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Biochemical Evaluation of Combination Drug Interaction Between Phenobarbitone and Halofantrine in Weaning Rats

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Abstract: Combination drug interaction between sodium phenobarbitone and halofantrine was studied biochemically in weaning rats. Groups were made according to their body weights. All the rats were sacrificed after 4 days of drug administration and the liver homogenates and blood prepared for biochemical and haematological investigations of cytotoxicity. Results showed that total protein contents, monooxygenase, acid phosphatase and alkaline phosphatase activities in the liver increased with increase in drug administered for all the tests compared with the drug free controls. In the haematological analysis, there was increase down the column with increase in drug for RBC, PCV and Hb while the reverse was the case for WBC (total and differential) compared with the controls. This would suggest that phenobarbitone had an overwhelming sparing effect on the depletion of the haematological parameters and induced their synthesis and lymphocytic maturation but inhibited eosinophil production. The result indicated that phenobarbitone affected the cytotoxic and haematological effects of halofantrine, an antimalarial drug.

Key words: Phenobarbitone, Halofantrine, total protein, monooxygenase, alkaline phosphatase, acid phosphatase, liver, RBC, WBC, Hb, PCV, blood, weaning rats

Introduction

Phenobarbitone, a sedative and hypnotic drug was found to exhibit anticonvulsant at dose level which did not heavily sedate the patient (Crossland, 1980). A barbiturate, phenobarbitone is a model inducer of various drug metabolising drug enzymes including monooxygenase, NADPH - Cytochrome C reductase (Akintonwa ***et al.***, 1993, Zimmermann, 1983). It then appears that if phenobarbitone affects the activities of many enzymes involved in drug metabolism, it will be an important factor in drug-drug interactions. The possible role of phenobarbitone in drug interactions and combination drug therapy had been predicted. Akintowa (1991) reported that if phenobarbitone had been administered in the ethylene-glycol in paracetamol disaster among Nigerian children, the disaster would have been avoided probably, by inducing the glucuronyl transferase pathway.

However, some side effects such as folate deficiency, hypocalcemia and coagulation effects in the newborn have been reported. (Crossland, 1980). The above notwithstanding, combination drug interaction involving not only phenobarbitone but other drugs is currently being investigated. Studies on the interactions between phenobarbitone and antimalarial like fansidar, paludrine (proguanil pal) (1,4-chlorophenyl-5-isopropyl bigaunide) and anagol (Maduka and Yelwa, 1996; Maduka and Mangzsha, 1997; Maduka ***et al.***, 1993); co-administration of pyrimethamine and paracetamol (N-acetyl - aminophenol) (Essien, 1991) have been reported. Essien (1991) reported that the above co-administration in the drug interaction protected rats against the lipid peroxidation effects, in hepatic and renal tissues. It was also reported that 0.64 mmol kg^{-1} body weight choloroquine administration intra-peritoneally killed the rats whereas with simultaneous administration of chloroquine, a 4-amino quinoline antimalarial and phenobarbitone at the same doses, the rats survived (Akintowa, 1991; Akintowa ***et al.***, 1993 and Akintowa, 1992). It is possible that phenobarbitone induced metabolism and increased the rate of clearance of the active metabolites of the drug involved.

Halofantrine (halfan) is a phenanthrene methanol antimalarial which is schizonticidal with a high degree of activity against the asexual erythrocytic stage of malarial infections caused by single or mixed infections of ***Plasmodium falciparum*** or ***Plasmodium vivax***. It has limited effect against the exoerythrocytic or gametocyte stages of malaria parasites (Smith Kline Beecham, 1998). The useful parameters of oxidant induced oxidative stress (Maduka ***et al.***, 1999) which are affected during cytotoxicities and malarial infections - the effect of halofantrine on parameters nor on the cytotoxic enzyme markers after combined drug administration in and around 0.64 mmol body weight of chloroquine or its equivalent antimalarial in experimental animals was not reported, thus limiting comparability. This study was, therefore, carried out to investigate the combined effect of co-administration of phenobarbitone and halofantrine as part of its toxicological evaluation. It was also with a view to find out the mechanism of the observed non-lethality with phenobarbitone action.

Materials and Methods

Drug administration

Male Wister strain weaning rats were stabilized on Pfizer laboratory chow and water for one week in a well ventilated room with 12 h light and 12 h dark cycle. They were then randomly divided into 5 groups each. The rats were treated with phenobarbitone and halofantrine each as a single intraperitoneal administration according to their body weights. Groups I, II and III rats were given 0.08 mmol kg^{-1}, o.16 mmol kg^{-1} and 0.32 mmol kg^{-1} respectively. Group IV rats received the highest doses of 0.64 mmol kg^{-1} body weight while group V served as the control and were not injected with the drug. All the experimental groups had free access to food and water throughout the period of the experiment. The rats were sacrificed after 4 days and their liver homogenates, blood were prepared and used for biochemical and physiological analyses. The procedure followed that described by Akintowa ***et al.*** (1993), Maduka and Yelwa (1996) and Maduka and Mangzsha (1997).

