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Short Communication *Pharmacology*

Erythrocyte Glutathione S-transferase Activity of Non-Malarious Male Human Volunteers Administered with Five Antimalarial Drugs.

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Abstract:
Investigation to ascertain the potencies of five antimalarial drugs (Fansidar, Halfan, Quinine, Coartem and Chloroquine phosphate) to alter/distort non-parasitized human erythrocyte (HbAA genotype) glutathione S-transferase (GST) activity was carried out. Apparently healthy and clinically confirmed non-malarious male human volunteers enrolled for this study. The incubation of human erythrocytes with 1-chloro-2, 4-dinitrobenzene (CDNB) resulted in almost quantitative conjugation of glutathione (GSH) to form S-(2, 4-dinitrophenyl) glutathione. The reaction formed the basis for the spectrophotometric determination of GST activity. Determination of GST activity was carried out before and after the five (5) drug treatments.The control values ranged between 3.27±0.13 iu/gHb and 3.40±0.05 iu/gHb. Generally, the erythrocyte GST activity was time dependent of the five antimalarial drugs showing biphase profile. The first phase showed decrease levels of relative GST activity within approximate time range: ($0 < t < 6$) hours after the drugs were administered to the volunteers. The second phase showed recovery effect of the erythrocyte GST activity from the inhibitory action of the drugs. The results of these findings suggested the capability of these drugs to bind to the human erythrocyte GST, accompanied with raised oxidant stress of the erythrocytes.

Keywords: Glutathione S-transferase (GST) activity, erythrocytes, antimalarial drugs, humans, 1-chloro-2, 4-dinitrobenzene (CDNB).

Introduction

Glutathione S-transferase (GST) activity occurs in highest level in the hepatocytes and present in a wide variety of tissues [1]. Erythrocyte GST is primarily for the protection of erythrocytes against electrophilic compounds, rather than serving a general protective function in the body [2, 3]. Harvey and Beutler, [2], further postulated that GST functions physiologically as a haemin-binding and/or transport protein in developing erythroid cells.

All antimalarial chemotherapeutic agents with established therapeutic benefits, when administered to humans, come in direct contact and interact with erythrocyte constituents, specifically, haemoglobin, diverse enzymes and membrane architectural components. Although general laboratory and clinical trials have shown that these drugs may exhibit little or no toxicity when administered according to recommended dose, there are well-documented reports on contraindications/adverse reactions of antimalarial drugs. However, on the contrary, specific and detailed underlying molecular account of antimalarials on diverse and distinct erythrocyte components are in most cases ignored, taken for granted and not exhaustively reported. Therefore, this present study intend to ascertain the potency of five (5) commonly prescribed antimalarial drugs (Fansidar™, Halfan™, Quinine, Coartem™, and Chloroquine phosphate) to alter/distort erythrocyte GST activity, required for cellular integrity and functionality.

Materials and Methods:

Anti-malarial Drugs: Five (5) antimalarial drugs were used in this study: Fansidar™ {Swiss (Swipha) Pharmaceuticals Nigeria Ltd}, Coartem™, (Beijing Norvatis Pharmaceutical Company, Beijing, China) Chloroquine phosphate (May and Baker, Pharmaceutical Company, Nigeria Plc), Halfan™ (Smithkline Beecham

Laboratories Pharmaceutical Company, France) and Quinine (BDH, UK).

Experimental Design: Twenty-five (25) non-malarious male (61-73kg) human volunteers of confirmed HbAA genotypes between the age bracket of 20-28years enrolled for this study. The human volunteers were administered with single dose of each of five antimalarial drugs, according to the following specifications (Table 1).

Blood samples were then withdrawn from the human volunteers at time intervals of 3 hours, 6 hours and 18 hours after treatment. Analyses of the blood samples were carried out to ascertain the erythrocyte GST activity. The determination of this erythrocyte parameter prior to the administration of the five antimalarial drugs constituted the control sample analysis.

Ethics: The institutional review board of the Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria, granted approval for this study and all human subjects/ volunteers involved signed an informed consent form. This conducted study was in accordance with the ethical principles that have their origins in the Declaration of Helsinki. Individual human volunteers were drawn from Imo State University, Owerri, Nigeria and environs. The research protocols were in collaboration with registered and specialized clinics and medical laboratories.

Table 1: Doses of Drugs (Fansidar, Halfan, Quinine, Coartem and Chloroquine Phosphate) administered to Human Male Participants.

