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Effect of Antimalarial Drugs on Polymerization of Sickle Cell Hemoglobin (HbS)

[Antimalaryal İlaçların Orak Hücre Hemoglobininin (HbS) Polimerizasyonu Üzerine Etkisi]

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ABSTRACT

Aim and Method: The effect of antimalarial drugs (Coartem™, Quinine, and Chloroquine phosphate) on polymerization of sickle cell hemoglobin (HbS) was studied spectrophotometrically using hemolysates of HbS containing erythrocytes treated with sodium metabisulphite in the presence of each antimalarial drug.

Results: Each drug caused significant ($p<0.05$) reduction in HbS polymerization: Coartem™ (17.05-31.07 %), Quinine (13.95-28.85 %) and Chloroquine phosphate (10.85-33.01 %).

Conclusion: We conclude that each of the three drugs reduced HbS polymerization and are a potential candidate for therapy and management of sickle cell disease.

Key Words: antimalarial drugs, polymerization, sickle cell, haemoglobin, sodium metabisulphite.

ÖZET

Amaç ve Metod: Antimalaryal ilaçların (Coartem™, Kinin ve Klorokin fosfat) orak hücre hemoglobininin (HbS) polimerizasyonuna olan etkisine spektrofotometrik olarak bakıldı. Bu amaçla HbS içeren eritrositlerden elde edilen hemolizat, antimalaryal ilaçların varlığında ve yokluğunda sodium metabisülfit ile muamele edildi.

Bulgular: Denenen bütün ilaçlar HbS'nin polimerizasyonunda belirgin ölçüde azalmaya neden oldu. Coartem™ (% 17.05-31.07), Kinin (% 13.95-28.85) ve Kolokin fosfat (% 10.85-33.01).

Sonuç: HbS polimerizasyonunda azalmaya neden olan bu üç ilacın orak hücre hastalıklarının tedavisinde potansiyel aday olduğu düşünülmektedir.

Anahtar Kelimeler: antimalaryal ilaçlar, polimerizasyon, orak hücre, hemoglobin, sodyum metabisülfit.

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Introduction

The sickling disorder was the first description of a molecular disease and accounts for the vast majority of clinically important disorders [1]. The sickle cell gene (β^S) occurs widely throughout Africa, parts of Asia, the Arabian Peninsula and parts of Southern Europe. Sickle cell anemia is caused by a single base mutation of adenine to thymine which results in a substitution of valine for glutamic acid at the sixth codon of the β-globin chain [2]. This substitution has a profound consequence on the structure of hemoglobin and its biologic function because substitution of polar glutamic acid residue by the non-polar valine molecule generates a sticky patch on inter-molecular contact region of each of the beta chain [3]. In conditions of reduced oxygen tension, HbS molecules form a relatively insoluble polymer through sequential steps of nucleation, growth and alignment of molecules into parallel microfibrils which produce membrane deformity and damage [4].

In spite of the full understanding of the pathology, physiology, and the molecular nature of the disease, a cure for sickle cell anemia still is unavailable. Strategies have focused mainly on prophylactic measures to alleviate the painful crises by the use of blood transfusions, painkilling drugs, intravenous fluids, oral antibiotics such as penicillin and the anticancer drug hydroxyurea [5]. Several attempts are in progress to finding anti-sickling agents that specifically bind to HbS. Such agents include: 5-hydroxylmethyl-2-furfural (5HMF), [6], the amino acids such as phenylalanine, lysine, and arginine, [7] and 2-imidazolines [8]. In clinical practice, hydroxyurea is commonly used as anti-sickling agent and has recently approved by the United States Food and Drug Administration as a drug [9].

Some anti-malarial drugs (Fansidar, Halfan, Quinine, Coartem and Chloroquine Phosphate) change red blood cell glutathione-S transferase activity, osmotic fragility index and content of methemoglobin [10-13]. Therefore, we have studied the effect of three anti-malarial drugs on HbS polymerization.