Fractionation of samples and tissue homogenization

All the experimental rats were sacrificed by cervical dislocation after 4 days, their livers excised and pooled into clean properly labeled beakers in ice-cold water. They were weighed and homogenized in 0.25 M sucrose solution (1:5w/v) with Elvehjhem homogenizer. Each homogenate was centrifuged at 3,000 g for 10 min. at 40°C in a refrigerated centrifuge. The supermatants were stored in ice and used in estimating total soluble protein contents, monooxygenase, acid phosphatase and alkaline phosphatase activities. Similarly, blood samples of all the groups were collected separately by cardiac puncture prior to death into clean properly labeled ethylene diamine tetraacetate (EDTA) bottles and then placed in ice-cold water bath. They were used for the determination of RBC, WBC counts (total and differential), PCV and Hb concentration following standard procedure.

Biochemical analysis

Protein contents of the test and control rats (Group 1 to 5) were determined spectrophotometrically at 540nm using Biuret method described by Jayaramen. (1984). Hepatic acid phosphatase activities were assayed by the Goldsby procedure (1972). Hepatic alkaline phosphatase activities were assayed by Verley (1980) procedure using disodium phenyl phosphate as the substrate while the relative monooxygenase activities were assayed by the Stroev and Makarowa (1989) method. Statistical comparison between the various tests groups and their control were done by students t-test (Mead and Curnow, 1983).

Physiological analyses

The haematological parameters namely RBC, WBC counts (total and differential), PVC and Hb conc. were determined following the standard procedure of Ibu and Adeniyi (1989).

Results

The result of the effect of phenobarbitone on the total soluble protein in the livers of the test and control were obtained. There was an increase in total soluble protein content with increase in the doses of drugs administered compared with the controls (Table 1). The result showed that total protein content ranged from 6.07"0.12 mg ml^{-1} liver homogenate for the 1st group (0.08 mmol kg^{-1} body weight) to 7.85 " 0.47 for the 4th test group (0.64 mmol kg^{-1} body weight) and 5.36 " 0.23 mg ml^{-1} liver homogenate in the controls. There were significant increases ($P<0.05$) for the test results compared with the controls suggesting induction of proteins by the phenobarbitone. Also, the increases were dose-dependent and significant among the various test groups ($P<0.05$).

The effect of phenobarbitone and halofantrine metabolism on the hepatic alkaline phosphatase activities were also observed (Table 2) The result showed that alkaline phosphatase activity (mg phenol min^{-1}) was increased significantly in all the tests compared with the control ($P<0.05$) suggesting enzyme induction. The alkaline phosphatase activity increases between group 1 and group 2 were not significant ($P>0.05$) suggesting that the doses were not administered enough for enzyme induction. However, group 3 and group 4 significant increase ($P<0.05$) of

Table 1: Total Soluble Protein content in Liver of Tests and Control Rats of Phenobarbitone and Halofantrine Combination

Treatments (mmol Kg^{-1})	Estimated total protein content (mg ml^{-1})
0.08 (group 1)	6.07±0.12ab
0.16 (group 2)	6.61±0.18ab
0.32 (group 3)	7.50±0.24ab
0.64 (group 4)	7.85±0.47ab
control(group 5)	5.36±0.23

Table shows the mean ± S.E. of 5 determinations

[a]Significantly different from the control (P<0.05)

[b]All the groups are significantly different (P<0.05) from each test

Table 2: The Effect of Phenobarbitone and Halofantrine Metabolism on Hepatic Alkaline Phosphatase and Acid Phosphatase Activities respectively

Treatments (mmol Kg^{-1})	Alkaline phosphatase activity (mg phenol min^{-1})	Acid phosphatase activity (mmol min^{-1})
0.08 (group 1)	0.16±0.005ab	0.08±0.02
0.16 (group 2)	0.18±0.01ab	0.09±0.03
0.32 (group 3)	0.20±0.05ac	0.15±0.10a
0.64 (group 4)	0.25±0.01a	0.39±0.15a
Control(group 5)	0.06±0.01a	0.05±0.01

Values are ± S.E., n=5

[a]Significantly different from control (P<0.05)

[b]Statistically different from group 4 (P<0.05)

[c]Statistically different from group 3 (P<0.05) or higher than group 3

Table 3: The Relative Hepatic Monooxygenase Activity after Treatment with Phenobarbitone and Halofantrine

Treatments (mmol Kg^{-1})	Relative monooxygenase activities (%)
0.08 (group 1)	75.2±12.3a,b
0.16 (group 2)	81.0±10.5a,b
0.32 (group 3)	161.9±13.1a,b
0.64 (group 4)	269.7±19.82a
Control(group 5)	53.10±13.1

Values are ± S.E. of 5 determinations

[a]Significantly different from control (P<0.05)

[b]Statistically different from group 4 (P<0.05)

about 80 and 100% compared with the control value 2.04% was observed. This would seem to suggest several fold increase in alkaline phosphate hence, enzyme induction in the liver.

