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Research Article

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Catalase Activity and Stability of Rat Erythrocytes in the Presence of Four Oral Antiretroviral Drugs

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Abstract This research investigates the effects of four oral antiretroviral drugs on erythrocyte catalase activity and the relative stability of the erythrocyte membrane in wistar rat. Sixty (60) rats with average body weight 100 grams were allowed to acclimatize for two weeks and were distributed into four (4) groups. Blood samples were collected from the experimental animals by cardiac puncture. Catalase activity was determined spectrophotometrically, allowing the enzyme to split H_2O_2 for different periods of time. 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 catalase activity decreases significantly ($p<0.05$) in the presence of lamivudine (from 17.13±3.01to 16.40±2.04) efavirenz (from 17.05±1.18 to 16.41±1.11) abacavir sulfate (from 16.36±0.28 to 15.60±2.18); nevirapine caused non-significant ($p>0.05$) decrease. All drugs investigated increased the mean corpuscular fragility of rat erythrocyte membrane. Efavirenz and abacavir caused significant ($p<0.05$) increases from 0.34±0.03 to 0.543±0.10 and 0.34±0.00 to 0.45±0.05 respectively, while the increases from 0.33±0.0 to0.40±0.02 and from 0.34±0.06 to 0.42±0.08 respectively in the presence of nevirapine and lamivudine were not significant ($p>0.05$). All the drugs investigated destabilized the rat erythrocyte. All drugs investigated may reduce the ability of the erythrocyte to withstand oxidative stress, and they have the capacity to increase the permeability of the erythrocyte membrane and can compromise the integrity and functionality of the erythrocyte.

Keywords Erythrocyte, Catalase, Osmotic fragility, Antiretroviral drugs, Nevirapine, Lamivudine, Abacavir sulfate, Efavirenz

Introduction

The biconcave disc shape of the erythrocyte provides a surface area to volume ratio that is optimal for gas exchange and tolerates high amounts of shear force. The erythrocyte is equipped with a specialized cytoskeleton which provides mechanical stability and flexibility. The erythrocyte membrane has elastic network of skeletal protein, which makes it to cope with fluid stresses [1]. When erythrocytes are placed in hypotonic solution in which osmolarity is diminished they are transformed to spheres. This phenomenon is put into practical use in the osmotic fragility test, which determines the release of haemoglobin from erythrocyte in hypotonic saline solution. Thus osmotic fragility index as defined by Oyewale and Ajibade, [2] is a measure of the resistance of erythrocyte to lysis by osmotic stress. The test is generally useful to ascertain the level of stability and functionality of plasma membrane [3], erythrocyte mean cell volume (MCV) and surface area-to-volume ratio (SAVR) and diagnosis of hereditary spherocytosis [4-5].

Catalase (E C. 1.11.1.6) is primarily an intracellular enzyme; itshighest concentrations in mammals are found in erythrocytes and liver and occasionally in the kidney [6]. It plays very important role in maintenance of human body ambiance mainly through regulation of hydrogen peroxide metabolism [7]. For instance, in red blood cells, catalase prevents the accumulation of hydrogen peroxide (H_2O_2) formed during oxygen transport [8]. It catalyzes the dissociation of hydrogen peroxide directly into H_2O and O_2. The altered catalase activity has been observed in number of disease conditions [9-10].

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 [11]. This study focuses on four reverse transcriptase inhibitors, namely nevirapine, efavirenz, lamivudine and abacavir sulfate. This research investigates the effects of four oral antiretroviral drugs on the erythrocyte catalase activity and fragility of rat erythrocyte.

Materials and methods

Antiretroviral drug 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: Sixty rats with average body weight 100 grams were allowed to acclimatize for two weeks and were distributed into four groups. Each group was divided into five subgroups. The first in each subgroup served as control while the other four groups served as tests to which four different dosages: 0.2, 0.4, 0.6 and 0.8mg/100g body weight (for nevirapine and lamivudine); and 0.6, 0.8, 1.0and 1.2mg/100g body weight (for efavirenz and abacavir sulfate) of the drugs were administered to the rats by intubation. Blood samples were collected by cardiac punction each week for analysis.

