**Invitro Effect of Papaverine on Lipid Peroxidation in Sickle Cell Anaemia**

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**Abstract**—Disruption in erythrocyte membrane Ca$^{2+}$ATPase activity in sickle cell anaemia is linked to increase in lipid peroxidation product, malondialdehyde (MDA). Some drugs are known to inhibit the Ca$^{2+}$ATPases therefore, the study was undertaken to evaluate the effect of Papaverine a known Ca$^{2+}$ channel inhibitor on the level of MDA and plasma calcium in sickle cell anaemia. The MDA concentrations of the sickle cell homozygous group before and after incubation (17.00±0.30nmol/l and 11.20±0.39nmol/l respectively) were significantly higher (p<0.05) when compared to groups AA (13.30±0.32nmol/l and 6.66±0.42nmol/l) and SS (11.20±0.39nmol/l and 6.80±0.44nmol/l). MDA concentration decreased significantly in all the groups after incubation with Papaverine. The SS group showed the least inhibition (35.29±0.36%) with Papaverine which is significant (p<0.05) when compared to groups AA (49.92±0.38%) and AS (39.29±0.40%). Plasma calcium concentrations before and after incubation with Papaverine were significantly higher (p<0.05) in SS group (12.8±0.42mg/dl and 9.00±0.14mg/dl respectively) when compared to groups AA (11.45±0.49mg/dl and7.14±0.21mg/dl) and AS (8.25±0.35mg/dl and 6.15±0.07mg/dl).

**Index Term**—Papaverine, erythrocyte, lipid peroxidation, malondialdehyde, calcium, ATPases

I. INTRODUCTION

Sickle cell anemia is an inherited disorder of hemoglobin synthesis, characterized by life-long severe hemolytic anemia and vasoocclusive crisis. It results from a point mutation in the genetic code such that glutamic acid is replaced by valine at 6th position of β-globin chain of hemoglobin (Hb) [1]. This substitution transforms normal adult hemoglobin (HbA) into sickle hemoglobin (HbS). In a low oxygen tension environment, the replaced valine can bind to a complementary hydrophobic site on beta subunit of another haemoglobin tetramer in a polymerization process that leads to the sickling of the red blood cells (RBCs). Polymerization of deoxygenated sickle hemoglobin (HbS) tetramers is central to the process of vasoocclusion [2]. Lipid peroxidation due to oxidative stress plays a major role in the pathophysiology of Sickle cell anaemia [1]. This leads to significant increase in plasma malondialdehyde (MDA) level in sickle cell anaemia [3][4][5]. Accumulation of MDA disrupts the structural pattern of human erythrocyte membrane phospholipid bilayer leading to formation of irreversible sickle cells and erythropagocytosis [6][7]. RBCs are particularly susceptible to peroxidative damage because they contain hemoglobin, one of the most powerful catalysts for initiation of peroxidative reaction [8]. However, under ambient oxygen tensions, sickle cells spontaneously generate superoxide radical O$_2^•$-, H$_2$O$_2$ and hydroxyl radical OH$^•$ approximately two times more, when compared to normal RBCs [5][9]. These reactive species are capable of damaging diverse bio-molecules and cell structures [10] in which lipids are probably the most susceptible [11]. These radical species can initiate lipid peroxidation on the erythrocyte membrane. Furthermore, accumulation of hydrogen peroxide (H$_2$O$_2$) decreases the half life of erythrocytes by increasing oxidation of polyunsaturated fatty acids of membranes constituents [12] and can oxidize haemoglobin to methaemoglobin [13][14][15]. Sickle cell anaemia is a hereditary disorder with higher potential for oxidative damage due to chronic redox imbalance in red cells that often results in clinical manifestation of mild to severe hemolysis in patients [16]. The membranes of sickle cells possess higher levels of denatured hemoglobins, hemin, and nonheme iron [17]. It is speculated that the nonheme iron component is free iron bound to the membrane, possibly chelated to anionic phospholipids. These iron compounds enhance the oxidative stress in sickle cell membranes, and this has been proposed as the cause for many of the changes that occur in sickle cell anemia, including dehydration and adhesivity [5]. The importance of membrane damage as a secondary factor contributing towards the pathophysiology of sickle cell anaemia is suggested by the retention of the sickled morphology in irreversibly sickled cell (ISCs) even when the haemoglobin is completely reoxygenated [18]. The altered characteristics of sickle cell membranes compared with those of normal erythrocytes include: increased haemoglobin binding to the membrane, decreased deformability, and elevated intracellular calcium levels, cellular dehydration, decreased lipid fluidity and altered membrane surface charge, defective cytoskeleton and altered phospholipid asymmetry [18]. These changes appear to be associated with the formation of dense irreversibly sickled cells, the hallmark of this disease [5]. Formation of these dense cells may be caused by a transient increase of intracellular calcium since alterations in Ca$^{2+}$ and Mg$^{2+}$-ATPase activities have been shown in sickle cell membranes, particularly the portion stimulated by calmodulin [19]. The high ATPase activities in HbS cells require the utilization of ATP leading to rapid depletion of energy stores and low ATP levels. This implies...
increases in the rate of oxygenation/deoxygenation and sickling or production of dehydrated erythrocyte [20][21].

