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Full Length Research Paper

ANTIOXIDANT – MEDIATED GLUTATHIONE LEVELS OF GLUCOSE – 6 – PHOSPHATE DEHYDROGENASE – DEFICIENT ERYTHROCYTES UNDER DRUG-INDUCED OXIDATIVE STRESS

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The effects of two antioxidants, ascorbic acid and α– tocopherol, on the glutathione (GSH) levels of glucose – 6 – phosphate dehydrogenase (G6PD) – deficient erythrocytes were studied before inducing oxidative stress with acetylphenylhydrazine (APHZ), and after inducing the stress, followed separately by ascorbic acid and α – tocopherol treatments. Before APHZ treatment, the mean GSH levels in the control and G6PD – deficient erythrocytes were 38.82 + 1.48 mg/100ml and 36.00 + 2.44mg/100ml respectively. Their respective mean post – APHZ levels were 37.00 + 2.35 mg/100ml and 17.00+ 0.01mg/100ml. Post – APHZ + ascorbic acid, the control's mean GSH level was 37.75 + 2.50mg/100ml and the mean level in the G6PD – deficient cells were 27.00+ 3.44mg/100ml, while their respective post – APHZ + α– tocopherol levels were 38.76 + 2.00mg/100ml (control) and 33.00 + 1.93mg/100ml (G6PD – deficient red cells). The results of this study indicate that the initial effect of an oxidant drug on erythrocytes is to lower their GSH levels. This effect was found to be more severe in G6PD – deficient red cells than in the non – deficient ones. Ascorbic acid and α– tocopherol could ameliorate the effect of this oxidant stressor, and possibly others, but the latter was more effective. Its fat – solubility is suspected to be responsible for this enhanced efficacy.

Keywords: Antioxidants; Glucose -6 –phosphate dehydrogenase; oxidative stress; ascorbic acid; α – tocopherol

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INTRODUCTION

The severe and fatal haemolytic anaemia that attended the introduction of primaquine in the 1920s to cure resistant or relapsing forms of Plasmodium vivax and P. falciparum brought this drug to a notorious clinical notice. A study in 1953 revealed the cause of this anaemia to be an X – linked lesion of the erythrocyte due to a deficiency or low activity of glucose – 6 – phosphate dehydrogenase (G6PD)[2]. This lesion, also called primaquine sensitivity, is now a generic reference to primaquine and many other forms of drug – induced haemolytic anaemia since it is inducible with over 100 known oxidant drugs in susceptible individuals [3]. The low activity of G6PD in susceptible erythrocytes is due to the accelerated breakdown of the mutant enzyme protein, leading to a defective pentose phosphate pathway and low levels of NADPH in such red cells [4].Consequently, oxidized erythrocyte glutathione (GSSG) cannot be effectively reduced to the erythro-protective thiol (GSH), thereby exposing the red cells to assault by free radicals and other oxidant species[5].

The primary line of defence against free radicals is a battery of enzymes: glutathione peroxidase (GPx), superoxide peroxidase (SOD) and catalase[6], while the secondary defence is provided by antioxidants, including vitamins A, C and E, as well as GSH, etc., [7]. The oxidative stress caused by an imbalance between the rates of production and removal of free radicals has been implicated in the pathogenesis and progression of many diseases and metabolic derangements [8].

In this study, the effects of two antioxidants, ascorbic acid and alpha – tocopherol, on the glutathione levels of G6PD – deficient erythrocytes in which oxidative stress was induced with 5mg of acetylphenylhydrazine (APHZ) per ml of whole blood are reported.

MATERIALS AND METHODS

Volunteers and Blood Samples: With the consents of the volunteers, the method of Beutler & Mitchell [9] was used to select 60 individuals in the study. They were 30 G6PD – deficient individuals and 30 G6PD – non-deficient ones who served as the control. About 5ml of venous blood was collected from each volunteer and transferred into a venoject tube containing Na- citrate, an anticoagulant.

Chemicals: All the chemicals used for this study were of analytical grades and products of reputable companies.

GSH Assay[10]: One ml of whole venous blood was drawn into 1ml of acid – citrate – D-glucose (ACD) solution made up of 16.0g of sodium citrate, 4.8g of citric acid, and 29.5g of glucose in one litre of distilled water. 5mg of acetylphenylhydrazine (APHZ) per ml of blood was added to induce oxidative stress, mixed, shaken to ensure thorough oxygenation, and incubated for 2hours at 370C in a water-bath. To test the effect of ascorbic acid on both APHZ – induced oxidative stress and the GSH levels of the erythrocytes, 0.08mg of ascorbic acid per ml of blood was added to the reaction mixture after the addition of APHZ, and incubated for 2 hours. For the effect of α – tocopherol, 0.1mg of vitamin E per ml of blood was added at that point to a separate, similarly - treated reaction mixture, and also incuzbated for 2 hours. After the incubation, 2ml of distilled water was added. Five minutes later, 5ml of 3% glacial metaphosphoric acid was added with agitation, followed with 3g of NaCl. The reaction mixture was shaken, filtered, and 2ml of the filtrate added to 6ml of saturated NaCl solution in a 25-ml cuvette at a temperature of 20 – 25oC. One ml of 2% sodium nitroprusside and 1ml of O.67M NaCN – 5M NaCO3 (1:1, v/v) solution were added to develop colour. A spectrophotometer reading was made at 525nm within one minute.