Drugs	Doses Administered
Fansidar:(pyrimethamine)	14.9 mg/kg
(sulphadoxine)	2.9 mg/kg
Halfan:(Halofantrine base)	13.9 mg/kg
Quinine	5.9 mg/kg
Coartem:(artemether)	1.2 mg/kg;
(lumefantrine)	7.2 mg/kg
Chloroquine Phosphate	14.9 mg/kg

Preparation of Erythrocyte Haemolysate: Five milliliters (5.0 ml) of human venous blood of HbAA genotype obtained from the volunteers by venipuncture was stored in EDTA anticoagulant tubes. The erythrocytes were washed by methods as described by Tsakiris *et al.,* [4]. Within 2 hours of collection of blood samples, portions of 1.0 ml of the samples were introduced into centrifuge test tubes containing 3.0 ml of buffer solution pH=7.4: 250mM tris (hydroxyl methyl) amino ethane–HCl(Tris-HCl)/140mMNaCl/I.0mMMgCl_2/10mMglucose).

The erythrocytes were separated from plasma by centrifugation at 1200xg for 10 minutes, washed three times by three similar centrifugations with the buffer solution. The erythrocytes were re-suspended in 1.0 ml of this buffer and stored at 4^0C. The washed erythrocytes were lysed by freezing/thawing as described by Galbraith and Watts, [5] and Kamber *et al.,* [6]. The erythrocyte haemolysate was used for the determination of glutathione-S transferase activity.

Determination of Erythrocytes Haemolysate Haemoglobin Concentration: A modified method of Baure, [7], based on cyanomethaemoglobin reaction was used for the determination of haemolysate haemoglobin concentration. The expressed values were in grams per deciliter (g/dl). A 0.05 ml portion of human red blood cell haemolysate was added to 4.95 ml of Drabkin reagent. The mixture was left to stand for 10 minutes and absorbance read at λmax=540 nm against a blank (Drabkin reagent only).

Determination of Erythrocyte Haemolysate Glutathione S-transferase Activity: Glutathione-S transferase activity was assayed spectrophotometrically by monitoring the conjugation of 1-chloro-2,4dinitro benzene (CDNB) with glutathione (GSH) at λmax=340nm at 37°C [8].

CDNB + GSH $\rightarrow$ CDNB – S – glutathione.

The enzyme assay was according to methods of Habig *et al.,* [8] with minor modifications [3]. The 1.0 ml in 2 % ethanol enzyme assay mixture contained 0.5 mM CDNB (0.02 ml), 1.0 mM GSH(0.05 ml), 0.68 ml of distilled water and 100 mM Phosphate buffer (K_2HPO_4/KH_2PO_4; pH=6.5) (0.2 ml).

The CDNB was mixed with the Phosphate buffer before use. The Phosphate buffer-CDNB mixture was incubated for 10 minutes at 37°C before the reaction started by adding GSH, followed

immediately by the addition of an aliquot (0.05 ml) of the haemolysate. In the control experiment, the rate of increase in absorbance at λmax = 340nm was measured for 10 minutes at 37°C against a blank solution containing the reaction mixture, in which; the haemolysate was substituted with distilled water. The enzyme assay was carried out before (control; t=0 hour) and after (tests; i.e. at t=3, 6 and 18 hours) the five (5) antimalarial drugs were administered to the human volunteers.

Statistical Analyses: The experiments were designed in a completely randomized method and data collected were analyzed by the analysis of variance procedure while treatment means were separated by the Least Significance Difference (LSD) incorporated in the Statistical Analysis System (SAS) package of 9.1 version. [9].

Calculation of Enzyme Activity: The expression below was used to evaluate erythrocyte glutathione-S transferase activity in international unit per gram haemoglobin (iu/gHb).

$$E_A = \frac{100}{[Hb]} \times \frac{0.D/min}{\sum} \times \frac{V_C}{V_H}$$

Where,

E_A = Enzyme activity in iu/gHb

[Hb] = Haemolysate haemoglobin concentration (g/dl)

0. D/min = Change per minute in absorbance at 340nm.

$\sum$ = Millimolar extinction coefficient = 9.6, in reaction in which 1mole of glutathione (GSH) is oxidized.

V_C = Cuvette volume (total assay volume) = 1.0ml.

V_H = Volume of haemolysate in the reaction system (0.05ml).

Results:

Table 2 shows the mean±(S.D) GST activity of erythrocyte haemolysate (HbAA genotype) obtained from non-malarious male volunteers before and after the administration of the five antimalarial drugs. The control values ranged between 3.27±0.13 and 3.40±0.05 iu/gHb.

