EFFECT OF FOLIC ACID AND VITAMIN B₁₂ ADMINISTRATION ON PHENYTOIN INDUCED TOXICITY IN RATS

Itemobong S. Ekaidem*, Monday I. Akpanabiatu, Friday E. Uboh and Offiong U. Eka

*Department of Chemical Pathology, University of Uyo, P.M.B. 1017, Uyo and
Department of Biochemistry University of Calabar, P. M. B. 1115, Calabar – Nigeria

ABSTRACT

Folic acid and vitamin B₁₂ are very important vitamins needed for normal cellular metabolic activities. The effects of folic acid and vitamin B₁₂ on liver integrity of growing Wistar albino rats following therapeutic dose of phenytoin administration were investigated. The activities of serum AST, ALT, ALP were investigated. Serum total protein level and lipid profile were also measured as indices of biochemical changes. The ingestion of phenytoin alone in rats significantly reduced serum protein while AST, ALT activities increased as compared to the control ($P < 0.05$). Supplementation of phenytoin with oral administration of 70 microgram/kg body wt of folic acid resulted in a significant reversal in serum total protein and suppression in serum AST and ALT activities. Vitamin B₁₂ supplementation did not afford any significant protection against the effect of phenytoin ingestion but rather phenytoin toxicity was exacerbated in this study. However, the combined effects of vitamin B₁₂ and folic acid ameliorated the effects of phenytoin on serum enzymes of experimental rats. The effect of combination of phenytoin with folic acid or folic acid and vitamin B₁₂ is an interesting finding. Supplementation of phenytoin with folic acid or combination of these vitamins may be recommended for the purpose of ameliorating the adverse biochemical changes which are associated with phenytoin therapy. Further work is ongoing to help elucidate the effects of phenytoin and these vitamins on oxidative stress inducing mechanism.

KEY WORDS

Phenytoin, Biochemical changes, Folic acid, Vitamin B₁₂, Serum enzymes.

INTRODUCTION

The effect of vitamins on enzyme systems and other cellular activity have become one of the most explored fields of scientific study. A wide variety of pharmacological and chemical agents are known to produce a range of acute or chronic liver diseases. Phenytoin, a hydantion anticonvulsant used widely in treatment of seizure (1, 2) is used for any generalized or partial seizure type, except for absence of seizures where it may worsen the condition. The complication of phenytoin therapy has called for serious concern since this drug is capable of causing alteration in tissue integrity (3, 4). It has been reported that hepatotoxicity of phenytoin is usually

accompanied by rashes, fever, lymphadenopathy and eosinophilia and these observations suggest that the mechanism of toxicity may be that of hypersensitivity (5-7). Phenytoin hypersensitivity syndrome is not fully understood and is not related to drug dosage or serum levels (8). However the cytotoxic activity and immunologic response are associated with the intermediate metabolites of phenytoin called arene oxides (7, 9). Phenytoin administration has been reported to result in altered folate and vitamin B₁₂ metabolism. Both serum and red cell levels of these vitamins have been altered in over 50% of patients on phenytoin therapy (10, 11). Serum folate decreases when phenytoin therapy is initiated alone with no folate supplementation and folic acid supplementation in folate deficient patients with epilepsy changes the pharmacokinetics of phenytoin, usually leading to lower serum phenytoin concentrations and possible seizure breakthrough (11).

Several suggestions have been made in an attempt to deduce the mechanism by which phenytoin alter folate metabolism

Address for Correspondence :

Dr. Monday I. Akpanabiatu

Department of Biochemistry University of Calabar,
P. M. B. 1115, Calabar – Nigeria
Email: akpanabiatu@yahoo.com

(11, 12). Porter and Meldrum (13), proposed that phenytoin causes mal-absorption of dietary folate by inhibiting a conjugase, an enzyme that converts the polyglutamate form of folate to the absorbable monoglutamate. It has been recommended that folic acid supplementation should be initiated each time phenytoin therapy commences. This is because of the hypothesized cofactor mechanism, decreased adverse effects associated with folate deficiency and better seizure control with no perturbation of phenytoin pharmacokinetics (11).

Phenytoin induced depletion of folate in rats have been reported to originate in the liver and it involved a mechanism that did not discriminate folate forms. Also it causes depletion of total hepatic folate to about 50% (12). There are several reports on the effect of drugs on liver function and phenytoin is included. Equally, incidence of convulsion from diverse causes, especially among the children and the use of phenytoin as a result is increasing. It has also been established that most of our locally sourced daily meals are grossly inadequate in essential micronutrients such as vitamins hence, the general practice of vitamin supplementation in our daily meals.

Presently literature on folate and vitamin B₁₂ interaction on phenytoin toxicity is scanty. The present study emphasizes the interaction of these agents in modulating or preventing the toxicity of phenytoin in experimental rats.

