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STUDIES ON HEPATIC GLUTATHIONE-S-TRANSFERASE SPECIFIC ACTIVITY FOLLOWING PRIMAQUINE PRETREATMENT IN WISTAR ALBINO RATS

V. C. IKPEAZU OKEZIE, DUNSTAN A. A. AKINTONWA , ASHANG B. UTU-BAKU

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ABSTRACT

The administraion of various doses of the antimalarial drug prmaquine,(PMQ), led to dose- dependent decrease in the specific activity of Glutathione-s- transferase in liver subfraction, whole homogenate (WH) and Postmitochondrial supernatant (PMS). The Glutathione-s- transferase specific activity values of 0.15±0.015 (WH); 0.26 ± 0.020 (PMS); 0.13± 0.01 (WH), 0.23 ± 0.013 (PMS) and 0.10 ± 0.010 (WH), 0.18 ± 0.02 (PMS) corresponding to 0.16 mmolPMQ/kilogram body weight (kgbw), 0.32 mmol PMQ/kgbw and 0.64 mmol PMQ/kgbw respectively indicate dose – dependent inhibition ofGlutathione-s- transferase in both subfractions studied. These values were significantly (P <0.01) less than the Dimethyl sulphoxide (DMSO) control groups. Similar observations were made on the effect of PMQ on rat liver protein. Further more, the RNA/protein ratio results, indicate dose – dependent increase from values of 1.3 (WH); 1.1 (PMS) to 1.6 (WH); 1.8 (PMS) corresponding to drug concentraions 0.16 mmol PMQ/kgbw and 0.64 mmol PMQ/kgbw respectively. These results were significantly (P< 0.01) higher than DMSO control values.

KEYWORDS: Primaquine, Pretreatment, Hepatic-glutathione-s-transferase, specific activity.

INTRODUCTION

Primaquine (PMQ), an 8- amino quinoline, is proven anti- malaria drug with both schizonticidal, gametocidal and sporontocidal effects (Clyde, 1981). A single dose of PMQ is capable of reducing parasitemia and PMQ is metaboliszed by 0- demethylation of the 6-methoxy group formed by hydroxylation at the 5 carbon and oxidation to give a quinine derivative, which can undergo oxidation. Despite the effectiveness of this drug as an antimalarial,its appliction in the field of Chemotherapy has suffered due to its side effects. The parent drug and and some of its metabolites have been implicated in the toxicity associated with PMQ therapy (Clyde 1981). For instance, oxidative effect of the drug on the red blood cells leads to the generation of superoxide ion (Akintonwa, 1984). Superoxide ion and other free radicals can interact with glutathione and hence affect membrane integrity via peroxidation mechanism (Goldberg ad Stema, 1976). However, there seems to be a division regading the exact species in PMQ metabolism that elicit these side effects especially its role in the promotion of methemoglobin formation in human red blood cells that are deficient in glucose-6-phosphatase dehydrogenase (Strother et al, 1981). The implication of drug metabolising enzymes in the mechanisms of drug toxicity has been reported (Oesh et al, 1987) Akintonwa, 1985). This view presupposes that if the Metabolites arising from the native compound through the action of phase 1 drug metabolizing enzymes, was the toxic entity, the presence of an inducer like phenobarbitone will enhance the preponderance of the toxic metabolites whereas the inhibition of such enzymes may alleviate the toxicity of the drug (Akintonwa, 1984). For instance, the N-acetylation of hydralazine by the enzyme N-acetyltransferase may lead to the generation of hydrocyl radicals, which are capable of hemolyzing erthrocytes (Akintonwa 1986). From the foregoing, it is clear that the role of drug metabolizing enzymes in the control of reactive metabolites may be more intricate than they were hitherto thought.

Many mutagenic, carinogenic and clinically active metabolites are controlled by several enzymes including deactivating and sequestering enzymes (Oesch, 1986). Therefore, a study of the effect of PMQ on some of these enzymes will create a podium for the elucidation of the mechanisms of its toxicity. Furthermore, the possible effect of the drug on other drugs which are amenable to Gluathione-s-transferase may be exposed.

MATERIALS AND METHODS

Thirty (30), Adult male albino rats of

V. C. IKPEAZU OKEZIE, Department of Applied Biochemistry, Enugu State, University of Science and Technology, Nigeria
DUNSTAN A. A., AKINTONWA Centre for Mechanistic and Theoretical Biochemistry, (CTMB) Calabar
ASHANG B.,UTU-BAKU Department of Chemical Sciences, The Polytechnic, Calabar

wistar strain which weighed between 150 – 200g were used for this study. The animals were maintained under room temperature and on pfizer rat feed throughout the duration of the experiment. PMQ was administered intraperitoneally (0.16,0.32 and 0.64mmol/kg body weight x 4 days) to the test group animals, while the control received by the same means, similar concentration of DMSO. At the end of the fourth day, the animals were killed by anaesthesia with chloroform.

