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The in Vitro Effects of The Antimalarial Drug Primaquine, on The Activities of Some Enzymes in Human Erythrocyte Lyzates

Abstract: The effect of primaquine on the enzymatic antioxidant defence mechanisms of erythrocytes were determined by measuring the activities of the below enzymes, before and after 30 minutes of incubation of hemolyzates with 1 mM primaquine at 37°C.

Among the enzymes studied glucose-6-phosphate dehydrogenase seemed the most sensitive enzyme; 57 % and 78 % inhibition at zero (due to the binding of primaquine to NADP binding site) and 30 minutes of incubation (due to the reactive oxygen species), respectively. Catalase, superoxide dismutase, and glutathione-S-transferase were less sensitive to primaquine-derived reactive oxygen species: 33, 11, and 0 % inhibition at the time zero and 39, 27, and 4 %, at the end of 30 minutes of incubation. Glutathione reductase and glutathione peroxidase were resistant to primaquine-derived reactive oxygen species.

The Primaquine-mediated toxicity, in vivo, due to the primaquine-NAD(P)H or/and primaquine-glucose-6-phosphate dehydrogenase interaction, will cause damage and the degree of the damage will depend on: i. concentration of the drug, PQ; ii. incubation period and; iii. the type of the system affected. The study of the interaction of reactive oxygen species with these enzymes, will help to better understand the role of protective enzyme systems in erythrocytes to oxidative stress.

Key Words: Primaquine, inhibition, glucose-6-phosphate dehydrogenase, catalase, glutathione peroxidase, superoxide dismutase, glutathione reductase, glutathione transferase.

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Introduction

Primaquine (PQ), an 8-aminoquinoline, is the only tissue schizontocide currently available for radical treatment of malarial infections but, the usefulness of the drug is limited due to its toxic side effects. For example, PQ causes hemolytic anemia in glucose-6-phosphate dehydrogenase (G6PD) deficient patients [1]. It has been demonstrated that the interaction between PQ and NADPH underlies many aspects of PQ toxicity. The interaction between PQ and NADPH and the auto-oxidation of PQ result in the formation of reactive oxygen species (ROS) which results in the oxidative alterations in erythrocytes [2-5].

Erythrocytes contain enzymes and antioxidants which protect them against the activated oxygen species: hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot}$), and hydroxyl ($^{\cdot}OH$) radicals. These activated oxygen species are generated within normal erythrocytes after the addition of PQ and other oxidant drugs [6,7].

Superoxide is detoxified by superoxide dismutase (SOD), by converting two molecules of superoxide to yield one molecule each of O_2 and H_2O_2 [6,7]. Catalase (CAT) and glutathione peroxidase (GSH-Px) protect the red cell against reactive oxygen species [8,9]. Glutathione reductase (GSSGGR) is an important enzyme for the regeneration of the most important reducing power of the erythrocytes, glutathione (GSH) [10]. Glutathione S-transferase (GST) which catalyzes the reaction of GSH as a nucleophile, with a variety of compounds bearing an electrophilic center plays an important role in the mercapturate pathway [11].

G6PD is the first enzyme of pentose phosphate pathway and which accounts for the most of NADPH synthesized in red cells. NADPH is necessary for both glutathione redox cycle [12,13] and the protection of CAT against inactivation by H_2O_2 [8].

There are several reports about the oxidative effect of PQ in erythrocytes but, the number of the parameters

measured in these studies are limited and the interrelations of these parameters are usually not been considered.

In order to reevaluate the oxidative effect of PQ on the enzymes responsible for the protection of erythrocytes against ROS, this study was performed. The activities of G6PD, CAT, GSH-Px, SOD, GSSGGR, and GST, before and after incubation of human erythrocytes with 1 mM PQ, for half an hour, at 37°C, were determined.

Materials and Methods

Materials

Primaquine (8-(4-methyl buthylamino)-6-methoxyquinoline), diphosphate salt; glucose-6-phosphate, disodium salt; NADP+, monosodium salt; bovine serum albumin, EDTA (Ethylenediaminetetraacetic acid), GSH, GSSG, 1-chloro-2,4-dinitrobenzene, and xanthine oxidase were from the Sigma Chemical Co., USA. 2-Mercaptoethanol, $CuCl_2$ and H_2O_2 were obtained from BDH and The Hopkins and William Ltd., England, respectively. All the other chemicals were analytical grade and obtained from either Sigma or Aldrich, U.S.A.

Methods

Preparation of packed red cells and hemolyzates for assays: Adult human venous blood (10 different healthy staff ages between 25-30 years: 4 men and 6 women), was drawn into EDTA-treated tubes and packed red cells were prepared washing with physiological serum (3 times) to eliminate leucocytes and pleteletes [14]. Different types of hemolyzates were prepared from packed red cells according to the type of enzyme going to be tested [14-16]. These hemolyzates were incubated with 1 mM PQ (30 min, at 37°C) and the activities of G6PD, GSGGGR, GSH-Px were determined by adding an aliquot of the enzyme from incubation medium to the cuvette and the change in absorbance were monitored [14]. CAT and SOD activities were measured by the method of Aebi [15], and Sun et. al. [16], respectively. All measurements were made on a Milton-Roy 3000 spectrophotometer.

