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Original Research Article

Impact of Four Oral Antiretroviral Drugs on Human Erythrocyte Catalase Activity (*In vitro*)

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Abstract: Catalases have powerful antioxidant support in the erythrocyte, thus the impact of four oral antiretroviral drugs on human erythrocytes catalase activity were investigated *in vitro*. Spectrophotometric method was used to determine the activity of erythrocyte catalase. The results revealed that catalase activity decreased in a drug dependent fashion. The decrease from 55.80±10.05 to 50.05±11.35 units/gHb in the presence of nevirapine was significant ($p<0.05$), while the decreases due to lamivudine (54.75±10.03 to 53.22±9.30), efavirenz (56.75±5.23 to 55.15±11.35[a]) and abacavir sulfate (55.75±13.30 to 54.96±10.15) were not significant ($p>0.05$). The decrease in the activity of catalase in the erythrocyte suggest that these drugs may cause imbalance in the erythrocyte's antioxidant defence system.
Keywords: Catalase, erythrocyte, nevirapine, lamivudine, efavirenz, abacavir sulfate.

INTRODUCTION

It is a known fact that hydrogen peroxide and some other derivatives of oxygen, are being recognized as toxic intermediates in a wide variety of human disorders. Research suggests that at low concentration, hydrogen peroxide acts as a cellular messenger in insulin signaling; whereas at high concentration, it is toxic, particularly in pancreatic cells which are catalase poor (Tirosh *et al*., 2005).

Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. Catalase is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. (Chelikani *et al.*, 2004) It is a very important enzyme in reproductive reactions.

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 (Tarnai *et al*., 2007). Catalase and glutathione peroxidase/ reductase system are known to clear the body of hydrogen peroxide. However, catalase is implicated as the major decomposer of hydrogen peroxide in the body. Gaetani *et al*., (1996) reported that the increased dependence on the glutathione peroxidase/reductase mechanism did not occur until more than 98% of the catalase had been inactivated.

Catalases have powerful antioxidant support; they are perhaps the single most efficient enzyme found in the cells of the human body, and has been shown to create a speedy reaction against hydrogen peroxide free radicals, turning them into water and oxygen (Hengge, 1999).

Erythrocytes acts as drug carriers in an organism and hence comes in direct contact with the drugs which in turn interact with the constituents of the erythrocytes such as diverse enzymes. The aim of this research is to investigate the impact of four (4) oral antiretroviral drugs on erythrocyte catalase activity.

MATERIALS AND METHODS

The drugs implicated for this investigation were obtained from the Pharmacy Department of the Federal Medical Centre, Bayelsa State. Nigeria. All reagents used were of analytical grade and do not need any further purification.

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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 of the drugs were incubated with the erythrocytes. Concentrations of nevirapine and lamivudine incubated with the erythrocytes were 0.2, 0.4, 0.6 and 0.8 mg / ml, while efavirenz and abacavir sulfate were 0.6, 0.8, 1.0 and 1.2 mg / ml.

Sample collection and preparation: Blood samples obtained by venipuncture were stored in EDTA anticoagulant tubes. The erythrocytes were washed by the method described by Tsakiris *et al.,* (2005). Exactly 1.0 ml portion of the sample was introduced into centrifuge tubes containing 3.0 ml of 250 mM tris (hydroxyl methyl) amino ethane–HCl buffer solution pH 7.4, containing 140 mM NaCl, 1.0 mM $MgCl_2$, and 10 mM glucose. The erythrocytes were separated from plasma by centrifugation at 1200g for 10 minutes, washed three times by three similar centrifugations with the buffer solution. The erythrocytes were resuspended in 1.0 ml of this buffer and stored at $4^{O}C$. The washed erythrocytes were lysed by freezing and thawing as described by Galbraith and Watts (1980). The erythrocyte haemolysate was used to determine the activity of the erythrocyte catalase.

Catalase Activity

Catalase activity was determined according to the method described by Sinha, (1971).

