THE CENTRAL AFRICAN JOURNAL OF MEDICINE

Vol. 40, No. 8 August, 1994

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CENTRAL AFRICAN
JOURNAL OF MEDICINE

Vol. 40, No.8,1994

Increased susceptibility of erythrocyte membrane lipids to peroxidation in sickle cell disease

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SUMMARY

Erythrocytes from normal subjects and from cases of sickle cell disease were exposed to hydrogen peroxide and the extent of membrane lipid peroxidation studied. Significantly increased peroxidation was observed in intact erythrocytes, isolated lipids from intact erythrocytes or their ghosts compared with normal controls. However, the extent of lipid peroxidation was less for isolated lipids of intact erythrocytes or their ghosts. This difference may be due to loss of factors enhancing peroxidation in intact erythrocytes in the extraction procedure. It is likely that damage to erythrocyte membranes caused by increased susceptibility to lipid peroxidation may be a factor in some of the haematological features of sickle cell disease.

INTRODUCTION

Although sickle cell disease (SCD) is well understood as an abnormality of the haemoglobin molecule, the complete mechanism by which this defect is expressed at the clinical level is not fully understood[1,2] even though secondary alterations in red cell metabolism and membrane structure and function have been described.[3] A considerable body of evidence suggests that defective function of the sickle cell membrane may contribute to some of the manifestations of the disease. The membrane defect is demonstrated in the permanently deformed morphology of the irreversibly sickled cell, binding of haemoglobin to the erythrocyte

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membrane, easy rupturing of the sickle cell, decreased filterability of sickle erythrocytes and increased cation leak from sickle cells when they are deoxygenated.[2,4] Equally, it has been established that the circulation of membrane damaged, rigid and irregularly shaped erythrocytes resulting in vascular occlusion are involved in this disease.[2]

However, the genesis of the membrane defect remains not fully known and relatively few examinations of the factors precipitating the collapse of the membrane have been made. Increased susceptibility of the membrane to peroxidant threats may be one of those factors. It is known that the erythrocyte membrane is rich in polyunsaturated fatty acids and that these molecules are themselves highly susceptible to peroxidation.

During its life span in the circulation the erythrocyte is exposed to small amounts of hydrogen peroxide (H_2O_2) generated as a result of normal metabolic process to greater amounts resulting from interactions of certain oxidant drugs and their metabolites with oxyhaemoglobin.[5] Intracellular H_2O_2 may cause peroxidation of membrane lipids with the formation of breakdown products, including oxygen radicals and malonyldialdehyde (MDA).[6] Membrane lipid peroxidation has been recognized as a deleterious process leading to cell damage in various tissues.[7] MDA itself has been found capable of reacting with aminophospholipids and proteins of membrane to produce cross linking of membrane constituents which can cause rigidity and decreased deformability of red blood cells.[6]

The present study was undertaken to examine the susceptibility of sickle cell membrane to lipid peroxidation and the possible role of lipid peroxidation in the damage to the membrane and reduced erythrocyte survival in sickle cell disease.

MATERIALS AND METHODS

Blood was drawn from healthy adult male volunteers or sickle cell patients who were in a steady state uncomplicated by infection, clinical or haematological crises, into ETDA vials and the plasma separated. Red blood cells were routinely washed in cold normal saline and suspended in phosphate-saline medium (1 vol. of potassium phosphate buffer, 0,1 M, pH 7,3 mixed with 9 vol. of 0,15 M NaCl). Saline washed, packed red cells were lysed in four times their volume of cold distilled water.

An aliquot of the lysate was used to determine the haemoglobin (Hb) content by the cynmethaemoglobin method described by Dacie and Lewis.[8] The lipids in the remainder of the lysate were extracted according to the method of Rose and Oklander.[9] Peroxidation of intact erythrocytes, isolated lipids of intact erythrocytes or their ghosts was carried out by exposure to hydrogen peroxide which was added directly to the medium as described by Kurien and Iyer.[10]

Erythrocyte ghosts were prepared according to Dodge *et al*[11]. The extent of peroxidation of the erythrocyte and its membrane lipids was measured by estimating the MDA formed using the thiobarbituric acid (TBA) reaction described by Stocks and Dormandy.[12] Data were analyzed by using student's t-test.