Determination of Osmotic Fragility: Osmotic fragility of the erythrocytes was determined by the method described by Benford and Kenned [12]. 20 lμ of blood was added to tubes containing 5 ml of phosphate buffered saline (pH 7.4) of serial concentrations ranging from 0 - 0.85% saline. The mixtures were allowed to stand for 60 minutes at room temperature (24°C) and then centrifuged (B. Bran Scientific and instrument company, England) at 1580 cpm for 5 minutes. The supernatant was decanted and its haemoglobin was determined spectrophotometrically at 540 nm using distilled water as a blank. The percentage of haemolysis in each concentration of buffered saline was calculated 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. 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 byParpart*et al.,* [13-14].

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

Catalase Activity

Catalase activity was determined according to the method described by Sinha [15]. It involves the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H_2O_2, Exactly 1ml of properly diluted haemolysate was added to a flat bottom flask containing 4ml of H_2O_2 solution and 5ml of phosphate buffer; pH 7.0. Exactly 1ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent at 60 seconds intervals and optical density measured with a spectrophotometer (Jenway, Model 6400) at 570nm.

Results and Discussion

The mean±SD of catalase activity, the mean corpuscular fragility and stability of rat erythrocytes in the presence of the four oral antiretroviral drugs investigated are reported in tables 1, 2 and 3. The activity of catalase *in vivo* decreased in a dose-dependent manner in the presence of nevirapine (table 1), though the group administered 0.8 mg/100g body weight decreased significantly ($p<0.05$) when compared to the control.

Lamivudine, efavirenz and abacair sulfate caused significant ($p< 0.05$) dose dependent decreased in catalase activity as presented in table 1.

The present study was designed to investigate the effect of four oral antiretroviral drugs on erythrocyte catalase activity and stability of the erythrocyte membrane when administered in the absence of the Human Immunodeficiency Virus (HIV).

It is generally accepted that hydrogen peroxide can be detoxified by catalase, which removes it whenhydrogen peroxide present at high concentration and glutathione peroxidase, which destroys it when it present at a steady state [16]. Erythrocyte catalase is the main regulator of hydrogen peroxide metabolism, thus any inherited or acquired deficiency in erythrocyte catalase may increase hydrogen peroxide concentration with both physiologic and toxic effects [17-18]. Snyder *et al*.,[19] have demonstrated that treatment of erythrocytes with H_2O_2 has an effect on the

lateral organization of membrane lipids even though the transbilayer lipid distribution remains unaffected. They concluded that the H_2O_2-induced changes in lipid packing were due to altered membrane protein-lipid interactions. Such modifications are potentially very important, since the membrane lipid microdomain structure has been linked to membrane function in erythrocytes [20].

The fact that catalase activity in this research decreased in the presence of the drugs investigated indicate that the drugs may induce oxidative stress likely through lipid peroxidation. This conclusion is logical due to the fact that the erythrocyte membrane is made more fragile by the drugs in this investigation.Kayode and Kayode [21] reported decreases in the catalase activities in the kidney in the presence of lamivudine, efavirenz and abacaiir sulfate suggesting that the organ may not be able to protect itself from cellular damage (especially those caused by hydrogen peroxide) in the case of hydrogen peroxide and hydroxyl radical–induced oxidative stress.

Table 1: Effects of Different Concentrations of nevirapine lamivudine, efavirenz and abacavir on Rat erythrocyte catalase activity.

Dosage (mg/100g body weight)	Catalase activity (units/gHb)		Dosage (mg/100g body weight)	Catalase activity (units/gHb)	
	Nevirapine	Lamivudine		Efavirenz	Abacavir
0.00	16.48±1.02^{a}	17.13±3.01^{a}	0.00	17.05±1.18^{a}	16.36±0.28^{a}
0.20	16.21±0.25^{a}	16.64±3.05^{b}	0.60	16.92±2.01^{b}	15.65±2.15^{b}
0.40	16.01±0.50^{a}	16.80±3.19^{a}	0.90	16.84±1.04^{b}	15.44±0.19^{b}
0.60	15.90±0.11^{a}	16.55±2.02^{b}	1.00	16.68±1.25^{b}	15.28±1.14^{c}
0.80	15.73±0.30^{b}	16.40±2.04^{b}	1.20	16.41±1.11^{b}	15.60±2.18^{b}

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

Table 2: Effects of Different Concentrations of Nevirapineand Lamivudine, onRat Erythrocyte Mean Corpuscular Fragility (MCF) Index.