Generally, the pattern of erythrocyte GST activity with time in the presence of the five antimalarial drugs showed two-phase profile. The first stage showed decreasing levels of relative GST activity within approximate time range :(6<t<0) hours after the drugs were administered to the volunteers. For instance, 6 hours after quinine was administered, erythrocyte GST decreased from 3.31±0.14 iu/gHb to 1.18±0.10 iu/gHb after 6 hours post treatment. This represented 35.6% of the enzyme activity compared to the control group.

Table 2*:* Effect of Antimalarial Drugs (Fansidar, Halfan, Quinine, Coartem and Chloroquine Phosphate) on Glutathione S-transferase Activity of HbAA Erythrocyte Haemolysate of Male Volunteers

DRUG	Time (hours)	GST Activity (iu/gHb)	Relative Activity (%)
FANSIDAR	0	3.40 ± 0.05^{a}	100.0
	3	3.02 ± 0.10^{b}	88.8
	6	2.62 ± 0.29^{c}	77.1
	18	$3.28 \pm 0.24^{a,b}$	96.5
HALFAN	0	3.37 ± 0.08^{a}	100.0
	3	2.08 ± 0.18^{c}	61.7
	6	2.01 ± 0.09^{c}	59.6
	18	3.00 ± 0.14^{b}	89.0
QUININE	0	3.27 ± 0.13^{a}	100.0
	3	1.48 ± 0.16^{c}	45.3
	6	1.00 ± 0.15^{d}	30.6
	18	2.89 ± 0.18^{b}	88.4
COARTEM	0	3.37 ± 0.07^{a}	100.0
	3	2.67 ± 0.20^{c}	79.2
	6	$2.79 \pm 18^{b,c}$	82.8
	18	2.97 ± 0.22^{b}	88.1
CHLORO-	0	3.29 ± 0.09^{a}	100.0
QUINE-P	3	2.48 ± 0.21^{b}	75.4
	6	2.01 ± 0.18^{c}	61.1
	18	3.11 ± 0.33^{a}	94.5

Means in the column with the same letter are not significantly different at $p < 0.05$ according to LSD.

The second phase exhibited characteristic recovery of erythrocyte GST activity from the inhibitory effects of the drugs. Between the 6th and 18th hours post treatments, erythrocyte GST activity increased from 2.03+/-0.12 iu/gHb to 2.69+/-0.37 iu/gHb, representing 19.8% increase in enzyme activity.

Discussion:

Over the duration of the *in vivo* experiment, the five administered antimalarial drugs caused profound disturbance by eliciting decreased level in erythrocyte GST activity approximately within 6hours (i.e., time range of $0 < t < 6$). Subsequently, as the experimental time approached the 18th hour, there was a progressive recovery of erythrocyte GST activity and the activity approached the control levels. Ayalogu *et al.*, [10], had previously documented that early short term inactivation of GST by gasoline may be responsible for the low activity of GST in rats injected with gasoline, which conformed to Chiapotto *et al*., [11], report on inactivation of GST by different concentrations of acetaldehyde. They further propounded that longer detoxification process resulting from the varying chemical composition of kerosene and crude oil (bonny light) together with biochemical changes that usually accompanied such process may have accounted for the subsequent increased activity of GST in the rats administered with kerosene and crude oil (bonny light) as the duration of the experiment progressed. All these accounts conformed to the present study.

In addition, Davies, [12], in his study of the effect of cholorothalonil on fish, had reported the reason for this pattern was as a result of depleting level of intracellular concentration of glutathione, one of the first lines of antioxidant defense mechanism against xenobiotics. Furthermore, Thomas and Wooford, [13], found that injection of paracetamol caused a minor decrease in glutathione in mullet liver after 3 hours, followed by rapid recovery and significant elevation by 24 hours. Haloacetonitriles were also found to initially cause a very rapid fall in rat liver glutathione (t_{min} can be as little as 30 minutes after ingestion), followed by a rebound by 18 hours [14].

In the light of these findings, it seems probable that the five administered antimalarial drugs caused initial decrease in glutathione concentration that rapidly stimulated/activated erythrocyte GST activity to give enhanced levels as the experimental time approached the 18th hour. Rapid compensation for depleted glutathione concentration by enhanced GST activity suggests utilization of glutathione in detoxification reactions,

Generally, the pattern of GST activity after the administration of the five antimalarial drugs was in two phases. Firstly, there was decreasing levels in the relative activity of the erythrocyte enzyme with time elicited by the capability of the drugs to bind to the enzyme [15,16]. The second stage was the recovery phase, whereby the buildup in reactive oxygen species (ROS) levels because of the first phase engendered positive activation of the enzyme, in an attempt to detoxify and neutralize the generated cytotoxic ROS. Therefore, this phase was essentially for restoration of homeostasis.

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