Glutathione and Lipid Peroxidation Profiles of Normal, G6PD-Deficient and Sickle Erythrocytes Exposed to Oxidative Stress

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Abstract

In this study, the glutathione (GSH) and lipid peroxidation profiles of normal, G6PD-deficient and sickle erythrocytes were assessed before (pre-APHZ) and after (Post-APHZ) induction of oxidative stress with acetyphenylhydraizine (APHZ). Lipid peroxidation was measured as the amount of malondialdehyde (MDA) produced per unit time (nmol hr^{1}). Before treating the erythrocytes with APHZ, the mean GSH levels were 39.82 ± 1.48, 36.00 ± 2.44 and 26.00 ± 2.32 mg /10 ml in normal subjects, G6PD-deficient subjects and sickle cell patients respectively. The post-APHZ treatment levels of GSH for the same categories of erythrocytes were 37.0 ± 3.25, 17.10 ± 0.011 and 13.00 ± 1.98 mg/ 100 ml respectively. This study revealed that in the absence of an oxidant stressor, normal red cells, and those of G6PD-deficient subjects maintain high levels of GSH and low levels of lipid peroxidation products (measured as malondialdehyde, MDA) and that the initial effects of exposure to oxidative stress are lower GSH and elevated MDA contents of the cells. This study further established that the erythrocytes of sickle cells patients, even without any exogenous oxidative challenge, have subsisting low levels of GSH and high levels of MDA, most probably because sickle cell complications give rise to high levels of redox species that constitute molecular threats to the integrity of red blood cells. It was also a finding in this study that the red cells of normal subjects respond less significantly to such oxidative stress-induced parameters of assessing red blood cell integrity-GSH content and lipid perodixation.

Keywords: Glutathione, Lipid peroxidation, G6PD-deficient, Sickle erythrocytes, Oxidative stress

Introduction

Oxidative stress on cells or tissues is frequently caused by free radicals (FRs) - species that contain an unpaired or odd number of electrons and may posses a positive, a negative, or no charge (Snyder et al., 1982). FRs-mainly reactive oxygen species (ROS), reactive nitrogen species (RNs), and lipid peroxyl radicals (LPR)-are derived either from normal essential metabolic processes in the body (internal sources) or from external /environment sources (Ho *et al.,* 2007). These FRs may be of organic or inorganic origin (Mason and Chignell, 1982) and are formed continuously in cells as a consequence of both enzymatic and non-enzymatic reactions (Bagchi and Puri, 1998). The enzymatic reactions which serve as sources of FRs include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis and cytochrome P_{450} system (Langseth, 1996). Accordingly, the internal sites/sources of FR generation are mitochondria, phagocytes, xanthine oxidase, reactions involving iron and other transition metal, arachidonate pathways (Ho et al., 2006). Free radicals also arise from non-enzymatic reactions of O_2 with organic compounds, including those initiated by ionizing radiation (UV), certain drugs, pesticides, anesthetics industrial solvents and ozone (Baumber *et al*., 2000). The human body employs two main lines of defence to counteract radical damages: a system of enzymes, and antioxidants (Uday *et al*., 1999). The primary line of defence, the enzymes, are glutathione peroxidase (GPx) superoxide dismutase (SOD), and catalase while the secondary line of defence is provided by the antioxidants and/or free radical scavengers such as α-tocopherol, ascorbic acid, retinol, β-carotene and glutathione (GSH). Oxidative stress engendered by an imbalance between the rate of FR production and enzymic and antioxidant defences in the body has been implicated in the pathogenesis and progression of many diseases (Ho et al., 2007; Ho et al., 2006; because the FRs mediate lipid peroxidation and destroy cellular proteins, lipids, carbohydrates and DNA (Eze, 1991). This study was therefore aimed at investigating drug-induced glutathione and lipid peroxidation profiles of normal, G6PF-deficient and sickle erythrocytes.

Materials and Methods

Subject: Glucose-6-phosphate dehydrogenase (G6PD) screening test procedures was used to analyze the 90 blood samples collected from 30 each of G6PD-deficient sickle cell patients (SCPs), G6PD-deficient but non-sickle cell subjects (NSCs) and normal subjects (NSs). The SCPs were selected from those attending routine checkups at the Federal Medical Center, Abakaliki, Nigeria, while the NSCs and NSCs were selected from the staff and students of the College of Health Sciences, Ebonyi State University, Abakaliki. They (NSCs and NSs) were not

taking any drug and the SCPs were not having any crisis at the times the blood samples were collected.