Papaverine is a vasodilator opium alkaloid. It acts as a calcium channel inhibitor that acts in a concentration dependent manner to inhibit voltage-dependent Ca$^{2+}$ inward current in L-type Ca$^{2+}$ channel thereby providing a realistic antisickling strategy [22]. Inhibition by Papaverine of abnormal calcium influx into the sickle cell due to disruption of erythrocyte membrane integrity by lipid peroxidation can provide a management strategy for sickle cell anaemia. One of the toxic end products of lipid peroxidation is malondialdehyde (MDA) [13]. Sickle erythrocytes contain increased amount of MDA; and an evidence of abnormal amino group cross-linking by MDA has been demonstrated in lipid extract of sickle erythrocyte membrane preparations [4]. This study therefore is aimed at ascertaining the effect of Papaverine, a Ca$^{2+}$ channel inhibitor, on the lipid peroxidation of sickle cell by monitoring the levels of MDA.

II. MATERIALS AND METHOD

A quantity, 5 ml of blood was aseptically collected from the antecubital vein in EDTA (0.47 mol/L K$_3$-EDTA) blood container. The blood was centrifuged at 1000g for 10min to separate the plasma. Plasma was used to analyze lipid peroxidation product malondialdehyde (MDA). Blood samples from three different groups of donors were used in this study. The haemoglobin genotypes were confirmed to be AA, AS and SS using the electrophoretic tank method with a standard AS blood sample as the control. The donors were age and sex-matched hematologically normal subjects. All the participants were included in the study after screening for haematological parameters. Subjects were excluded having past 3 month history of anaemic condition and serious illness.

1. Determination of Haematological Parameters and Plasma Calcium level

Erythrocyte Sedimentation Rate was determined using the method of Westergreen. Micromethod with the aid of a microhaematocrit tube as described by Dacie and Lewis (1994) [23] was used to determine the Packed Cell Volume. Haemoglobin level was determined using the Cyanide Haemoglobin Method described by Cheesbrough (2000) [24]. Red blood cell and platelet counts were ascertained using standard methods. A Randox calcium kit was used to measure plasma calcium level.

2. Determination of Lipid Peroxidation Products

The extent of lipid peroxidation of was determined using the thiobarbituric acid reactive substances (TBARS) assay method described by Wallin et al., 1993 [25]. The TBARS are expressed in terms of MDA equivalents. Tubes containing the blood plasma were added with 0.55ml 1% w/v TBA and 0.4% w/v NaOH solution were heated at 90°C in a water bath for 40 minutes and then cooled in ice. To reduce turbidity, 0.1ml of 20% w/v sodium dodecyl sulphate was added and mixed. After centrifugation at 10000rpm for 5 minutes, the absorbance of the clear supernatant was read at 532nm. TBARS were quantified using a standard curve of MDA equivalents, generated by acid hydrolysis of 1, 1, 3, 3,-tetraethoxypropane (TEP)

3 Inhibition Experiment

A quantity, 1ml of each of the three blood samples (AA, AS, SS) in different dialysis bags were incubated in a Papaverine solution (2mg in 300ml of distilled water) at 37°C for 30 minutes. Subsequently, the surface of the bags were rinsed and transferred into an incubation buffer at 37°C. After three (3) hours lipid peroxidation product, malondialdehyde (MDA) and Calcium level were determined.

Statistics

Values are expressed as mean ± SD, significance of the mean differences between the groups was assessed by two way analysis of variance.

RESULTS

Table I

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<thead>
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<th>Parameter</th>
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<th>AS</th>
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<td>47</td>
<td>37</td>
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<td>ESR (mm/hr)</td>
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<td>40 - 52</td>
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<td>Haemoglobin (g/dl)</td>
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<td>RBC Count (/mm$^3$)</td>
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<td>Platelet Count (*/mm$^3$)</td>
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Fig. 1. Plasma Calcium Level Before and After Incubation with Papaverine
There was significant reduction in calcium levels in all groups after incubation with Papaverine. The level of Calcium in the SS group increased significantly (p<0.05) when compared to groups AA and AS before and after incubation with Papaverine.

**DISCUSSION**

In our study, the plasma MDA level is significantly elevated in sickle cell anaemia which agrees with previous findings [1][3][4]. The MDA concentrations of the SS group before and after incubation (17.00±0.30nmol/l and 11.20±0.39nmol/l respectively) are significantly higher (p<0.05) when compared to the AA (13.30±0.32nmol/l and 6.66±0.42nmol/l) and SS (11.20±0.39nmol/l and 6.80±0.44nmol/l). This is due to increased lipid peroxidation in sickle cell homozygote [1]. MDA concentration decreased significantly in all the groups after incubation with papaverine. SS group showed the least percentage of inhibition when compared to other groups.

**CONCLUSION**

Due to its effect on the erythrocyte membrane Ca\(^{2+}\)-ATPase, Papaverine, a vasodilator opium alkaloid has been shown in this study to decrease lipid peroxidation. Therefore, it may provide antisickling strategy for the management of sickle cell anaemia.

**REFERENCES**