	NEVIRAPINE		LAMIVUDINE	
Conc (mg/ml)	MCF (g/100ml)	Relative stability (%)	MCF (g/100ml)	Relative stability (%)
0.00	0.33±0.00^{a}	100	0.34±0.06^{a}	100
0.20	0.36±0.03^{a}	-9.09^{D}	0.36±0.05^{a}	-5.88^{D}
0.40	0.37±0.06^{a}	-12.12^{D}	0.38±.0.05^{a}	-11.76^{D}
0.60	0.41±0.01^{b}	-24.24^{D}	0.41±.0.02^{b}	-20.58^{D}
0.80	0.40±0.02^{b}	-21.218^{D}	0.42±0.08^{b}	-23.53^{D}

D = destabilized. 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 3: Effects of Different Concentrations of Efavirenz and Abacavir sulfate onRat Erythrocyte Mean Corpuscular Fragility (MCF) Index.

	EFAVIRENZ		ABACAVIR	
Conc (mg/ml)	MCF (g/100ml)	Relative stability (%)	MCF (g/100ml)	Relative stability (%)
0.00	0.34±0.03^{a}	100	0.34±0.00^{a}	100
0.60	0.35±0.03^{a}	-2.94^{D}	0.35±0.05^{a}	-2.94^{D}
0.80	0.37±0.01^{b}	-8.82^{D}	0.39±0.05^{b}	-14.71^{D}
1.00	0.38±0.06^{b}	-11.76^{D}	0.40±0.03^{b}	-17.65^{D}
1.20	0.43±0.10^{c}	-26.47^{D}	0.45±0.05^{c}	-32.35^{D}

D = destabilized. 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 ($p<0.05$)

The mean corpuscular fragility (MCF) index represented and interpreted level of erythrocyte membrane stability. The mean corpuscular fragility (MCF) is the concentration of PBS solution that caused 50% haemolysis of the erythrocyte.

Results of the present investigation showed that all the drugs investigated increased the MCF of the erythrocyte membrane. The increases due to nevirapine and lamivudine were not significant ($p>0.05$). However abacavir sulfate

and efavirenz increased the fragility of the erythrocyte significantly ($p<0.05$) as can be seen in tables 2 and 3. The destabilizations of the erythrocytes due to all four drugs investigated were dose dependent.

All the drugs tend to increase the permeability of rat erythrocyte membrane, as can be seen in the increases in the MCF (tables 2 and 3). The drugs investigated increased the permeability of the membrane, thus increasing its fragility. Wodu *et al*., [22] reported that nevirapine, efavirenz; lamivudine and abacavir sulfate may be involved in the modification of the physical condition of the erythrocyte membrane, which resulted in change in the permeability of the membrane. Thus the drugs investigated may be involved in the modification of the physical condition of the erythrocyte membrane. Increase of water into the cell leads to an increase in hydrostatic pressure on the inner membrane that ultimately end in haemolysis [23]. Kuchel *et al.,* [24] reported that erythrocyte fragility is increased n patients with haemolyticanaemia. The ability of the drugs to stabilize or destabilize the rat erythrocyte membrane revealed that all drugs investigated have varying capacity to destabilize the erythrocyte, thus compromising the integrity of its membrane. The destabilization is in the order abacavir sulfate>lamivudine>efavirenz>nevirapine. It was reported by Wodu *et al*., [22] that human erythrocytes *in vitro* were stable at certain concentrations of nevirapine, efavirenz; and lamivudine. However they were destabilized by abacavir sulfate