Chemicals: All the chemicals used in this study were of analytical grade (Anal. AR).

Glutathione assay: Oxidative stress was induced in the erythrocytes by adding 5 mg of acetylphenylhydrazine (APHZ) per ml of whole blood. Glutathione concentrations were determined according to the acid-citrate-D-glucose (ACD) method of Beutler (1966). These measurements were done pre-and post-APHZ treatments.

Lipid Peroxidation Assay: MDA levels of whole blood samples were determined per-and post-APHZ treatment at 5 mg APHZ/ ml by the method described by Siroev and Makarova (1989).

Data analysis: The difference in treatment mean values was analysis using analysis of variance.

Results

Glutathione levels: Before APHZ treatment, the mean GSH level in normal subjects (Fig. 1) was greater than that the mean level in G6PD-deficient subjects (39.82 vs 26.00 mg /100 ml) (Fig. 2), and the level in G6PD-deficient subjects was greater than that of sickle cell patients (36.00 vs 26.00 mg/ml). Incubating the red cells with APHZ caused an insignificant ($p > 0.05$) in reduction in the mean GSH level of normal subjects (39.82 vs 37.00 mg/100 ml) when compared with the significant drop ($p < 0.05$) in G6PD-deficient red cells (36.00 vs 17.10 mg/100 ml), and sickle red cells (from 26.00 to 13.00 mg/100 ml).

Lipid peroxidation (MDA): In the absence of APHZ, minimal lipid peroxidation occurred in normal (Fig. 3) and G6PD-deficient subjects (3.01 and 4.00 nmol/hr respectively) (Fig. 4). The lipid peroxidation level in sickle cell patients was high (210.91 nmol/hr) under the same condition. Upon incubation with APHZ, mean MDA level still remained low in normal subjects (5.90 nmol/hr) when compared with G6PD-deficient subjects (260.10 nmol/hr) and sickle cell patients (284.29 nmol/hr).

Discussion

Prior to inducing oxidative stress in the three categories of erythrocytes-those of normal, G6PD-deficient and sickle cell patients (Fig. 1) normal and G6PD-deficient red cells both had high mean glutathione contents of 9.82 and 36 mg/100ml respectively. This was suggestive of a functional pentose phosphate pathway (PPP), the main source of NADPH that maintains glutathione in the reduced form-GSH. This was also indicative of the existence of low levels of oxidant stressors, particularly free radicals which are usually kept in check or impaired.

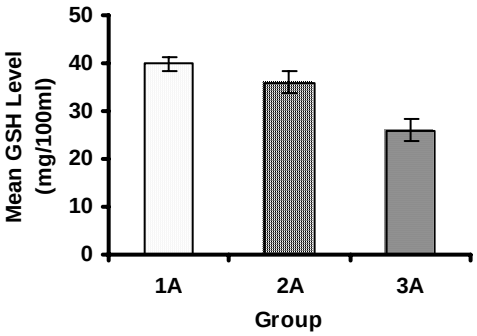

Fig. 1: Glutathione levels in normal subjects, G6PD-deficient subjects, and sickle cell patients before APHZ treatments

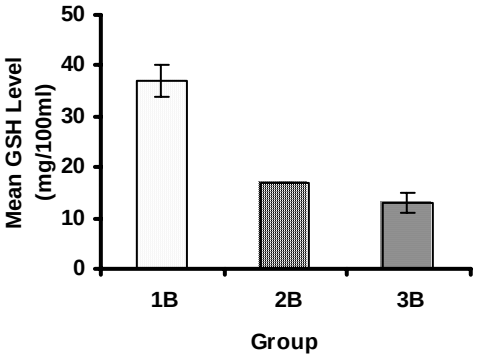

Fig. 2: Glutathione levels in normal subjects, G6PD-deficient subjects, and sickle cell patients after APHZ treatments

At this baseline (pre-APHZ) however, the mean GSH level of sickle erythrocytes was much lower (26 mg/100ml) than those of the other two categories. It is reasonable to infer that even in the absence of oxidative challenge; there is a subsisting high level of endogenously derived reactive species which continually deplete the GSH levels of these cells. Although normal red cells should have optimal G6PD activity, which generates NADPH to promptly reduce the GSSG that is continually generated, fig 2 indicates that APHZ caused an initial fall, though insignificantly ($p > 0.05$), in the mean GSH level of those cells, but insignificantly ($p < 0.05$) in G6PD-deficient and sickle red cells. From the APHZ-induced drops in the GSH levels of these cells, it can be inferred that at least one of the initial effects of an oxidant drug on red cells is to lower their GSH levels. Since red cell glutathione is maintained in the reduced state (GSH) by the NADPH generated by G6PD, it is also reasonable to infer that the initial effect of an oxidant drug is to lower the activity of the enzyme, so that less NADPH is available to reduce GSSG to GSH. This accounts, at least in part, for the reduced levels of this intracellular thiol when the cells were exposed to APHZ.