Table 4: The results of the physiological analysis of the blood of test and control rats

Treatments (mMol Kg^{-1})	RBC count ($\times 10^6$)	WBC Count	PVC	Hb Conc. (g/100 ml)	Differential Leucocytes Counts (%)			
					Neutrophils	Eosinophil	Monocytes	Lymphocytes
0.08 (Group 1)	3.95	6475	26.3	6.8	21	10	20	49
0.16 (Group 2)	4.57	6700	28.0	7.2	16	10	18	56
0.32 (Group 3)	5.01	8500	31.15	7.1	24	10.5	20	45
0.64 (Group 4)	5.17	9825	32.5	6.9	24	6	10	60
Control (Group 5)	5.0	6762	25.7	8.6	33.5	13.5	19	50

The alkaline phosphate activities increased with increase in the doses of the drug administered from group 1 to group 4 compared with the control rats ($P<0.05$), again suggestive of enzyme induction (Table 2). Treatment with phenobarbitone increased acid phosphate activities by 23.23, 36.47, 44.11 and 100% for groups 1, 2, 3 and 4, respectively compared with the control values suggesting an overwhelming effect by phenobarbitone.

The relative enzyme activities increased from groups 1 to 4 compared with the controls (Table 3). Though the increases from groups 1 and 2 were not significant, the activities increased significantly from group 3 and 4 ($P<0.05$) compared with group 5 (controls) again suggesting several fold enzyme induction by phenobarbitone. This same trend of result was essentially obtained in the acid phosphatase activity assays showing that both the acid phosphatase and alkaline phosphatase responded similarly to the metabolism of the two drugs. The result also indicated protection of the liver by phenobarbitone.

The RBC counts increased steadily from group 1 through group 2, 3 and then 4 after an initial fall from control through group 1 and then to group 2 (Table 4). In group 3 increases in RBC counts were observed compared with groups 5 (controls) suggesting an initial inhibition at lower doses of the drug administered followed by a gradual increase from 0.32 mol kg^{-1} drugs. This indicates dose dependent effect. Similarly, the PVC values increased from group 1 to group 4 compared with the controls but the increase seemed to be significant only from group 3 (0.32 mmol kg^{-1}). Eosinophil was synthesized in all the groups and likewise lymphocytic maturation appeared to have been stimulated (Table 4).

Discussion

Combination drug interaction is currently in vogue in drug - drug interaction used in the management or therapy of some diseases such as malaria, diabetes and lipid borne diseases. Phenobarbitone had demonstrated an overwhelming influence in such cases. For instance, phenobarbitone would have induced the glucuronyl transferase pathway to avoid the ethylene glycol disaster of the Nigerian children reported in 1991(Akintowa, 1991). The earlier report on combination drug interaction Yelwa, 1996; Maduka and Mangzsha, 1997) tried to demonstrate a modulating role by phenobarbitone in the metabolism of fansidar and proguanil, antimalarial.

The result showed that phenobarbitone had induced some marker enzymes of cytotoxicity namely alkaline phosphatase and acid phosphatase activities in the liver during halfan metabolism in a dose-related pattern. Phenobarbitone also spared tissue depletion of haematological parameters - RBC, Hb and PVC suggesting a biological response to halfan metabolism through

maturation of red cell factors. Similarly, eosinophil production was reduced suggesting minimal hypersensitivity reactions associated with 4 - amino quinoline therapy. The experimental observations of induced lymphocytic maturation also suggested some positive effects on the cell-mediated immune response. The results of this study were not at par with earlier works on phenobarbitone in combined therapy with some anti-malarial. During malaria infection, the haematological status of individuals were compromised with attendant elevation of the phosphatase and serum transaminase (Smith Klin Beecham, 1998). Elevated activities of the transaminase and phosphatase were toxic responses manifested during cytotoxic disorders. Amelioration of the above responses and particularly, induced PCV, RBC and Hb by phenobarbitone being reported in this study would lay credence to the fact that phenobarbitone and indeed, allied barbiturates might be important in the management of malaria.

The role of phenobarbitone, an enzyme inducer was just one example of possible efficacy of combined drug interaction in drug metabolism. The combined effect of garlic and wild honey on hypoglycemic effects and garlic in supplementation with vitamins E and C reducing garlic-induced anaemia have been reported (Umar ***et al.***, 1998, 2002). The mechanism of phenobarbitone observed overwhelming effect has not been worked out in combination during interaction ***in vivo*** but may include enzyme induction as well as induction of haematological parameters which are known to be peroxidized during oxidative stress by oxidants (Maduka ***et al.***, 1999). The possible mechanism of the observed effects of phenobarbitone and possibly pin-point the particular points of action on the various metabolic pathways are being studied.

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