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ACTIVATION OF HUMAN ERYTHROCYTE GLUTATHIONE - S - TRANSFERASE (EC.2.5.1.18) BY SOME ANTIHISTAMINES

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ABSTRACT

Various concentrations (0.05mg%, 0.10mg%, 0.20mg%, 0.30mg%, 0.40mg% and 0.50mg%) of each of the antihistamines – cyproheptadine, chlorpheniramine and klemastin (all H – 1 antagonists), were tested on their possible effect on human erythrocyte (red cell) glutathione – S – transferase (EC. 2.5.1.18) activity. The result indicated significant ($P < 0.001$) activation of the red cell enzyme by all the H – 1 antagonists according to the order: Chlorpheniramine > Cyproheptadine> klemastin.
The activation of human erythrocyte glutathione S – transferase (GST) by the antihistamines was also observed to be concentration – dependent i.e. higher concentrations produced greater activation of the enzyme. For instance, at cyproheptadine concentrations of 0.20mg% and 0.30mg%, red cell GST activity was increased by 4.99 folds (498.85%) and 6.98 folds (694.28%) respectively. Similarly, at the same concentrations (0.20mg% and 0.30mg%), klemastin activated human erythrocyte GST by 2.87 fold (387.36%) and 3.59 fold (359.20%) respectively, while chlorpheniramine produced activations of 7.82 fold (782.18%) and 8.93 fold (893.10%) respectively.
These results may point to a possible binding-deterioration of these drugs by the human erythrocyte GST.

KEYWORDS:

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).

Antihistamines are drugs which inhibit the combination of histamine with histamine receptors and consequently ameliorate and/or eliminate the symptoms of allergies (Frances, 1992 ; Sloan, 2000). These drugs are termed H – 1 or H – 2 receptor antagonists depending on which type of histamine receptor is involved. H – 1 receptor antagonists are used for treating allergies, and H–2 receptor antagonists are used to treat peptic ulcer disease and related conditions (Burkhalter, 1977; Frances, 1992). H – 1 receptor antagonists have been available for many years and include agents such as diphenhydramine, chlorpheniramine, klemastin, cyclizine, cyproheptadine, promethazine and terferadine. Their primary therapeutic use is to antagonize the effects of histamine that is released from cells by antigen-antibody reactions; they can thus inhibit histamine – induced effects, such as bronchoconstriction, skin reactions such as wheals and itching, and nasal inflammation (Burkhalter, 1977; Frances, 1992). The effects of the antihistamines are better realized if they are administered before contact with the relevant antigen. H – 1 receptor antagonists, such as diphenhydramine doxylamine and chlorpheniramine are also used in the treatment of motion sickness (Sloan, 2000). The principal adverse effect of the H – 1 antagonists is sedation while the other effects ascribed to their anticholinergic actions include dry mouth, blurred vision and urinary retention (Burkhalter, 1977). However, the effects of these drugs (H – 1 antagonists) on some key metabolic enzymes such as the red cell GST, remained speculative.

Glutathione–S–transferases (EC.2.5.1.18) are 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– tranferases (GSTs) catalyse the conjugation of electrophilic groups of hydrophobic drugs and xenobiotics to form glutathione (GSH) thiol esters. The thiol esters are in turn converted to mercapturic acid following a sequential action of gamma (M) – 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 GST, protect cellular constitutents from electrophiles and toxic xenobiotics.

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The location of GST in erythrocytes is ideal for the removal of circulating xenobiotics (Marcus *et al* 1978). Recently, the red cell enzyme was found to be involved in the binding deterioration of the antimalaria drug, chloroquine (Ayalogu *et al* 2002). 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 antihistamines, chlorpheniramine, klemastin and cyproheptading 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

Cyproheptadine, klemastin and chlorpheniramine were bought from SmithKline–Beecham pharmaceuticals, France. 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, pH7.0) 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 Zijlstra, 1961).

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. The CDBN 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 preincubated 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 againt a blank containing the reaction mixture without haemolysate.

