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Research Article

Osmotic Fragility Index and Stability of Human Erythrocytes in the Presence of Four Oral Antiretroviral Drugs

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Abstract: The relative stability of human erythrocyte membrane in the presence of four oral antiretroviral drugs was investigated *in vitro*. Blood collected by venipuncture from 60 confirmed healthy humans were used for the study. The osmotic fragility of the erythrocytes was determined by measuring the release of haemoglobin from blood added to tubes containing serially diluted phosphate buffered saline (PBS, pH 7.4). Results revealed that increase of mean corpuscular fragility was not significant ($p>0.05$) in the presence of nevirapine, lamivudine and abacavir sulfate, while, efavirenz cause a significant ($p<0.05$) increase from 0.49±0.01 to 0.53±0.04mg/100ml. The erythrocytes were stable at 0.2mg/ml of nevirapine; 0.6mg/ml efavirenz; and 0.2 and 0.4mg/ml lamivudine. Results show that all drugs have the capacity to increase the permeability of the erythrocyte membrane and can compromise the integrity of the erythrocyte.
Keywords: Erythrocyte, Antiretroviral drugs, Nevirapine, Lamivudine, Abacavir sulfate, Efavirenz

INTRODUCTION

The biconcave disc shape of the erythrocyte provides a surface area to volume ratio, optimal for gas exchange and tolerates high amounts of shear force. The erythrocyte is equipped with a specialized cytoskeleton providing mechanical stability and flexibility. The membrane of erythrocyte has elastic network of skeletal protein that makes it to cope with fluid stresses [1]. When erythrocytes are placed in hypotonic solution with diminished osmolarity, they are transformed to spheres. This phenomenon has practical use in the osmotic fragility test, which determines the release of haemoglobin from erythrocyte in hypotonic saline solution. Omotic fragility index is defined the measurement of the resistance of erythrocyte to lysis by osmotic stress [2, 3]. The test is useful to determine the level of stability and functionality of plasma membrane [3], erythrocyte mean cell volume (MCV) and surface area-to-volume ratio (SAVR) and the diagnosis of hereditary spherocytosis [4-6].

Antiretroviral drugs are drugs used for the treatment of infection by retroviruses primarily HIV that causes AIDS. Different classes of antiretroviral drugs are available that acts at different stages in the progression of HIV infection [7, 8]. This study focuses on four reverse transcriptase inhibitors, namely nevirapine, efavirenz, lamivudine and abacavir sulfate.

MATERIALS AND METHODS

Antiretroviral drug, nevirapine, efavirenz, lamivudine and abacavir were obtained from the Pharmacy Department of the Federal Medical Centre, Yenagoa, Bayelsa state, Nigeria.

All other chemicals used in this experiment were products of sigma chemicals, England. Distilled water was used all through the experiment.

Experimental design

Blood samples collected from 60 healthy individuals were distributed into four groups. Each group was divided into five sub-groups. The first in each sub-group served as control while the other four groups served as tests to which four different concentrations (0.2, 0.4, 0.6 and 0.8mg/ml for nevirapine and lamivudine; and 0.6, 0.8, 1.0 and 1.2mg/ml for efavirenz and abacavir sulfate) of the drugs were incubated with the erythrocytes.

Ethics

The institutional review board of the Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria, granted approval for this study and all participants involved signed an informed consent form. This study was conducted according to the ethical principles that have their origins in the Declaration of Helsinki. Individuals drawn were from Niger Delta University, Bayelsa State, Nigeria and environs.

Collection and preparation of sample

Blood samples were collected by vein puncture and the erythrocytes were washed by methods as described by Tsakiris *et al*. [9]. Within 2 hours of collection of blood samples, portions of 1.0 ml of the samples were introduced into centrifuge test tubes that contain 3.0 ml of buffer solution pH = 7.4 (250 mm tris-HCl / 140 mm NaCl / i.0 mm $MgCl_2$ / 10 mm glucose). The separation of the erythrocytes were from plasma was carried out by centrifuging at 1200 g for 10 min, washed three times by 3 similar centrifugations with the buffer solution. The erythrocytes were re-suspended in 1.0 ml of this buffer solution. The test carried out with the washed and intact erythrocytes.

Determination of osmotic fragility

Osmotic fragility of the erythrocytes was determined by the method described by Benford and Kenned [10]. 20µl of blood was added to tubes containing 5 ml of phosphate buffered saline (pH 7.4) of serial concentrations that range from 0 - 0.85% saline. The mixtures were allowed to stand for 1 hour at room temperature (24°C). After that they were centrifuged at 1580g for 5 minutes. The supernatant was decanted and its haemoglobin content was determined at 450 nm spectrophotometrically by using distilled water as a blank. The percentage of haemolysis in each concentration of buffered saline was determined assuming 100% haemolysis in the concentration with the highest absorbance.

Stability evaluation

The corresponding concentration of PBS solution that caused 50% haemolysis of erythrocyte is known as the mean corpuscular fragility (MCF) index [3, 11]. MCF was extrapolated from the osmotic fragility curve.