RESULTS

As seen in Table I, a significantly higher level of MDA formation was observed when intact red cells from SCD patients were exposed to H_2O_2 added directly to the red cell suspension, compared to that of erythrocytes from normal subjects, ($p < 0,001$). The same was true of isolated lipids from intact red cells or their ghosts, but the amount of MDA formed was less. The formation of MDA, a secondary fragmentation product of polyunsaturated fatty acid peroxides, is a specific, sensitive and extensively used measure of lipid peroxidation.[12]

Table I: Lipid peroxidation in normal and sickle erythrocytes (n moles of MDA/g Hb).[1]

Lipid Source	n moles MDA (13)*	p
Intact red cell normal		
Normal	198,6 + 24	
Sickle cell	408,7 + 33	< 0,01
Isolated lipids from intact red cells		
Normal	78,5 + 11	
Sickle cell	145,8 + 12	< 0,01
Isolated lipids from red cell ghosts		
Normal	63,9 + 10	
Sickle cell	187,8 + 14	< 0,01

1. Values are expressed as means ± SEM.
**** Number of cases studied given in parenthesis.***

DISCUSSION

Certain abnormalities that occur in SCD are difficult to relate directly to the presence of HbS or the sickling phenomenon.[1] It is now known that a combination of intrinsic erythrocyte abnormalities and other factors are likely to be epiphenomenologic. In this study it is shown that the susceptibility to peroxidation of intact erythrocyte and isolated lipids of intact erthrocytes or their ghosts is appreciably increased in SCD compared to normal subjects. This is suggestive that in SCD the erythrocyte membrane is more vulnerable to lipid peroxidation and that this increased vulnerability may be a feature which contributes to the easy collapse of the erythrocyte membrane in SCD.

The increased vulnerability may be due to disorganization of the Hb molecule, which as a haem-protein, is a powerful catalyst of lipid peroxidation,[13] distortion of normal Hb-membrane and membrane-membrane relations, and plasma and erythrocyte vitamin E depletion.[14] In fact, increased TBA – reactive materials have been demonstrated to precede haemolysis of sickle erthrocytes[15] and abnormal cation leaks that develop in SCD damaged membranes.[16,17]

It appears from the above consideration that in SCD H_2O_2 may be generated in increased amounts and not physiologically well managed. If its behaviour in this study is a guide, H_2O_2 is a potent oxidant initiating the production of a steady supply of free radicals which degrade membrane phospholipids, followed by the collapse of the membrane.

The disparity in the extent of peroxidation between lipids of intact erthrocytes compared to isolated lipids of intact erythrocytes or their ghosts as measured by MDA formation (Table I) may be related to loss of unsaturated lipids in the extraction procedure. This observation that the extent of peroxidation was significantly higher for intact erythrocytes than for lipids isolated from intact erythrocytes or their ghosts is consistent with the report of Stocks and Dormandy[12] that isolated lipids yield much less MDA compared to lipids of intact red cells. However, MDA formation was still higher for isolated lipids from sickle erythrocytes compared to normal erythrocytes.

From the above consideration it is plausible that in SCD, the primary event of crystallization of HbS is aided by damage to the cell membrane caused by increased peroxidation of membrane constituents among other factors. Excessive and symmetric fragmentation and loss of cell membrane due to peroxidation may precipitate irreversible sickling of erythrocytes containing HbS. These cells will not return to a normal configuration even upon oxygenation. In fact, these cells can be lysed and the solubilised Hb removed, and the membrane will remain in the sickled configuration[2] indicating permanent damage to the membrane.

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