Table 1: GSH levels in erythrocytes before and after APHZ and antioxidant treatments.

Red Cell Type	Pre – APHZ (mg/ 100 ml)	Post – APHZ (mg/ 100ml)	Post – APHZ + Ascorb acid (mg/ 100ml)	Post – APHZ + α– Tocopherol (mg/ 100ml)
Control	39.82 ±1.48	37.00 ± 2.35	37.75 ± 2.50	38.76 ± 2.00
G6PD Deficient	36.00 ±2.44	17.00 ± 0.01	27.00 ± 3.44	33.00 ± 1.93

DISCUSSION

Prior to the induction of oxidative stress with APHZ in the red cells studied, G6PD – deficient erythrocytes had a lower mean GSH level than the control (Table 1). This was suggestive of a subsisting oxidant challenge, even in the absence of an exogenous oxidative stress.

Although the control red cells should have an optimal G6PD activity to generate NADPH to reduce GSSG to the erythro – protective GSH, there was a significant fall ($p< 0.05$) in the mean GSH level of these cells upon exposure to APHZ. However, when the APHZ – treated red cells were exposed to ascorbic acid, an antioxidant; there was a significant elevation ($p< 0.05$) of the mean GSH level of the erythrocytes. Exposure of the APHZ – treated cells to α– tocopherol also caused a significant ($p<0.05$) elevation of their mean GSH level, such that the difference between their mean pre – and post – APHZ treatment levels became insignificant ($p>0.05$). It is inferable from the above changes that, the initial effect of an oxidant drug (e.g. , APHZ) in erythrocytes is to lower their GSH levels. Since red cell GSH is maintained in the reduced state by the NADPH generated in the G6PD – catalyzed reaction in the pentose phosphate pathway, it is also reasonable to infer that the initial effect of an oxidant drug is to lower the activity of this enzyme, so that less NADPH is available to reduce GSSG to GSH. This must have accounted, at least in part, for the reduced mean level of this intracellular thiol (GSH) when the cells were exposed to APHZ ab initio.

Alternatively, the lowering of GSH level could have occurred through the activation of γ – glutamyltranspeptidase, an enzyme located on the external surfaces of red cells, and known to initiate the degradation of GSH in vivo 11. Irrespective of the mechanism of down – regulating the red cell GSH levels upon exposure to oxidant drugs, it was observed that the two antioxidants, ascorbic acid and α – tocopherol, mediated their up-regulation although to varying extents. The effect of ascorbic acid, although significant ($p< 0.05$) , was less profound than that of α – tocopherol. It is probable that the latter's fat-solubility enabled it to so - penetrate the erythrocyte membrane lipids and scavenge the offending redox species that, the mean GSH level of the control red cells was elevated close to their pre – APHZ treatment level.

The treatment of the G6PD – deficient red cells with APHZ caused a significant fall ($p< 0.05$) in their mean GSH level. As speculated for the "G6PD –normal" cells, the activity of G6PD needed to generate NADPH to reduce GSSG to GSH was lowered by APHZ. Similarly, APHZ could also have activated γ - glutamyltranspeptidase that initiates the degradation of G6PD enzyme protein (Goldberg & Rock, 1992). Both ascorbic acid and α – tocopherol elevated the mean GSH levels of the erythrocytes but that of G6PD – deficient ones still remained significantly lower ($p<0.05$) than the pre – APHZ level. It is most likely that overwhelming levels of redox species were generated and sustained when the red cells were treated with APHZ.

In conclusion, the effect of the oxidant drug (APHZ) was found to be more severe in G6PD – deficient erythrocytes than in the non – deficient ones. Irrespective of red cell type however, this study revealed that the initial effect of the oxidant drug (and possibly others) is to lower the activity of G6PD probably by accelerating the γ – glutamyltranspeptidase - mediated degradation of its enzyme protein. Furthermore, ascorbic acid and α – tocopherol ameliorated the effect of the oxidant but the latter was more effective, probably because it is fat – soluble. By implication, the co – administration of ascorbic acid or α – tocopherol with oxidant drugs, especially oxidant anti-malarial drugs, such as 4 – and 8 – aminoquinolines, to which G6PD – deficient individuals react adversely, will stabilize or restore erythrocyte GSH levels.

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