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IN VITRO EFFECTS OF ASPIRIN AND PARACETAMOL ON HUMAN ERYTHROCYTE GLUTATHIONE-S-TRANSFERASE ACTIVITY.

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ABSTRACT

The activity of glutathione-S-transferase of human erythrocytes was monitored spectrophotometically in the presence and absence of two analgesics (aspirin and paracetamol). Five different concentrations (1.0, 3.0, 5.0, 7.0, 9.0 and 10.0 mg/ml) of each analgesic were used which covers the reported therapeutic and toxic concentration range of the drugs. The drugs were observed to significantly ($P < 0.05$) inhibit the enzyme. Inhibitions greater than 42.0% were observed at 10mg/ml concentrations of the two drugs. Enzyme inhibition studies demonstrated a competitive inhibition pattern for both aspirin and paracetamol. The inhibition pattern was interpreted as indicative of a higher degree of inhibition at reduced levels of the enzyme's substrate, glutathione (GSH).

KEY WORDS: Aspirin, Paracetamol, Erythrocyte, Glutathione S-transferase, Analgesic.

INTRODUCTION

Glutathione S-transferases (EC. 2.5.1.18) are a family of proteins having multiple detoxification functions (Habig *et. al.*, 1974; Keen *et. al.*, 1976, Jacoby, 1978). These enzymes, present in the cytosol of most cells, catalyze the reaction of glutathione with a large group of hydrophobic compounds bearing an electrophilic center (Pabst *et. al.*, 1974).

Human erythrocytes apparently contain a form of glutathione S-transferase (GST) that differs from the forms found in other tissues (Boyland and Chasseaud, 1968). It has been suggested that red cell GST functions primarily as a haem-binding or transport protein (Jacoby, 1978). The second major function of the erythrocyte GST is believed to be the protection of cellular constituents from electrophilic xenobiotic chemicals (Kamisaka *et al*, 1975). It is thought that the preferential reaction of an electrophilic agent with reduced glutathione (GSH) through the enzymatic action of GST prevents its reaction with other cellular nucleophiles (Kamisaka *et al*, 1975). In addition to the protective enzymatic properties of the glutathione S-transferases, they may also detoxify certain extremely reactive substances by the direct covalent binding of the electrophilic agent to the protein (Jacoby, 1978).

Aspirin, also known as acetylsalicylic acid ($C_9H_8O_4$), is a white crystalline compound of molecular weight 180.12 daltons. It is used in the treatment of fever and headaches. It also has wide applications in dermatology (Hardman and Limbird, 1996). The fatal dose of any salicylate is estimated to be 0.2 to 0.5g/kg body weight. Clinically, the principal manifestations of salicylate poisoning are hypernea and disturbed acid-base balance (Harman and Limbird, 1996).

Aspirin has broad spectrum of pharmacological activities. In therapeutic concentrations (0.5 to 2.0μM), salicylates have been noted to uncouple oxidative phosphorylation in the mitochondria, and at much higher concentrations they may inhibit cellular oxidative enzymes, prevent the activation of kinin-forming enzymes, and antagonize peripheral effects of kinins. (Burgen and Mitchel, 1978, Katzung, 2001). The therapeutic concentrations of aspirin range from 0.6g to 4g daily depending on body weight (Katzung, 2001).

Paracetamol, also known as acetaminophen ($C_8H_9NO_2$), is a white crystalline compound of molecular weight 151.16 daltons. The compound is useful for its antipyretic and analgesic effects, but unlike aspirin, it has no anti-rheumatic or anti-inflammatory activity (Goodman and Gilman 1988; Katzung, 2001). However, because of its lack of gastrointestinal side effects, paracetamol is used as a substitute for salicylates in patients with peptic ulcer diseases or who for other reasons cannot tolerate aspirin (Hardman and Limbird, 1996).

The therapeutic and toxic concentrations of paracetamol have been reported to range from 1 to 10mg paracetamol/ml plasma (Hardman and

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Limbird, 1996). Toxic doses can injure the liver, kidney, heart and central nervous system. Liver damage from paracetamol develops within hours as a result of oxidation to toxic metabolites which damage the liver following the depletion of the detoxifying agent, glutathione (Goodman and Gilman 1988; Katzung, 2001).

The reported effect of paracetamol on hepatic glutathione concentration (Goodman and Gilman 1988; Katzung, 2001) as well as the speculated effect of aspirin on cellular oxidative enzymes (Burgen and Mitchel, 1978; Katzung, 2001) informed the current investigation of the possible effects of these analgesics on human erythrocyte glutathione S-transferase, a notable detoxifying enzyme which has glutathione (GSH) as its substrate.