Materials and Methods

5 ml of venous blood obtained from sicklers by venipuncture was stored in EDTA anticoagulant tubes. Blood samples were from patients (HbSS) attending clinics at the Federal Medical Center (FMC), Imo State University Teaching Hospital (IMSUTH), Orlu, St. John Clinic/ Medical Diagnostic Laboratories, Avigram Medical Diagnostic Laboratories, and Qualitech Medical Diagnostic Laboratories.

The erythrocytes were washed as described by Tsakiris et al., [14]. Within 2 hrs of collection, 1 ml portion of the blood were introduced into centrifuge tubes containing 3 ml of 250 mM Tris-(hydroxyl methyl) amino ethane–HCl (Tris-HCl) buffer solution pH=7.4, 140 mM NaCl, 1 mM $MgCl_2$, 10 mM glucose and centrifuged at 1200 x g for 10 min and washed three times with the buffer solution. They were re-suspended in 1 ml of the buffer and stored at 4 ^{0}C. Erythrocytes were lysed by freezing/thawing as described by Galbraith and Watts [15] and Kamber et al. [16].

We used the antimalarial drugs: CoartemTM (Beijing NORVATIS Pharmaceutical Company, Beijing, China), Chloroquine phosphate (MAY and BAKER, Pharmaceutical Company Nigeria, Plc), and Quinine (BDH, UK). 2 mg of each drug was dissolved in 100 ml of distilled water.

HbS polymerization was assessed as described previously [17]. The level of polymerization was ascertained by increasing absorbance of the assay mixture. A 0.1 ml of hemolysate containing HbS was introduced into a test tube and followed by 0.5 ml of phosphate buffered saline solution (PBS, 9 g NaCl, 1.71 g $Na_2HPO_4.2H_2O$, 2.43 g $NaH_2PO_4.2H_2PO_4.2H_2O$ per liter of distilled water, pH=7.4) and 1 ml of distilled water. The mixture was transferred into a cuvette and 3.4 ml of 2 % sodium metabisulphite solution was added. The absorbance of the assay mixture was recorded at every 30 seconds for 180 seconds at λ max= 700 nm (control sample). This procedure was repeated by substituting the distilled water by 1 ml of the drug solution (test sample).

Percentage polymerization was calculated from =

$$\frac{At/c \times 100}{Ac_{180^{th}\ second}}$$

Where:

At/c =Absorbance of test/control sample at time = t second.

$Ac_{180^{th}\ second}$ = Absorbance of control sample at the 180th second.

The experiments were designed in a completely randomized method and data collected were analyzed by the analysis of variance procedure while treatment means were separated by the Least Significance Difference (LSD) incorporated in the Statistical Analysis System (SAS) package of 9.1 version (2006).

Results and Discussion

The change mean $\pm$ S.D in absorbance of the control and test the samples are presented in Table 1 and Figure 1 respectively.

The results presented in Table 1 showed increasing absorbance of the assay mixture in the control and test samples as the experimental time progressed. However, the absorbance of the polymerization mixture in the presence of the three antimalarial drugs was not significantly different ($p<0.05$) from the control sample at the 30th second. This indicates that polymerization of HbS molecules occurred in the control sample and in the presence of the three antimalarial drugs (Figure 1). For instance, within the experimental time of 30-180 seconds, the polymerization range was between 33.33-82.95 %,

Table 1. Changes in absorbance of the control and test samples with time

Time(Seconds)	Control	Absorbance(λ=700nm) Coartem™	Quinine	Chloroquine phosphate
0	0.00	0.00	0.00	0.00
30	0.052± 0.02[a]	0.043±0.004[a]	0.037±0.04[a]	0.042 ±0.02[a]
60	0.103 ± 0.01[b]	0.071±0.005[a]	0.074±0.04[a]	0.069±0.02[a]
90	0.113 ±0.02[b]	0.087±0.008[a]	0.091±0.04[a]	0.086±0.02[a]
120	0.122±0.01[b]	0.097±0.012[a]	0.100±0.05[a]	0.098±0.02[a]
150	0.126 ±0.02[b]	0.104±0.015[a]	0.106 ±0.05[a]	0.107±0.02[a]
180	0.129±0.01[b]	0.107±0.015[a]	0.111±0.05[a]	0.115±0.02[a]

Means ±S.D in the row with the same letter are not significantly different at $p< 0.05$.
Four samples (n=4) were used in each determinations.