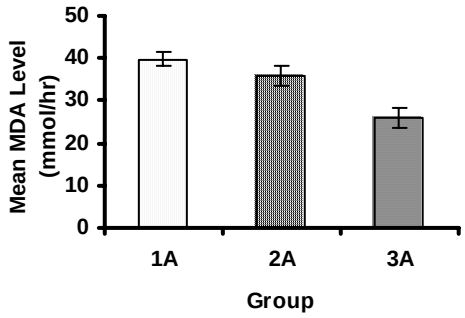

Fig. 3: Lipid peroxidation levels in normal subjects, G6PD-deficient subjects, and sickle cell patients before APHZ treatments

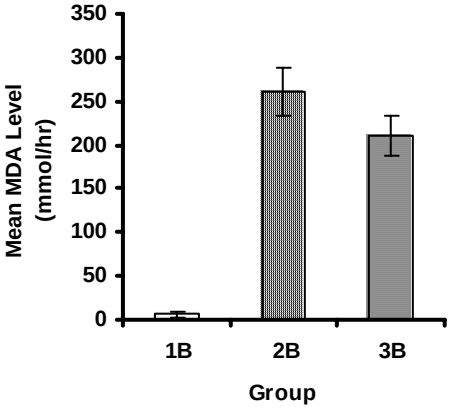

Fig. 4: Lipid peroxidation levels in normal subjects, G6PD-deficient subjects, and sickle cell patients before APHZ treatments

Alternatively, this effect (lowering of GSH level) could be due to the activation of γ-glutamyltranspeptidase, an enzyme located on the external surfaces of red cells and which initiated with degradation of GSH in vivo (Voet and Voet, 1994). The significant ($p < 0.05$) fall in the mean GSH level of G6PD-deficient erythrocytes was expected in view of the documented low activity of G6PD in these cells upon exposure to oxidative stress and, by extension, low level of NADPH needed to keep GSH in its reduced form. Much as low G6PD activity and subsequent low NADPH level could also account for the lowering of the mean level of GSH in sickle erythrocytes, the very low level of GSH in these cells pre-APHZ treatment demands other explanations. This finding was agreement with the studies of Ho et al., (2007) in which they concluded that sickle cell complications cause the red cells to produce very high levels of reactive oxygen and nitrogen species which overwhelm the enzymic and antioxidant defences against those redox species.

The lipid peroxidation profiles of the three categories of red cells used in this study mirrored those of GSH. Before the red cells were treated with APHZ, the lipid peroxidation products (MDA) in normal and G6PD-deficient subjects were low. This was a converse reflection of the high level of GSH (and possibly other defense against free radical damages) in these cells. On the other hand, a high pre-APHZ level of MDA (210.90 vs 3. 01 and 4.00 nmol/hr) (fig 3) existed in sickle erythrocytes, and this was very much in agreement with the much lower mean level of GSH (26 vs 39 and 36 mls) in these cells. In the same way that mean GSH levels of normal and G6PD-deficient cells dropped upon exposure to APHZ, there were respectively insignificant ($p > 0.05$) upregulation of mean MDA level in normal subjects, but significant ($p < 0.05$) rises in the mean MDA levels of G6PD-deficient and sickle red cells.

This study has shown that in the absence of an oxidative challenge, the red cells of normal and G6DP-deficient subjects maintain high levels of GSH and low levels of lipid peroxidation products (MDA) respectively. It has also shown that sickle erythrocytes, even in the absence of oxidant stressors, have a subsisting high level of MDA and a low level of GSH. It was also a finding in this study, that the initial effect of an oxidant substance (drug) on red cells is to lower their GSH levels and elevate their MDA levels, and that normal red cells, with efficient pentose phosphate pathways, counteract these effects while those of G6PD-deficient subjects and sickle cell patients do not.

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