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COMPARATIVE *IN-VITRO* EFFECTS OF SOME ANTI-MALARIA DRUGS ON HUMAN ERYTHROCYTE GLUTATHIONE-S-TRANSFERASE (EC. 2.5.1.18) ACTIVITY

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ABSTRACT

The *in-vitro* effects of the anti-malaria drugs chloroquine, camoquine (amodiaquine), mefloquine (lariam), quinine, halfan (halofantrine) and fansidar (sulfadoxine + pyrimethamine), on human erythrocyte gluatathione-S-transferase (GST) activity was spectrophotometrically investigated at 37°C, pH6.5 and at milligram percent (mg%) concentrations (0.10mg%, 0.20mg%, 0.40mg%, 0.60mg %, 0.80mg% and 1.00mg%) of the drugs.

Quinine and its congeners, chloroquine, camoguine (4-amino quinoline) and mefloquine, were all observed to significantly ($P<0.001$) increase the activity of human erythrocyte glutathione-S-transferase in a concentration dependent manner and according to the order: chloroquine > quinine > mefloquine> camoquine. For instance at 1.00mg% concentration of the drugs, human erythrocyte GST activity was increased by 13.97, 8.98 7.98 and 6.98 folds in the presence of chloroquine, quinine, mefloquine and camoquine respectively.

Halfan (halofantrine) and fansidar (non-quinoline antimalarials) produced no significant change in the activity of human erythrocyte GST at the various concentrations tested. The activation of this enzyme by quinine and its derivatives could point to a possibility of these drugs to raise the oxidant stress of the red cells in the course of their therapeutic actions.

Key Words: Malaria, Chloroquine, Camoquine, Halfan, Quinine, Fansidar, Mefloquine, Erythrocytes, Glutathione-S-transferase.

INTRODUCTION

Drugs, even when taken in the right doses, elicit a number of pharmacologic effects at the site of action. This may include deleterious effects in addition to the desired clinical effects. (Katzung, 1982).

Chloroquine, camoquine, mefloquine, quinine, halfan and fansidar are among the many drugs in common use for the treatment of malaria (WHO, 1973). Like any other drugs, these antimalarials have some side effects which, however, may be unknown to the users. This is moreso when they are used in the treatment of other disease. For instance, chloroquine is used in the treatment of rheumatoid arthritis or discoid lupus erythematosus (Olatunde, 1970). One of the most notable serious consequences of this is damage to the retina (Meyer, 1964 and Peters, 1968). Some antimalarials also show their side effects on the gastrointestinal tract, cardiovascular system and skeletal muscles (Olatunde, 1970). High doses of chloroquine could lead to hyper excitability and convulsion within two hours (Katzung, 1982). Death may result due to cardiovascular collapse from extreme vasodilation and hypotension (Olatunde, 1970). There is a reported finding that chloroquine and fansidar, at milligram

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doses, activated human/rat serum alpha-amylase *in-vitro* and in *vivo* (Ayalogu *et al* 2000a). It has also been reported that the pyrimethamine component of fansidar when given in a single dose of 25mg daily for seven weeks caused the development of megaloblastic anaemia which readily disappeared after stopping the drug (Olatunde, 1970).

Chloroquine and its congeners exert their action on the malaria parasite by blocking the enzymatic synthesis of DNA and RNA (Pearlman, 1975). The drug forms a complex with DNA thereby preventing it from acting as a template for its own replication or transcription to RNA. Chloroquine was reported to exhibit no toxic effect when used in low doses required for chemosuppression of malaria (Katzung, 1982).

The plasmodial effect of fansidar is due to its ability to block folic acid synthesis in the protozoa (Katzung, 1982).

Halfan (halofantrine) is now employed primarily as an alternative to quinine, mefloquine and their congeners for treatment of acute malaria attacks caused by chloroquine – resistant and multi-drug-resistant strains of *Plasmodium falciparium* (Lawrence *et al.* 1999). Reported side effects of halfan include gastrointestinal disturbances, pruritis and hazardous dysrhythamia (Lawrence *et al,* 1999).

Recently, chloroquine, at an optimum concentration of 41.65mg%, was noted to increase human erythrocyte GST activity by 46.15% in vitro (Ayalogu *et al* 2000b). In the same study, fansidar, at a concentration of 26.24mg% was observed to decrease GST activity by 46.61%.