Standard curve: Hydrogen peroxide, ranging from 10 to 100 µmoles were added to 2ml of dichromate/acetic acid. The mixture was heated for 10 minutes in a boiling water bath. After cooling at room temperature, the volume of the reaction mixture was made to 3ml with distilled water and the optical density measured with a spectrophotometer at 570nm. The concentrations of the standards were plotted against absorbance.

Assay: 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.

Catalase activity was calculated using the relationship below:

H_2O_2 consumed=800µmoles– H_2O_2 remaining

Thus,

$$\text{Catalase activity (units / g Hb)} = \frac{H_2O_2 \text{ consumed}}{\text{Hb (g / dl)}}$$

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.

Statistical Analysis

The SPSS statistical analysis system was used for analysis of the data. All the assays were made in triplicate determinations. The data collected were presented as means ± standard deviations. The statistical significance was assessed by one-way analysis of variance. Significant differences ($P \leq 0.05$) among treatments were detected using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Oxidative stress results in the production of free radicals (hydrogen peroxide inclusive); and catalase decomposes this toxic hydrogen peroxide to less toxic substances. The importance of the enzyme catalase in living cells has been well known for many years. Catalase is one of the biological antioxidant that prevent the uncontrolled formation of free radicals and activated oxygen species or inhibit their reactions with biological structures. The importance of erythrocyte catalase activity cannot be over emphasize. Tarnai *et al*., (2007) reported that the main regulator of hydrogen peroxide metabolism is erythrocyte catalase, as such any deficiency in erythrocyte catalase may accumulate hydrogen peroxide concentration with both physiologic and toxic effects.

Table1: Human erythrocyte catalase activity in the presence of four oral antiretroviral drugs.

CONC (mg/ml)	Catalase activity (units/gHb)		CONC (mg/ml)	Catalase activity (units/gHb)	
	Nevirapine	Lamivudine		Efavirenz	Abacavir S
0.00	55.80±10.05[a]	54.75±10.03[a]	0.00	56.75±5.40[a]	55.75±13.30[a]
0.20	53.12±6.01[b]	53.90.±6.01[a]	0.60	56.12.±8.05[a]	56.64±11.12[a]
0.40	52.30±5.20[b]	53.43±5.20[a]	0.80	56.23±5.43[a]	55.12±7.23[a]
0.60	52.27±9.35[b]	53.22±9.30[a]	1.00	55.82±9.10[a]	55.03±8.40[a]
0.80	50.05±11.35[c]	53.14±12.35[a]	1.20	55.15±11.35[a]	54.96±10.15[a]

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

Results in the present study revealed that the decreases in catalase activity in the presence of efavirenz, abacavir sulfate and lamivudine were not significant ($p>0.05$) as shown in Table 1, whereas the decrease due to nevirapine was significant ($p< 0.05$).

In agreement with the present findings Adjene *et al.*, (2011) reported that efavirenz decrease catalase actively in superior colliculus The superior colliculus and lateral geniculate body in the brain constitutes the intracranial visual relay centres.

The treatment of erythrocytes with H_2O_2 has an effect on the lateral organization of membrane lipids even though the trans-bilayer lipid distribution remains unaffected; H_2O_2-induced changes in lipid packing were due to altered membrane protein-lipid interactions. (Snyder *et al*., 1985). Earlier studies by Wodu *et al*., (2016) had revealed that efavirenz, lamivudine, nevirapine and abacavir sulfate decreased erythrocyte catalase activity in wistar rats thus indicating that these drugs may induce lipid peroxidation. This they infer made the erythrocyte membrane more fragile. Kayode and Kayode (2015) reported decreases in the catalase activities in the kidney in the presence of lamivudine, efavirenz and abacavir 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.

It is important to consider the effects of these drugs on the erythrocyte since Salzer, and Prohaska, (2001) reported that such modifications are potentially very important, because the membrane lipid microdomain structure has been linked to membrane function in erythrocytes.

CONCLUSION

Antiretroviral therapy has been of tremendous benefit to humans living with the HIV virus. However, as these drugs come in contact with the erythrocyte, they modify its makeup. Thus it could be concluded that the decrease in erythrocyte catalase activity observed was highest in the presence of nevirapine, which implies that it may have more potential to reduce the erythrocyte's antioxidant defense system.

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