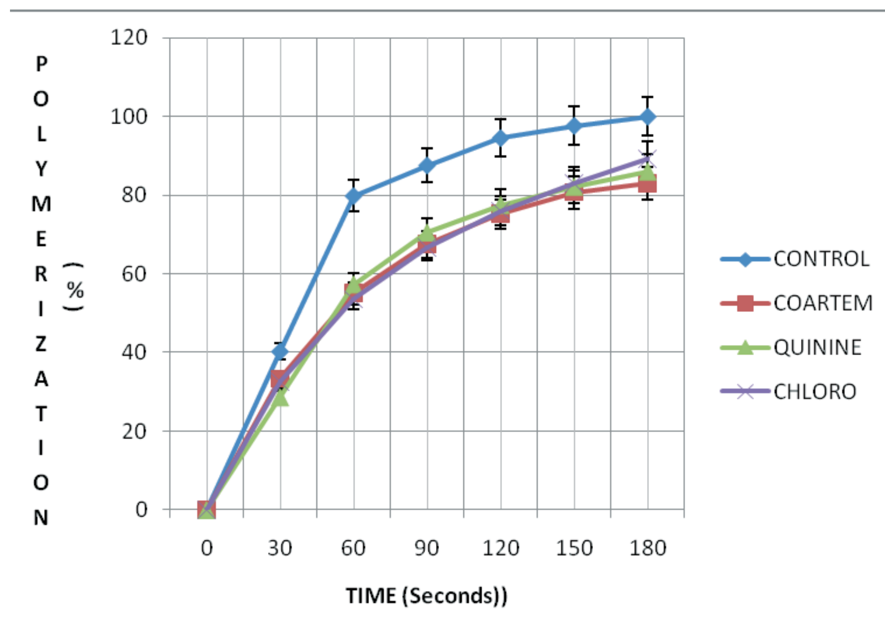

Figure 1. Percentage polymerization of HbS in the presence of antimalarial drugs

Table 2: Percentage reduction of HbS polymerization in presence of antimalarial drugs with time:

Drug/ Time (sec)	Percentage reduction of polymerization 30	60	90	120	150	180
Coartem™	17.31±0.09	31.07±0.08	23.01±0.08	20.49±0.06	17.46±0.08	17.05±0.08
Quinine	28.85±0.07	28.54±0.10	19.47±0.07	18.03±0.08	15.87±0.06	13.95±0.08
Chloroquine P	19.23 ±0.08	33.01±0.06	23.89±0.05	19.67±0.10	15.08±0.11	10.85±0.06

28.68-86.05 % and 32.56-89.15 % upon the introduction of Coartem, Quinine and Chloroquine phosphate respectively.

The results presented in Table 2 showed the three antimalarial drugs caused significant ($p<0.05$) reduction in HbS polymerization in the following range: Coartem™ (17.05-31.07 %), Quinine (13.95-28.85 %) and Chloroquine phosphate (10.85-33.01 %).

Whereas Chloroquine phosphate caused maximum reduction in HbS polymerization at the 60th second (percentage inhibition= 33.01±0.06 %), this fell gradually so that at the 180th second it was 10.85±0.06 %. Generally, there was a decrease in capacity of the antimalarials to affect HbS polymerization between the 60th second and the 180th second.

This study showed that polymerization of HbS molecules was reduced upon introduction of each of the antimalarial drugs in a pattern similar to that caused by phenylalanine [18,7], methanol and water soluble extracts of dried fish (tilapia) and dried prawn (*Astacus red*) [19], and methanol and water soluble extracts of *Cyperus esculentus* (tiger nut sedge) [20]. These observations reflect the capability of these anti-sickling agents to bind and shield the contact points of HbS monomers required for polymerization.

These three antimalarials that we used have been implicated in alteration of certain red blood cell characteristics so as to compromise its physiochemical integrity and functionality [10-13]. Our present findings showed that they exhibit anti-polymerization properties.

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