Glutathione-S-transferases (EC. 2.5.1.18) are a group of enzymes, which use reduced glutathione (GSH) and a wide variety of hydrophobic compounds as substrates (Jacoby, 1978). They are present in rats and human liver, pigeon, locust gut, housefly and other sources (Ketley, *et al*, 1975). Functionally, The glutathione-S-transferases (GSTs) catalyse the conjugation of electrophilic groups of hydrophobic drugs/xenobiotics to form glutathione (GSH) thiol esters. The thiol esters are inturn converted to mercapturic acid following a sequential reaction of gamma (γ)-glutamyl-transpeptidase, dipeptidase and acetylase (Boyland and Chasseaud, 1969) Glutathione-S-transferases help to detoxify certain extremely reactive substances by direct covalent binding of the electrophilic agent to protein (Jacoby, 1978). Thus GSTs protect cellular constituents from electrophiles and toxic xenobiotics. The location of GST in erythrocytes is ideal for the removal of circulating xenobiotics (Marcus *et al* 1978). The red cell GST also functions physiologically as a haemin-binding and/or transport protein in developing erythoid cells (Keilin, 1960). It has, however, been suggested that the occurrence of GST in the erythrocytes is primarily for the protection of erythrocytes against electrophilic compounds rather than serving a general protective function in the body (Harvey and Beutler 1982). The present study is to examine and/or elucidate comparatively, the *in vitro* effects of the antimalarials, chloroqunine, quinine, camoquine, mefloquine, halfan and fansidar on the activity of human erythrocyte glutathione-S-transferase, an enzyme which plays a vital role in the functional integrity of the erythrocytes.

MATERIALS AND METHODS

Fansidar, chloroquine, quinine and mefloquine (lariam) were bought from Roche pharmaceuticals, Nigeria. Halfan was bought from SmithKline-Beecham pharmaceuticals, France while camoquine was bought from Parke-Davis pharmaceuticals Senegal. Other chemicals used were from BDH and M&B, London.

Sample Collection and Preparation

Blood samples were collected, from ten (10) healthy volunteers of ages 18-25 years and of both sexes (6 males and 4 females), into citrate anticoagulant tubes. Erythrocytes were isolated from the blood samples by centrifugation at 10,000g for fifteen minutes using bench centrifuge (MSE Minor). Following

careful siphoning of the plasma (with a pasteur pipette), the erythrocytes were washed thrice with 10 volumes of normal saline and diluted 1:20 with a stabilizing solution (2.7mM EDTA, 0.7mM 2-mercaptoethanol, pH 7.0) as described by Beutler (1984). The samples were then frozen and thawed for immediate use. Portions (0.02ml) of the prepared samples (haemolysates) were made use of for the determination of haemoglobin concentration of haemolysate using Drabkin's solution (Drabkin and Austin, 1935; Van-Kampen and Ziglstra 1961).

Assay of Erythrocyte GST.

Glutathione-S-transferase was assayed spectrophotometrically by monitoring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with glutathione (GSH) at 340nm at 37°C (Habig *et al* 1974). The 3ml assay mixture contained 0.5mM CDNB, 1mM GSH and 100mM phosphate buffer, pH 6.5. CDNB was dissolved in ethanol and added to the phosphate buffer before use. The ethanol concentration in the assay mixture was 2%. The phosphate buffer-CDNB mixture was pre-incubated for 10min at 37°C and the reaction was started by adding GSH, followed immediately by an aliquot (0.15ml) of the haemolysate. The rate of increase in absorbance at 340nm was measured for 10min at 37°C against a blank containing the reaction mixture without haemolysate.

Effect of the Antimalaria Drugs

Red cell glutathoine-S-transferase activity was determined in the presence of varied concentrations (0.00mg%, 0.10mg%, 0.20mg%

Table 1: *In-vitro* effect of chloroquine (CQ) on Human erythrocyte glutathione-S-transferase activity (E), at 37°C pH 6.5

[CQ]mg%	E(UFL) $\bar{X} \pm SD$, n = 10	fold increase in E
0.00*	1.74 ±0.06	0.00
0.10	1.74±0.04	0.00
0.20	8.68±0.08	3.99
0.40	12.15±0.05	5.98
0.60	15.63±0.06	7.98
0.80	19.10±0.08	9.98
1.00	26.04±0.07	13.97

*Control

Table 2. *In-vitro* effect of camoquine (CAM) on human erythrocyte glutathione-S-transferase activity (E) at 37°C, pH 6.5.

[CAM)]mg%	E(UFL) $\bar{X} \pm SD$, n = 10	Fold increase in E
0.00*	1.74 ±0.06	0.00
0.10	1.74±0.05	0.00
0.20	3.47±0.03	0.99
0.40	6.94±0.21	2.99
0.06	8.68±0.01	3.99
0.80	12.15±0.05	5.98
1.00	13.89±0.07	6.98

*Control

Table 3: *In-vitro* effect of mefloquine (MEF) on human erythrocyte glutathione-S-transferase activity (E) at 37°C pH 6.5

[MEF]mg%	E(U/L) X ± SD, n = 10	fold increase in E
0.00*	1.74 ±0.06	0.00
0.10	1.74±0.06	0.00
0.20	3.47±0.08	0.99
0.40	10.42±0.04	4.99
0.06	10.42±0.00	4.99
0.80	12.15±0.02	5.98
1.00	15.63±0.02	7.98

*Control

Table 4. *In-vitro* effect of quinine (QN) on human erythrocyte glutathione-S-transferase activity (E) at 37°C pH 6.5

[QN)]mg%	E(U/L) X ± SD, n = 10	fold increase in E
0.00*	1.74 ±0.06	0.00
0.10	5.21±0.01	1.99
0.20	5.21±0.01	1.99
0.40	8.68±0.08	3.99
0.06	10.42±0.040	4.99
0.80	12.15±0.00	6.98
1.00	17.36±0.06	8.98

*control

0.40mg%, 0.60mg%, 0.80mg% and 1.00mg%) of each of the anti-malaria drugs: chloroquine, camoquine, mefloquine, quinine, halfan and fansidar.