MATERIALS AND METHODS

Growing adult male and female albino rats of the Wistar strain (135 - 150g) were obtained from the Biochemistry Department Animal house in the Faculty of Basic Medical Science, University of Calabar, Calabar. The animals were kept in a well ventilated standard laboratory condition in the experimental section of the animal house, at room temperature. The animals were fed with normal rat formular (Pfizer Livestock Co. Ltd., Aba, Nigeria). Both the experimental and control animals had free access to both rat chow and water during the experimental period. The animals were randomly divided into 5 groups of 8 rats each. Group 1 animals served as the control and were gavaged normal saline (1.0 ml). Groups 2, 3, 4, and 5 received phenytoin, phenytoin + folic acid, phenytoin + vitamin B₁₂ and phenytoin + vitamin B₁₂ + folic acid respectively.

Administration of phenytoin, folic acid and vitamin B₁₂ : Commercially available phenytoin capsules were obtained from Parke-Davis Hoofireg; Vitamin B₁₂ and folic acid tablets were obtained from Vitabiotics (Nig) Ltd, Lagos, Nigeria. The

choice of commercial products was to reflect the condition of those under the medication of these drugs. Phenytoin: 5mg/kg body weight of rat, folic acid: 70 microgram/kg body weight of rat and vitamin B₁₂: 15 microgram/kg body weight of rat. Phenytoin and folic acid were administered daily by oral intubations whilst vitamin B₁₂ was administered intraperitoneally twice a week. Treatment lasted for 4 weeks.

The animals were sacrificed and blood samples collected into plain sample tubes for sera preparation. The blood samples were allowed to clot at room temperature for 2 h. Sera were separated by centrifugation at 3000 g for 5 min using bench top centrifuge (MSE Minor, England). Sera were separated into sterile plain tubes and stored in the refrigerator for analysis. All analysis on serum samples were completed within 24 h of sample collection.

Biochemical determinations : Serum protein, lipid profile and hepatic enzymes of clinical significance were measured in serum samples spectrophotometrically, using Randox kits (Randox Laboratory Ltd., Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured according to the method of Reitman and Frankel (13). Serum alkaline phosphatase activity was determined by a kinetic method according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (14). Serum total protein was analyzed using biuret kit method (15). Total cholesterol, triglyceride and HDL-cholesterol were measured by enzymatic colorimetric endpoint methods (16). LDL-cholesterol was obtained by calculation using the formula provided in Randox HDL-cholesterol kit booklet. The absorbances of all the tests were determined using spectrophotometer (HAICH, DR 3000, Germany). Statistical analysis was carried out by employing student's t-test, a probability of 0.05 being used as a level of significance.

RESULTS

The effect of folic acid and vitamin B₁₂ administration on phenytoin induced changes in total serum protein and liver function enzymes activities and changes in serum lipid profiles of albino Wistar rats are shown in Table 1 and 2. The activities of AST, ALT and ALP were significantly higher (36.39, 21.37 and 22.28 % respectively) while total serum protein was significantly lower (30.15 %) in phenytoin treated rats as compared to the control ($P \leq 0.05$). Serum enzymes activities and total protein concentration in groups 3 and 5 did not change significantly ($P \geq 0.05$). Following recovery the supplementation of phenytoin with folic acid in group 3 resulted

Table 1. Effect of folic acid and vitamin B₁₂ administration on phenytoin induced liver enzyme activities and serum protein level.

Group	Total serum protein (gm/dl)	Aspartate amino transferase (u/L)	Alanine amino transferase (u/L)	Alkaline phosphatase (u/L)
Control (1)	8.69 ± 0.31	34.67 ± 8.50	23.63 ± 4.23	118.10 ± 20.95
Phenytoin (2)	6.07 ± 0.47*	54.50 ± 5.95**	33.17 ± 3.39*	151.97 ± 30.83**
Phenytoin+ folic acid (3)	8.35 ± 0.45	35.17 ± 6.38	24.00 ± 4.24	130.96 ± 31.36*
Phenytoin+ vit. B ₁₂ (4)	6.31 ± 0.51*	73.17 ± 5.56**	39.50 ± 5.91*	192.03 ± 34.64**
Phenytoin+ folic acid +vit. B ₁₂ (5)	8.32 ± 0.49	37.00 ± 6.60	26.33 ± 4.08	139.35 ± 28.25*

Mean ± SD of 8 determinations. * p < 0.05, **p < 0.01 compared to control; (Student's t-test).

in a decrease in serum AST (35.47 %), ALT (20.45 %), ALP (13.83 %) except serum total protein which increased by 27.31 % when compared with group 2 that were given phenytoin only. Combining phenytoin with B₁₂ showed further increase in enzymes activity when compared with the group on phenytoin therapy only. However, no significant difference was observed in serum total protein. The concomitant effects of supplementing phenytoin administration with folic acid and vitamin B₁₂ reduced the activities of AST, ALT and ALP by 32.11, 12.73 and 8.30 % respectively to about the control values. Also serum total protein levels were restored.