The destabilization of the erythrocytes by these reverse transcriptase inhibitor class antiretroviral drugs may be due to the production and accumulation of reactive oxygen species which likely overwhelm the capacity of the antioxidant defense to maintain and sustain membrane integrity of the erythrocytes. Reactive oxygen species are generated as by-products of oxidative metabolism particularly in mitochondria of aerobic cells as well as in erythrocyte corresponding to spontaneous oxidation of hemoglobin to methemoglobin [25]. Gutteridge *et al*.,[26] reported that extensive lipid peroxidation in biological membranes cause loss of fluidity, decrease in membrane potential, increased permeability to ions and eventual rupture leading to release of cell and organelle contents. Likely these antiretroviral drugs may induce oxidative stress leading to increased lipid peroxidation which may increase the permeability of the erythrocyte membrane to ions and eventual lead to rupture and release of cell and organelle contents. Erythrocyte cell membrane is also prone to lipid peroxidation owing to its high content of polyunsaturated lipids [27] and it has been extensively used to investigate the role of oxidative membrane damage in various pathological conditions [28].

Studies have shown that HIV infection is accompanied with a progression in the oxidative stress process [29-31], thus any antiretroviral drug whose administration potentially induces oxidative stress in non-infected animals would only increase the oxidative damage in HIV- infected animals. Therefore, the findings of the presentstudy suggest that these antiretroviral drugs have deleterious effect on the erythrocyte and thus may compromise the integrity and functionality of rat erythrocyte membrane.

References

1. Mohandas, N. and Gallagher, P. G. (2008). Red cell membrane: past present and future. Blood 112 (10) 3939-3948
2. Oyewale, J. O and Ajibade, H. A. (1990). The osmotic fragility of erythrocyte of turkey. Vet. Archi. 60:91-100.
3. Krogmeier, D. E., Mao, I. L. and Bergen, W. G. (1993) Genetic and non-genetic effects of erythrocyte osmotic fragility in lactating Holstein cow and its association with yield traits. Journal of Diary Science. 76: 1994-2000.
4. Alderich, K. J., Saunders, D. K., Sievert, L. M, and Sievert, Y. G. (2006) Comparison of erythrocyte osmotic fragility among amphibians, reptiles, birds and Mammals. Transactions of the Kansas Academy of Sci. 109(3): 149 – 158.
5. Kumar, S. (2002). An analogy for explaining erythrocyte fragility: concepts made easy. Advan. Physiol. Educ. 26: 134 – 135.
6. Deisseroth, A., D' ounce, A. L. (1970) Catalase: physical and chemical properties, mechanism of catalysis, and physiological role. Physiol. Rev. 50, 319-375.
7. Goth, L, Rass, P and Pay, A. (2004) Catalase enzyme mutations and their association with diseases. Mol. Diagn. 8:141-9.
8. Foulkes, E. C, and Lemberg, R. (1949) The formation of choleglobin and the role of catalase in the erythrocyte. Proc R SocLond B Biol Sci. 136:435–48.
9. Koepke, J. I., Wood, C. S., Terlecky, L. J., Walton, P. A. and Terlecky, S. R. (2008) Progeric effects of catalase inactivation in human cells. Toxicology and Applied Pharmacology. 232: 99–108.