Effect of the Antihistamines

Red cell glutathione–S–transferase activity was determined in the presence of varied concentrations (0.05mg%, 0.10mg%, 0.20mg%, 0.40mg% and 0.50mg%) of each of the antihistamines: cyproheptadine, klemastin and chlorpheniramine. The drugs were dissolved in the phosphate buffer prior to the assay.

Statistical Analysis:

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

RESULTS

The activity of human erythrocyte glutathione–S–transferase, as determined for 10 samples, increased significantly ($P<0.001$) in a concentration – dependent manner in the presence of the antihistamines: chlorpheniramine, klemastin and cyproheptadine (Tables 1, 2, and 3). For instance, at 0.50mg% concentration of the drugs, erythrocyte GST activity was raised by 16.96 folds (1695.98%), 3.99 folds (398.85%) and 21.06 folds (2106.32%) in the presence of cyproheptadine, klemastin and chlorpheniramine, respectively, in comparison with the control. Comparatively, for each concentration, chlorpheniramine exerted the highest activation of the enzyme while klemastin has the least activatory effect on the erythrocyte GST (Tables 1, 2, and 3).

Table 1: Effect of Klemastin (KM) on red cell Glutathione – S – transferase activity (E) at 37°C, pH 6.5.

[KM] (mg%)	E (U/L) $\bar{X} \pm$ SD, n = 10	% E $\bar{X}$, n = 10	Fold increase in E $\bar{X}$, n = 10
0.00*	1.74 ± 0.00	100.00	0.00
0.05	3.13 ± 0.01	179.89	0.80
0.10	4.08 ± 0.01	234.48	1.35
0.20	6.74 ± 0.03	387.36	2.87
0.30	7.99 ± 0.02	459.20	3.59
0.40	8.16 ± 0.04	468.97	3.69
0.50	8.68 ± 0.02	498.85	3.99

* Control

Table 2: Effect of Cyproheptadine (CH) on red cell Glutathione – S – transferase activity (E) at 37°C, pH 6.5.

[CH] (mg%)	E (U/K) $\bar{x}$±SD; n = 10	% E $\bar{x}$; n = 10	Fold increase in E $\bar{X}$., n = 10
0.00*	1.74 ± 0.00	100.00	0.00
0.05	3.47 ± 0.02	199.43	0.99
0.10	5.47 ± 0.02	314.37	2.14
0.20	10.42 ± 0.03	598.85	4.99
0.30	13.89± 0.02	798.28	6.98
0.40	20.83± 0.04	1197.13	10.97
0.50	31.25± 0.04	1795.98	16 96

* Control.

Table 3: Effect of Chlorpheniramine (CP), on red cell glutathione – S – transferase activity (E) at 37°C, pH 6.5.

[CP] (mg%)	E (U/K) $\bar{x}$ ±SD; n = 10	% E $\bar{x}$; n = 10	Fold increase in E $\bar{X}$.; n = 10
0.00*	1.74 ± 0.00	100.00	0.00
0.05	3.84 ± 0.03	220.69	1.21
0.10	5.75 ± 0.02	330.46	2.30
0.20	15.35 ± 0.03	882.18	7.82
0.30	17.28± 0.04	993.10	8.93
0.40	25.20± 0.05	1678.16	15.78
0.50	38.39± 0.04	2206.32	21.06

* Control.

DISCUSSION

A lot of work has been done on the properties and functions of liver and kidney forms of glutathione – S – transferase (GST) 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 – tranferase 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 antihistamine (H – 1 antagonist) drugs, cyproheptadine, klemastin and chlorpheniramine, according to the order: chlorpheniramine > cyproheptadine > klemastin. The activation of this human erythrocyte enzyme (GST) by these H–1 antagonists 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 antihistamines 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 intracellularly 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 GST in the protection of cellular constituents from xenobiotics (Boy land and Chasseand, 1969).

In his work, Jacoby (1978) suggested that the glutathione – S – transferases help to detoxity 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 antihistamines studied in this work and according to the order: chlorpheniramine > cyproheptadine > klemastin.

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