The effect of vitamins (folic acid and B₁₂) administration on phenytoin induced changes in serum lipid profile is shown in Table 2. There were significantly (P ≤ 0.05) increased serum total cholesterol (22.34 %), LDL-cholesterol (50.58 %), VLDL-cholesterol (29.17 %) and triacylglycerol (26.47 %) levels by phenytoin administration in group 2 animals. However, folic acid supplementation resulted in normal lipid profile. In groups 4 and 5 serum triacylglycerol (29.25 and 25.00 %) and VLDL (29.17 and 24.44 %) increased significantly while in group 5 LDL-cholesterol showed significant (P ≤ 0.05) decrease (40.17 %) when compared to control.

DISCUSSION

Treatment with phenytoin after for 4 weeks showed a significant alteration in the value of serum total protein indicating that cellular integrity may have been affected since proteins are building blocks in the body. Decreased protein level is also a sign of increased catabolism than anabolism due to alteration in the synthesis of protein in the liver of phenytoin treated rats. The activities of AST, ALT and ALP were significantly increased in phenytoin treated rats as compared to the controls (P ≤ 0.05). Phenytoin therapy have resulted in raised activities of serum AST and ALT (14). The changes in these enzymes as found in this study show that phenytoin is capable of affecting both mitochondrial and cytosolic enzyme activities. Since ALP is important in general metabolic activities, its increased level after phenytoin administration signals an increase in cellular metabolism.

Phenytoin supplementation with folic acid significantly decreased the influence of phenytoin on liver enzymes by reversing AST and ALT to values slightly above that of control. Alkaline phosphatase activity also decreased but to a level higher than that of control. Folic acid supplementation resulted in complete reversal of the effect of phenytoin on serum protein.

Table 2. Effect of folic acid and vitamin B₁₂ administration on serum lipid profile during phenytoin induced toxicity in rats

Group	Total-cholesterol (mmol/L)	Triglycerol (mmol/L)	HDL-cholesterol (mmol/L)	VLDL-cholesterol (mmol/L)	LDL-cholesterol (mmol/L)
Control (1)	2.12 ± 0.34	0.75 ± 0.14	1.57 ± 0.19	0.34 ± 0.01	0.209 ± 0.02
Phenytoin (2)	2.69 ± 0.43*	1.02 ± 0.19*	1.78 ± 0.13	0.48 ± 0.03*	0.422 ± 0.18*
Phenytoin + folic acid (3)	2.16 ± 0.36	0.84 ± 0.35	1.60 ± 0.12	0.37 ± 0.012	0.183 ± 0.014
Phenytoin + vit. B ₁₂ (4)	2.23 ± 0.48	1.06 ± 0.18*	1.53 ± 0.14	0.48 ± 0.05*	0.22 ± 0.00
Phenytoin + folic acid + vit. B ₁₂ (5)	2.20 ± 0.31	1.00 ± 0.27*	1.64 ± 0.15	0.45 ± 0.07*	0.125 ± 0.012*

Mean ± SD of 8 determinations. * P < 0.05, compared to control (Student's t-test).

Folic acid plays vital roles in several enzymes activities in the body and folate is essential for one carbon metabolism. This is however, not unconnected to the fact that folic acid causes a fall in serum phenytoin level. Also the hydroxylase, an enzyme involved in the metabolism of phenytoin is folate dependent (15).

Phenytoin is reported to cause change in the metabolism of folate and vitamin B₁₂ (11). However, the effect of vitamin B₁₂ supplementation did not influence the toxicity of phenytoin as the raised level of the enzymes activities, which were raised by phenytoin remained almost the same. The level of serum protein also followed the same trend. This results showed that the metabolism of phenytoin may not require vitamin i.e. vitamin B₁₂ is not a coenzyme in phenytoin metabolism. The mechanism by which vitamin B₁₂ is altered by phenytoin is therefore a subject that presents unanswered questions.

The observation of increase in the activities of liver function enzymes in response to concomitant supplementation of phenytoin with folic acid and vitamin B₁₂ is an interesting finding. Though the ameliorating effect of this combination was significantly less than that of folic acid, it could be seen from the result that the presence of vitamin B₁₂ showed no effect on the influence of folate in phenytoin metabolism. The levels of serum AST, ALT and ALP were significantly higher than the control (P < 0.05). The serum total protein concentration of group 5 (phenytoin + folic acid + vitamin B₁₂) remained the same as in the control, indicating little or no alteration in metabolic pathways.