MATERIALS AND METHODS

Reduced glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (CDNB) and absolute ethanol were purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A). Aspirin and paracetamol were purchased from May and Baker, Lagos, Nigeria. All other reagents were of the purest analytical grades commercially available.

Sample collection and preparation

Blood samples were collected by venupuncture (using syringes) from ten volunteers of both sexes aged between 17 and 28 years, into citrate anticoagulant tubes. Erythrocytes were isolated from the whole blood samples, washed thrice with 10 volumes of normal saline and diluted 1:20 with a stabilizing solution (2.7mM EDTA, pH 7.9, 0.7mM 2-mercaptoethanol) as described by Beutler (1984). The haemolysates were pooled and then standardized for haemoglobin using the method of Van-Kampen and Zijlstra (1961).

Enzyme assay

Glutathione S-transferase was assayed spectrophotometrically by monitoring the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with glutathione (GSH) at 340nm and 37°C (Anosike *et. al.*, 1991). The 1ml assay mixture contained 0.5mM CDNB, 1mM GSH and 100mM potassium 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% (v/v). The phosphate buffer-CDNB mixture was preincubated for 10 mins at 37°C and the reaction was initiated by addition of GSH, followed immediately by an aliquot (0.05ml) of the haemolysate. The rate of increase in absorbance at 340nm was measured for 8 mins at 37°C against a blank containing the reaction mixture without haemolysate.

Experimental studies of effect of Aspirin and Paracetamol

Glutathione S-transferase activity was determined in the presence and absence of different concentrations of aspirin (1.0, 3.0, 5.0, 7.0, 9.0 and 10.0 mg/ml). Similar determinations were made using different concentrations of paracetamol (1.0, 3.0, 5.0, 7.0, 9.0 and 10.0 mg/ml) in place of aspirin. The drugs were dissolved in the 100mM phosphate buffer, pH 6.5, and triplicate determinations were made for each concentration. The concentrations used cover the reported therapeutic and toxic concentrations of the drugs (Hardman and Limbird, 1996; Katzung, 2001).

Determination of inhibition pattern

GST activity was determined using the Lineweaver-Burk plot (Lineweaver and Burk, 1934) of the initial velocity rates at varying concentrations of GSH at fixed concentrations of CDNB and at different concentrations (1.0, 5.0 and 9.0 mg/ml) of aspirin. Similar assays were done at similar concentrations (1.0, 5.0 and 9.0mg/ml) of paracetamol.

Statistical Analysis

Student's t-test of statistical significance (Brokes *et al*, 1979) was used to analyse the resultant data for statistical significance. Data were also analyzed by one-way analysis of variance (ANOVA) using Duncan's (1955) multiple range test.

RESULTS

The activity of human red cell glutathione-S-transferase (GST) decreased notably with increasing concentrations of aspirin (i.e. in a concentration-dependent manner). Similar results were obtained in the presence of paracetamol. The results are shown in Tables 1 and 2. The results of the inhibition pattern experiment showed a competitive inhibition pattern by both aspirin and paracetamol on human erythrocyte GST. Figures 1 and 2 are the Lineweaver-Burk plots showing the inhibition pattern. The enzyme was equally inhibited by aspirin and paracetamol with inhibition constants of 1.33mM, 0.83mM and 0.40mM at 1mg/ml, 5mg/ml and 9mg/ml concentrations respectively (Figures 1 and 2).

DISCUSSION

Aspirin and paracetamol are two of the

Table 1: Activity of Glutathione S-transferase (E) in the presence of Aspirin

[Aspirin] mg/ml	*E (iu/g Hb)	%E*
0.0	0.26±0.00	100.00±0.00
1.0	0.24±0.01^{+}	92.30±3.85^{+}
3.0	0.21±0.02^{+}	80.77±7.69^{+}
5.0	0.18±0.01^{+}	69.23±3.85^{+}
7.0	0.16±0.00^{+}	61.54±0.00^{+}
9.0	0.12±0.01^{+}	46.15±3.85^{+}
10.0	0.11±0.02^{+}	42.30±7.69^{+}

*mean±SD; n = 5
$^{+}$ Statistically significant from the control at 95% (P = 0.05) confidence level

Table 2. Activity of Glutathione S-transferase (E) in the presence of Paracetamol

[Paracetamol] mg/ml	*E (iu/g Hb)	%E*
0.0	0.26±0.00	100.00±0.00
1.0	0.24±0.00^{+}	92.31±0.00^{+}
3.0	0.20±0.03^{+}	76.12±11.54^{+}
5.0	0.18±0.02^{+}	69.23±7.69^{+}
7.0	0.14±0.01^{+}	53.85±3.84^{+}
9.0	0.12±0.01^{+}	46.15±3.84^{+}
10.0	0.11±0.00^{+}	42.31±0.00^{+}

*mean±SD; n = 5
$^{+}$ Statistically significant from the control at 95% (P < 0.05) confidence level.