The relative capacity of the four antiretroviral drugs to stabilize or destabilize the erythrocyte was evaluated using the relationship as expressed by [12].

$$\text{Relative stability (\%)} = \frac{MCF_{control} - MCF_{test}}{MCF_{control}} \times 100$$

RESULTS AND DISCUSSION

The mean corpuscular fragility (MCF) index represented and interpreted level of erythrocyte membrane stability. The Mean ± S.D. MCF values of the human erythrocyte in the presence of the four antiretroviral drugs are presented in Table 1 & 2.

Result shows that nevirapine, lamivudine and abacavir sulfate increased the fragility of the erythrocyte as can be seen in the non-significant ($p>0.05$) increase in the MCFs of the erythrocytesin (Table 1 & 2). Efavirenz on the other hand caused a significant ($p<0.05$) increase in the MCF. The destabilized of the erythrocytes were concentration dependent. The concentration 0.2 mg/ml of nevirapine did not show any capacity to destabilize the erythrocyte. The erythrocytes were stable at 0.6mg/ml efavirenz; and 0.2 and 0.4mg/ml lamivudine.

Table-1: Erythrocyte Mean Corpuscular Fragility (MCF) Index in the presence of Nevirapine and Lamivudine

	NEVIRAPINE		LAMIVUDINE	
Conc (mg/ml)	**MCF (g/100ml)**	**Relative stability (%)**	**MCF (g/100ml)**	**Relative stability (%)**
0.00	0.47 ± 0.01^{a}	100	0.48 ± 0.10^{a}	100
0.20.	0.47 ± 0.02^{a}	0	0.46 ± 0.10^{a}	4.17^{S}
0.40	0.48 ± 0.02^{a}	-2.13^{D}	$0.47\pm.0.01^{a}$	2.08^{S}
0.60	0.50 ± 0.01^{b}	-6.38^{D}	$0.51\pm.0.02^{b}$	-8.51^{D}
0.80	0.50 ± 0.00^{b}	-6.38^{D}	0.53 ± 0.02^{c}	-12.77^{D}

D = destabilized, S = stailized.Values are recorded as MEAN±SD of triplicate determinations. Means in same column with same superscript letters are not statistically different at 95% confidence limit ($p<0.05$)

Table-2: Erythrocyte Mean Corpuscular Fragility (MCF) Index in the presence of Efavirenz and Abacavir

	EFAVIRENZ		ABACAVIR	
Conc (mg/ml)	**MCF (g/100ml)**	**Relative stability (%)**	**MCF (g/100ml)**	**Relative stability (%)**
0.00	0.49 ± 0.01^{a}	100	0.48 ± 0.00^{a}	100
0.60	0.47 ± 0.01^{b}	4.08^{S}	0.49 ± 0.05^{a}	-2.08^{D}
0.80	0.52 ± 0.03^{c}	-6.12^{D}	0.51 ± 0.05^{a}	-6.25^{D}
1.00	0.52 ± 0.05^{c}	-6.12^{D}	0.52 ± 0.03^{b}	-8.33^{D}
1.20	0.53 ± 0.04^{c}	-8.16^{D}	0.53 ± 0.05^{b}	-10.42^{D}

D = destabilized, S = stailized.Values are recorded as MEAN±SD of triplicate determinations. Means in same column with same superscript letters are not statistically different at 95% confidence limit ($p<0.05$)

From the present study, all four drugs tend to increase the permeability of the erythrocyte membrane, as can be seen in the increases in the mean corpuscular fragility. The mean corpuscular fragility (MCF) is the concentration of PBS solution that caused 50% haemolysis of the erythrocyte. All drugs may be involved in the modification of the physical condition of the erythrocyte membrane, which resulted in change in the permeability of the membrane.

Study of Rabini *et al*. [13] had reported that the increase of water into the cell leads to an increase in hydrostatic pressure on the inner membrane, ultimately ending in haemolysis. It has been reported that erythrocyte fragility has been found to increase in patients with haemolytic anaemia [14].

The ability of the drugs to stabilize or destabilize the human erythrocyte membrane revealed that all drugs have varying capacity to destabilize the human erythrocyte, with abacavir sulfate having the highest and lamivudine having the least.

The destabilization of the erythrocytes by these reverse transcriptase inhibitor class of antiretroviral drugs may be because of the production and accumulation of reactive oxygen species that likely overwhelm the capacity of the antioxidant defense in order to maintain and sustain membrane integrity of the erythrocytes [6]. Reactive oxygen species are generated as by-products of oxidative metabolism particularly in mitochondria of aerobic cells [15] as well as in erythrocyte corresponding to spontaneous oxidation of hemoglobin to methemoglobin [16]. Extensive lipid peroxidation in biological membranes leads to loss of fluidity, decrease in membrane potential, increased permeability to ions and eventual rupture that leads to release of cell and organelle contents [17]. These antiretroviral drugs may induce oxidative stress that leads to increased lipid peroxidation [18].

Erythrocyte cell membrane is also prone to lipid peroxidation due to to its high content of polyunsaturated lipids [19]. It has been extensively used for the investigation the role of oxidative membrane damage in different pathological conditions [20, 21].

CONCLUSION

The findings of the present study suggest that these antiretroviral drugs have deleterious effect on the erythrocyte and are thus agents of membrane destabilization.

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