Statistical Analysis

Student's t-test of statistical significance (Brokes *et al* 1979) was used to analyze the resultant data for statistical significance.

RESULTS

The activity of human erythrocyte GST, as determined for 10 samples, increased significantly ($P<0.001$) and in a concentration-dependent manner in the presence of quinine, chloroquine, camoquine and mefloquine and according to the order chloroquine > quinine > mefloquine>camoquine (Tables 1, 2, 3 and 4).

Halfan (halofantrine) and fansidar, non-quinoline antimalarials, produced no significant change in the activity of human erythrocyte GST at the various concentrations tested (Tables 5 and 6).

DISCUSSION

A lot of work has been done on the properties and functions of liver and kidney forms of glutathione-S-transferase in both experimental animals and man (Boyland and Chasseaud 1969, Ketley *et al* 1975, Awasthi *et al* 1981, Harvey and Beutler 1982) However, the physiological role of this enzyme in the human erythrocyte is not yet fully and conclusively defined. It has been suggested that red cell glutathione-S-transferase functions intracellularly to prevent superoxide-induced haemolysis in addition to curbing the toxic effect of red cell electrophiles/oxidants (Anosike *et al* 1991). The present study has revealed significant ($P<0.001$) *in-vitro* activation of the human erythrocyte GST by the antimalaria drugs quinine, chloroquine, camoquine and mefloquine according to the order: chloroquine> quinine>

Table 5. *In-vitro* effect of halofantrine (HF) on human erythrocyte glutathoine-S-transferase activity(E) at 37°C, pH 6.5

[HF]mg%	E(U/L) $\bar{X} \pm$ SD, n = 10	fold increase in E (%)
0.00*	1.74 ±0.06	0.00
0.10	1.74±0.02	0.00
0.20	1.74±0.04	0.00
0.40	1.74±0.04	0.00
0.06	1.74±0.01	0.00
0.80	1.74±0.01	0.00
1.00	1.74±0.03	0.00

*Control

Table 6. *In-vitro* effect of fansidar (FD) on human erythrocyte glutathoine-S-transferase activity (E) at 37°C, pH 6.5.

[FD]mg%	E(U/L) $\bar{X} \pm$ SD, n = 10	fold increase in E
0.00*	1.74 ±0.06	0.00
0.10	1.74±0.02	0.00
0.20	1.74±0.01	0.00
0.40	1.74±0.02	0.00
0.06	1.74±0.03	0.00
0.80	1.04±0.01	-0.40
1.00	0.69±0.04	-0.60

*Control

mefloquine> camoquine. The activation of this enzyme (GST) by quinine and its congers could suggest a possibility of these drugs to raise, in varied proportions, the oxidant stress of the red cells in the course of their targeted therapeutic actions. In other words, these quinolines could either be acting as electrophiles/oxidants or be involved in the direct generation of red cell electrophiles with the resultant increase in erythrocyte GST activity. This possibility agrees with the suggestion of Anosike *et al* (1991) that red cell GST functions intracellulaly to prevent superoxide induced haemolysis as well as the toxic effects of electrophiles. It is also in agreement with the suggested role of red cell GSTin the protection of cellular constitutents from xenobiotics (Boyland and Chasseaud, 1969).

In his work, Jacoby (1978) suggested that the glutathione-S-transferases help to detoxify certain extremely reactive substances by direct covalent binding of the electrophilic agent to protein. It could therefore be concluded that human erythrocyte GST is involved in the binding deterioration of the quinoline antimalarials and according to the order: chloroquine > quinine > mefloqluine > camoquine.

It has been reported that chloroquine and its congeners exert their action on the erythrocytic malaria parasite by blocking the enzymatic synthesis of DNA (Pearlman, 1975). The drug forms a complex with DNA thereby preventing it from acting as a template for its own replication or transcription to RNA.

However, the finding in this work of the effect of these quinolines on erythrocyte GST could suggest that these drugs in addition to their reported role in inhibiting the replication of plasmodium, could also be acting therapeutically in creating an unfavourable environment for malaria parasite proliferation/survival by raising the oxidant stress of the infested erythrocytes.

Contrary to observed effect of quinine and its congeners, the other antimalarials used in this work, halfan and fansidar, had no significant *in-vitro* effect on human erythrocyte GST activity This could suggest little or no binding deterioration of these drugs by the erythrocyte GST. It could also imply that these drugs, at the concentration used, do not have negative impact on the redox status of the human erythrocyte. It

is hoped that this work would serve as a springboard for a more conclusive *in-vivo* analysis to further establish the individual impact of these antimalarials on the redox status of human erythrocyte.

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