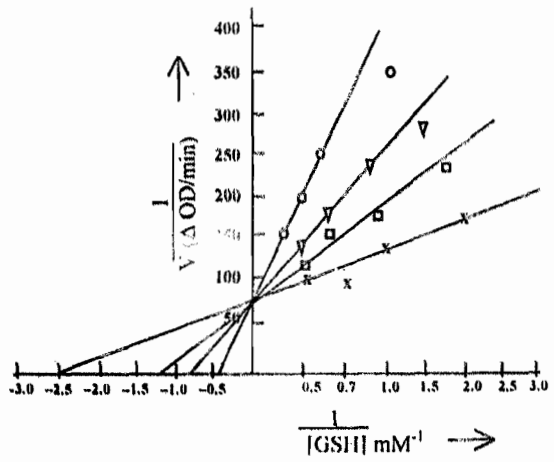

Fig. 1: Lineweaver-Burk plot of inhibition of human erythrocyte glutathione S-transferase by Aspirin.
o—o Control; ∇—∇ 1mg/ml of aspirin; □—□ 5mg/ml. Of aspirin; x—x 9mg/ml. of aspirin.

common analgesics used in the treatment of fever, headache and pains, and usually also in dermatological therapy (Burgen and Mitchel, 1978,

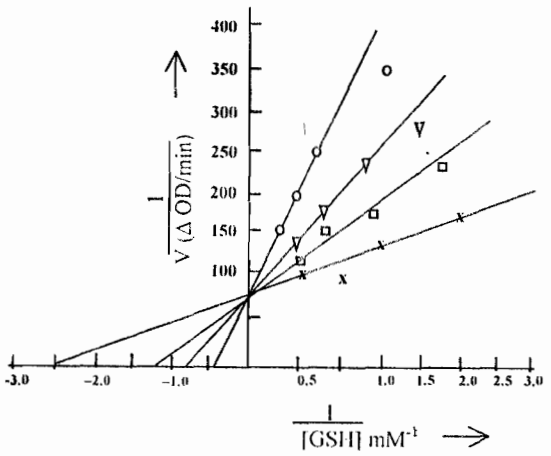

Fig. 2: Lineweaver-Burk plot of inhibition of human erythrocyte glutathione S-transferase by paracetamol.
o—o Control; ∇—∇ 1mg/ml of paracetamol; □—□ 5mg/ml. Of aspirin; x—x 9mg/ml. of paracetamol.

Katzung 2001; Hardman and Limbird, 1996). Incidentally, these drugs are also among the drugs most often abused in their usage without recourse to their possible biochemical and physiological side effects. The present *in vitro* study has established inhibitory effect of the drugs on human erythrocyte glutathione S-transferase (GST). Glutathione S-transferases are a family of proteins, having multiple detoxification functions (Habig *et. al.*, 1974). They are reported to catalyze the reaction of glutathione (a tripeptide) with a large group of hydrophobic compounds (most especially xenobiotics) bearing an electrophilic center (Pabst and Jacoby, 1973; Anosike, *et. al.*, 1991).

Human erythrocyte GST is noted to perform two major functions. The first primary function is as a heam-binding or transport protein (Jacoby, 1978), while the second major function is the protection of cellular constituents from electrophilic xenobiotic chemicals (Kamisaka, 1975). Thus, it could be concluded that human red cell GST plays a major role in the stability/functional status of the erythrocyte. An inhibition of this enzyme, as noted in this study, would therefore imply a disturbance of red cell stability/function. It is possible that increased exposure of red cells to aspirin and paracetamol could precipitate red cell destruction (haemolysis) by electrophilic xenobiotic/free radicals generated by the system.

This can account for the reported hepatic damage by these drugs at reduced GSH concentrations (Burgen and Mitchel, 1978; Goodman and Gilman 1988; Katzung, 2001). The foregoing argument could further be substantiated by the observed competitive inhibition of the enzyme by the drugs (aspirin and paracetamol),

implying a higher inhibition rate, and thus more profound destructive effect at reduced glutathione concentration.

It is therefore advisable that the administration/intake of these drugs be done with utmost caution and only when necessary so as to avoid a possible degenerative effect on cellular systems, most especially on the red blood cells.

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