10. Goyal, M. M. and Basak, A. (2010) Human catalase: looking for complete identity. Protein Cell; 1: 888–897.
11. Who (2003) Scaling up retroviral therapy in resource limited setting. World Health Organisation, Rome.
12. Benford, M, and Kennedy, H. E. (2007). The effect of EDTA, heparin and storage on the erythrocyte osmotic fragility, plasma osmolality and haematocrit of adult ostriches (Struthiocamelus). VetenaryArhive77(5), 427-434,
13. Parpart, A. K., Lorenz, P. B., Parpart, E. R., Gregg, J. R and Chase, A. M. (1947). The osmotic resistance (fragility) of human red cells. Journal of clinical investigations. 26, 636-638.
14. Chikezie, P. C (2007) Osmotic fragility index of HbAA erythrocytes in the presence of aqueous extracts of three medicinal plants (Aframomummelegueta, Garina kola and CymbopogonCitracus). Global J Pure Applied Sci. 13(4): 496-499.
15. Sinha, A. K. (1972) Colorimetric assay of catalase Anal. Biochem. 47, 389-394
16. Casado, A., Dela, T. R., Lopez-Fernandez, M., Carrascosa, D., Casado, M. C. and Ramirez, V. (2011) Superoxide dismutase and catalase blood vessels in patients with malignant diseases. Cancer Lett. 91, 19-23.
17. Sebastian, M., Hans-Dieter, .R. and Wolfgang, S. (1997) Direct Evidence for Catalase as the Predominant H_2O_2 -Removing Enzyme in Human Erythrocytes. Blood, 90 (12) 4973-4978
18. Tarnai, I., Csordás, M., Sükei, E., Shemirani, A. H., Káplár, M., and Góth, L. (2007) Effect of C111T polymorphism in exon 9 of the catalase gene on blood catalase activity in different types of diabetes mellitus. Free Radic. Res. 41, 806-811
19. Snyder, L. M., Fortier, N. L., and Mohandas. N. (1985). Effect of hydrogen peroxide exposure on normal human erythrocyte deformability, morphology, surface characteristics, and spectrin-hemoglobin cross-linking. J. Clin. Invest. 76:1971–1977.
20. Salzer, U., and R. Prohaska. 2001. Stomatin, flotillin-1, and flotillin-2 are major integral proteins of erythrocyte lipid rafts. Blood. 97:1141– 1143.
21. Kayode, A. A. A. and Kayode, O. T. (2015)Effect of Selected Antiretroviral Drugs on Malondialdehyde (MDA) and Catalase Levels in Healthy Rat Tissues. SMU Medical Journal, 2 (1) 101-100
22. Wodu, E., Uwakwe, A. A., Monanu, M. O. (2015) Osmotic fragility index and stability of human erythrocytes in the presence of four oral antiretroviral drugs. Scholars Journal of Applied Medical Sciences. 3(2E):884-887
23. Rabini, R. A., Petruzzi, E., Straffolani, R., Tesel, M., Fumelli, P., Pazzagli, M. and Mazzanti, L. (1997) Diabetes mellitus and subjects aging: a study on ATP content and ATP related enzyme activities in human erythrocytes. European Journal of clinical Investigation. 27:327-332.
24. Kuchel, P. W., Coy, A. and Stibs, P. (1997) NMR diffusion-diffraction of water revealing alignment of erythrocytes in a magnetic field and their dimension and membrane transport characteristics. Magnetic research and therapy. 7(31):31
25. Seven, A., Seymen, O., Hatemi, S., Hatemi, H., Yigit, G. and Candan, G. (1996) Antioxidant status in experimental hyperthyroidism: effect of vitamin C supplementation.Clinchimacta; 256:65-74.
26. Gutteridge, J. M. C. (1995) Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clinchem; 41:1819-1828.
27. Yalcin, A. S., Kılınc, A., Gulcan, G., Sabuncu, N., Emerk, K. (1989) Cumenehydroperoxide induced in vitro peroxidative changes in human red blood cell membranes. Biochem Arch; 5:147-52.
28. Toker, K., Ozer, N. k., Yalcin, A. S., Tuzuner, S., Gogus, F. Y. and Emerk, K. (1990). Effect of chronic halothane exposure on lipid peroxidation, osmotic fragility and morphology of rat erythrocytes. J ApplToxicol; 10: 407-9.
29. Papadopulos-Eleopulos, E, Hedland-Thomas, B, and Causer, D, (1991) Changes in thiols and glutamate as consequences of simian immunodeficiency virus infection, Lancet; 11, 1013
30. Allard, J. P., Aghdassi, E., Chau, J., Tam, C., Kovacs, C. M., Salit, I. E. and Wallmsley S. L. (1998) Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects, AIDS. 12, 1653-1659.
31. Ngondi, J. L, , Julius, O., David, M. F., Lucein, H. E. and Mbanya, D. (2006) The effect of different combination therapies on oxidative stress markers in HIV infected patients in Cameroon, AIDS Research and Therapy., 3:19.