synthesis in red cell under hypoxia accompanied with inhibition of catalase and glutathione reductase enzymes and by this effect the red cells were more susceptible to hemolysis. The results of this study are in agreement with the previous studies that mentioned above and clearly show that ceftriaxone directly induced an increase hemolysis percent by 5.5% in a concentration of 200 μg mL^{-1}. After intravenous administration of cefotetan, minute

concentration of the drug is tightly bound to red cell for weeks after the last dose (Davenport *et al.*, 2004). In about 8% of individuals who received cefotetan cur the antibodies were detected in their sera. Moreover, cephalothin also modified the red cell membrane which rendered the protein to attach non-immunologically to red cell membrane (Garratty, 2009). Therefore, it is possible to explain the finding of these results on the basis of cephalosporins bound to red cells and the degree of hemolysis is related to the magnitude of the binding.

CONCLUSION

It concludes that 3rd generation cephalosporins in high concentrations directly altered the red cell membrane function, *in vitro*, by the evidence of shifting the osmotic fragility curve and a high CH50. The results of this study highlight a precaution in the prescription of 3rd generation cephalosporins in patients with hereditary hemolytic anemia.

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