The whole homogenate (WH) was obtained using a hand driven homogenizer in ice cold 0.25M STKM (sucrose, Tris, KCL, and $MgCL_2$ buffer PH 7.4) . The other subfractions were obtained using an MSE centrifuge with a centrifugal radius of 15.5cm, according to the methods of Akintonwa and Itam (1988) as adopted from Conn and stumpf (1976).

Marker enzymes and protein recoveries were used to examine the subfractionation technique (Potter, 1955). Total protein assay was by Biuret method (Donninger, et al, (1972). RNA was assayed using the methods of Fleck and Berg (1965). Glutathione s-transferase activity was estimated based on the principles of substrate analysis using trichlofenvisphos as substrate (Akintonwa and Ikpeazu, 1997). The unit of glutathione –s-transferase activity was defined as the amount of enzyme required to produce a decease in substrate (nmol/minutes).

DATA ANALYSIS

All experiments reported in this study were carried out in triplicates and the mean reported at each point. The student 't' test was used in the comparison of sample means with the control mean as a first approach (Winer, 1962).

RESULTS

The results show that hepatic WH and PMS possessed significant Glutathione-s-transferase activity respectively (Table 1). PMQ administraion significantly ($P<0.01$) impaired the activity of Glutathione-s-transferase relative to the doses administered. When 0.16 mmolPMQ/Kgbw was given, the percentage decrease (% - Δ) was 6.2 (WH),18.7 (PMS) and at 0.32mmolPMQ/Kgbw the % Δwas 18.7 (WH) and 34.0(PMS). The percentage decrease 44.4% (WH) and 53.8% (PMS) was even more pronounced at higher concentration of 0.64 mmol/PMQ/Kgbw.

The results presented in Table 2 indicated that PMQ administration led to dose- dependent decreases in rat liver total protein mg/liver. The total protein values of 326 ± 22.2 (WH) and 166 ± 15.0 (PMS) coressponding to 0.16 mmolPMQ/Kgbw is significantly lower than the DMSO control values of 362 ± 12.8(WH) and 207 ± 12.7 (PMS). Further increases in drug concentration led to greater decreasees in

TABLE 1 THE EFFECTS OF VARYING DOSES OF PRIMAQUINE ON GLUTAHIONE-S-TRANSFERASE SPECIFIC ACTIVITY IN RAT LIVER.

TREATMENT	0.16mmol/Kg b.w		0.32mmol/Kg b.w		0.64mmol/Kg b.w	
SUBFRACTIONS AND WHOLE HOMOGENATE	WH	PMS	WH	PMS	WH	PMS
DMSO (control)	0.16 ± 0.013,	0.32 ± 0.015	0.16 ± 0.013,	0.35 ± 0.012	0.18 ± 0.015,	0.39 ± 0.013
PMQ	0.15 ± 0.015,	0.26 ± 0.020	0.13 ± 0.01,	0.23 ± 0.013	0.10 ± 0.010 ,	0.18 ± 0.02

Each value represents group mean (nmol/min/mg protein) ± SD. (n = 10)

WH and PMS = Rat Liver Subfraction whole Homogenate and post mitochondrial supernatant respectively.

TABLE 2 THE EFFECTS OF VARYING DOSES OF PRIMAQUINE ON RAT LIVER TOTAL PROTEIN.

TREATMENT	0.16mmol/Kg b.w		0.32mmol/Kg b.w		0.64mmol/Kg b.w	
SUBFRACTIONS AND WHOLE HOMOGENATE	WH	PMS	WH	PMS	WH	PMS
DMSO (control)	362 ± 12.8,	207 ± 12.7	380 ± 25.5,	212 ± 9.1	426 ± 10.9,	174 ± 16.9
PMQ	326 ± 22.2,	166 ± 15.0	270 ± 31.4,	146 ± 11.1	260 ± 20.9 ,	116 ± 28.1

Each value represents group mean mg/liver (n = 10)

WH and PMS = Rat Liver Subfraction whole Homogenate and post mitochondrial supernatant respectively.

TABLE 3 THE EFFECTS OF PRIMAQUINE PRETREATMENT ON RAT LIVER RNA.

TREATMENT	0.16mmol/Kg b.w		0.32mmol/Kg b.w		0.64mmol/Kg b.w	
SUBFRACTIONS AND WHOLE HOMOGENATE	WH	PMS	WH	PMS	WH	PMS
DMSO (control)	4.5 ± 0.5,	1.8 ± 0.07	4.2 ± 0.22,	2.0 ± 0.01	4.0 ± 0.23,	2.2 ± 0.19
PMQ	4.3 ± 0.51,	1.9 ± 0.09	4.2 ± 0.27,	2.0 ± 0.09	4.2 ± 0.23 ,	2.2 ± 0.19

Each value represents group mean mg/liver ± SD (N = 10)

WH and PMS = Rat Liver Subfraction whole Homogenate and supernatant respectively.