Results and Discussion

Oxidant drugs increase oxidative stress in red blood cells by generating free radicals and decreasing the activities of antioxidant enzymes. It has been shown that the oxidative effects of PQ have been the formation of a charge transfer complex between PQ and (NAD[P])H, and auto-oxidation of PQ leading to the oxidation of the reduced pyridine nucleotides and generation of ROS (H_2O_2, $O_2^{.-}$, $.OH$) [2-4]. Thus, these observations explain the various aspects of PQ toxicity in erythrocytes.

In the present study, erythrocyte hemolyzates were incubated with 1 mM PQ, at 37°C, for 30 minutes and the effect of PQ on the activities of erythrocytes, G6PD, CAT, SOD, GSH-Px, GSSGGR and GST, was determined. Hemolyzates were used instead of intact erythrocytes because, i. erythrocytes are hemolyzed by [PQ] ≥ 1 mM; ii. entrance of PQ into erythrocytes is difficult (transport problem). The effect of PQ on the activities of G6PD, SOD, CAT, GSH-Px, GSSGGR, and GST in human erythrocyte lyzates is summarized in the below Table.

It has been demonstrated that PQ reacts with NADPH and this interaction lowered the cellular NADPH level [2,4]. The 57% inhibition of G6PD, following 1 mM PQ addition, could be explained by the binding of primaquine to the NADP+ binding site on G6PD and additional 21% inhibition, during 30 minutes incubation, might be due to the oxidation of the two very reactive thiol groups responsible for the activity of the enzyme [17,18].

In erythrocytes, it is possible that PQ exists in various redox states and can form H_2O_2 either from the dismutation of superoxide radicals or from the oxidation of the NADPH:PQ complex by superoxide radicals, which will cause the oxidation of NADPH [2] or thiols [18]. It has been demonstrated that glucose partially maintains NADPH concentration in the presence of PQ by increasing the flux through the pentose monophosphate pathway [19]. It has also been shown that the inhibition of G6PD caused by PQ can be completely reversed by excess NADP+ [20].

However, SOD and CAT were affected less from the incubation of hemolyzates with the drug, PQ. Inhibitions at zero and 30 minutes of incubation with PQ (1 mM, 37°C) for SOD and CAT were; 33, 11 and 39, 27 per cent, respectively (Table).

Among these three enzymes; SOD, is a Cu^{+2} dependent enzyme and it will be inactivated if the cupper at the active of the enzyme is reduced to Cu^{+1} by H_2O_2. No inactivation of SOD, in the presence of CAT, was observed [21]. In this study, inhibition of SOD activity in hemolyzates incubated with PQ, was observed. This contradiction could easily be explained by the low concentration of the PQ in earlier works. Here, the rate of H_2O_2 production is probably much higher than its consumption by endogenous CAT in the PQ-treated erythrocyte lyzates.

Enzymes	Control (-PO)	0 min (+PQ) % Inhibition	30 min (+PQ) % Inhibition
G6PD (U/g Hb)	100 (12.99±1.31)	57 (5.62±1.43)*	78 (2.60±0.82)*
SOD (U/mg protein)	100 (217±29)	33 (146±48)*	39 (126±49)*
Catalase (k/g Hb)	100 (291±30)	11(259±31)*	27 (201±20)*
GST (U/g Hb)	100 (7.5±2.1)	0 (7.5±2.1)*	4 (7.2±2.0)*
GSSGGR (-FAD) (U/g Hb)	100 (5.31±1.31)	0 (5.31±1.31)*	0 (5.31±1.31)*
GSSGGR (+FAD) (U/g Hb)	100 (7.84±1.35)	0 (7.84±1.35)*	0 (7.84±1.35)*
GSH-Px (U/g Hb)	100 (7.91±3.34)*	0 (7.91±3.34)*	0 (7.91±3.34)*

Table 1. The Effect of PQ on the Activities of G6PD, SOD, CAT, GST, GSSGGR (±FAD) and GSH-Px in Erythrocyte Lyzates.

* Values in parenthesis are absolute values: Means±SD (n=10).

The second protective enzyme, CAT contains four tightly bound molecules of NADPH which are not essential for activity [8], but protects the enzyme against inactivation by its substrate, H_2O_2 [9, 22, 23].

Glutathione-related enzymes, (GST, GSSGGR, GSH-Px) are the least (or none) affected by ROS. Among these enzymes only GST was sligthly inhibited (4%) by the incubation with PQ for 30 min, at 37°C. These enzymes seem more resistant to oxidation by H_2O_2.

As a result we can say that the prooxidant drug PQ causes some deleterious effects on the antioxidant enzyme systems in erythrocytes. PQ-NAD[P]H interaction might be responsible for the PQ-mediated toxicity, in erythrocyte lyzates. The degree of the damage will depend on: i. concentration of the drug, PQ; ii. incubation period and; iii. the type of the system affected. A more detailed study, using hemolyzates or drugs with no transport problem or/and study which undertakes direct interaction of ROS with these enzymes, will help to better understand the role of protective systems in erythrocytes against oxidative stress.

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