TABLE 4 THE EFFECTS OF PRIMAQUINE PRETREATMENT ON RAT LIVER RNA/PROTEIN RATIO.

TREATMENT	0.16mmol/Kg b.w		0.32mmol/Kg b.w		0.64mmol/Kg b.w	
SUBFRACTIONS AND WHOLE HOMOGENATE	WH	PMS	WH	PMS	WH	PMS
DMSO (control)	1.2	0.9	1.1	0.9	0.9	1.2
PMQ	1.3	1.1	1.5	1.4	1.6	1.8

WH and PMS Rat Liver Subfractions whole Homogenate and post mitochondrial supernatant respectively

the liver total protein of the treated animals viz 270 ± 31.4 (WH),146 ± 11.1 (PMS) and 260 ± 20.9 (WH); 116 ± 28.1 (PMS), corresponding to 0.32 and 0.64 mmolPMQ/Kgbw respectively.

Table 3 shows the RNA results. There were no significant differences in the RNA values, irrespective of concentrations. The control values 4.5 (WH); 1.8(PMS) and 4.2 (WH);2.0 (PMS) are comparable with test values of 4.3 (WH); 1.9 (PMS) and 4.2(WH); 2.0 (PMS) corresponding to 0.16 and 0.32 mmol/PMQ/Kgbw respectively. Similar values 4.2 (WH); 2.2 (PMS) were obtained at the highest drug concentration of 0.64 mmol PMQ/Kgbw.

When the RNA Protein ratios were computed (Table 4) the profile indicated an increasing trend. RNA/protein ratio values of 1.3 (WH) and 1.1 (PMS) corresponding to 0.16mmol PMQ/Kbw suggests significant increases compared with corresponding control values of 1.2 (WH) and 0.9 (PMS) ($P < 0.01$).

The results obtained from the groups pretreated with higher drug concentration (0.32 or 0.64 mmol PMQ/kgbw) indicate further increases in relation to increasing drug concentrations viz: 1.2 (WH); 1.4 (PMS) and 1.6 (WH); 1.8 (PMS) respectively. The corresponding control values were 1.1 (WH), 0.9 (PMS), 0.9 (WH) and 1.2 (PMS).

DISCUSSION

The basis for the use of RNA/protein ratio approach as an index of enzyme induction (Utu-Baku 1986); induction/inhibition has been reported by other workers (Akintonwa and Archibong, 1987; Attah, 1990). This simple and cheap procedure can be applied as a preliminary assay to assess the effect of biocides on hepatic drug metabolizing enzyme system. The proliferation of the smooth endoplasmic reticulum coupled with associated increases in protein synthesis which accompanies enzyme induction, accounts for the observed decreases in RNA/protein ratio on event of enzyme induction, especially since RNA turnover is stable under these conditions (Wright et al, 1978). The increasing trend obtained in the RNA/protein ratio data presented in this study, could be due to the inhibitory effects of the drug PMQ on protein synthesis. Hence higher doses of the drug led to significantly lower protein values compared to the result obtained from smaller doses. When GST specific activity was monitored in the PMQ pretreated rats, similar dose-dependent decreases were observed, suggesting enzyme inhibition phenomenon predicted by the RNA/protein ratio data. Expectedly, RNA/protein ratio cannot be sufficient as a conclusive index of enzyme induction/inhibition therefore specific enzyme assays must be used to collaborate the RNA/protein ratio data. The toxicological and chemotherapeutic significance of such assessment cannot be over emphasized, especially with regards to key detoxifying enzymes like GST.

The Glutathione-s-transferase (GST) enzyme is amenable to induction and inhibition (Oesch, 1987, Attah, 1990). Other workers have reported the selective impact of inducers like 3 – methyl cholanthrene on drug metabolizing enzyme systems. Back et al (1983) have reported the effects of chloroquine and PMQ in antipyrine metabolism. While PMQ inhibits the metabolism of antipyrine in man, chloroquine does not. Similarly, Attah (1990) has reported the inhibitory effects of chloramphenicol in rat hepatocellar nitroreductase activity.

The implication of the present result is at least two fold: the inhibition of GST by PMQ indicates, that concomitant administration of PMQ with other established inhibitors of GSTmay impair the detoxification of biocides like, benzo(α)pyrene and styrene oxide which are substrates for the enzyme with toxicological implications. Tardiff and Dubios (19680, have reported enhanced toxicity of many drugs due to the inhibition of hepatic drug metabolism by alkylating agents, cyclophosphamide and piporoman. Besides this, however, certain inhibitors, have been employed for beneficial therapeutic goals. For instance, proniacid and pargyline are inhibitors of monoamine oxidase which are used to relieve tuberculosis, mental depression and angina pectoris (Back et al (1983).

Further understanding of drug- drug interaction at the metabolic level will enliven the available literature of mutiple drug therapy and probably enhance the judicious application of drug in chemotherapy.

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