It is evident from Table 2 that phenytoin induced increase in serum lipid profile was significant except in HDL-cholesterol. This result tally with the report on increase serum cholesterol and triglyceride levels in healthy volunteers and epileptic patient treated with phenytoin (16). This shows that phenytoin may have some influence on lipid metabolism and its atherosclerotic risk could be ameliorated by folic acid / vitamin B₁₂ or both. The influence of vitamin B₁₂ and folic acid on phenytoin altered lipid metabolism may not be unconnected with their co-operative action on 5-deoxyadenosylcobalamin, a coenzyme of L- methylmalonyl-CoA mutase which catalyses the conversion of L-methylmalonyl CoA to succinyl-CoA, an important reaction in energy production from fat and protein.

The mechanism by which folic acid supplementation reduced liver dysfunction is not clear but liver tissues have been known of hepatocytes proliferation as a means of combating xenobiotic toxicity. The roles of folic acid in DNA synthesis and energy metabolism are well known facts. The picture of

serum enzymes in this study suggest that more damage may have been done to the mitochondria than the cytoplasm and the pathology in this work may be more of the heart than the liver (17-19).

Our results are in harmony with that of earlier workers but we have also found that vitamin B₁₂ exacerbated the toxicity of phenytoin and also decreased the ameliorating effect of folic acid on phenytoin toxicity. Since it is observed that vitamin B₁₂ decreases the effect of folic acid in phenytoin metabolism in this study, we therefore suggest the use of folic acid only in the supplementation of phenytoin in treating seizures. This we believe will give a better clinical result than concomitant administration of phenytoin, folic acid and vitamin B₁₂ or phenytoin and B-complex.

REFERENCES

1. Merrit HH, Putman TJ. Sodium diphenylhydantoin in treatment of convulsion disorders. *J Am Assoc* 1938;3: 1068-73.
2. Smythe MA, Umstead GS. Phenytoin hepatotoxicity: a review of the literature. *Ann Pharmacother* 1989;23:13-8.
3. Vittorio CC, Muglia JJ. Anticonvulsant hypersensitivity syndrome. *Arch Int Med* 1995;155:2285-93.
4. Bethesda MD. Hydrantoin. In: McEvoy, G.K, editor. American hospital formulary service drug information 98. American Society of Health System Pharmacists. 1998. p. 1750-55.
5. Howard PA, Engen PL, Dunn MI. Phenytoin hypersensitivity syndrome: a case report. *DICP. Ann Pharmacother* 1991; 25: 929-32.
6. Garnett WR. Epilepsy. In: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Walls, BG, Posey LM eds., *Pharmacotherapy: a pathophysiologic approach* 3rd ed. Stamford, CT: Appleton & Lange; 1997. p. 1187-91.
7. Havill S. Anti-convulsant hypersensitivity syndrome. *Prescribers update* 1998;16: 28-31.
8. Gupta R. Phenytoin adverse drug reactions and monitoring. *P & T forum. University of Virginia Health system Newsletter.* 1999;4(5): 1-7
9. Gennis MA, Vemuri R, Burns EA, Hill JV, Miller MA, Spielberg SP. Familial occurrence of hypersensitivity to phenytoin. *Am J Med* 1991;91:631-4.
10. Froscher W, Maree V, Laage M, Wolfersdorf M, Straub R, Rothmeier J, Steinert T, Fiaaux A, Frank U, Grupp D. Folate deficiency, anticonvulsant drugs and psychiatric morbidity. *Clin Neuropharmacol* 1995;18(2): 165-82.

11. Lewis DP, VanDyke DC, Willhite LA, Stumbo PJ, Berg MJ. Phenytoin-folic acid interaction. *Ann Pharmacol* 1995; 29:726-35.
12. Carl GF, Hudson FZ, McGuire BS. Phenytoin induced depletion of folate in rats originates in liver and involves a mechanism that does not discriminate folate form. *J Nutr* 1997;127(127):2231-8.
13. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate transferase and glutamate pyruvate transferase. *Am J Clin Pathol* 1957; 28:56-63.
14. Rec. GSCC (Deutsche Gesellschaft fur klinische chemie); *J Clin Chem. Clin Biochem* 1972;10: 182.
15. Tietz NW. *Clinical Guide to Laboratory Tests*. 3rd ed. Philadelphia PA: W.B. Saunders Company; 1995. p. 518-9.
16. Trinder P. Quantitative determination of triglyceride using GPO-PAP method. *Ann Biochem* 1969;6:24-7.
17. Porter RJ, Meldrum B, Meldrum BS. Anti-epileptic drugs. In: Katzung, B.G. editor. *Basic and clinical pharmacology*. 7th ed. Stanford: Appleton & Lange;1998. p. 386-408.
18. Richens A, Perucca E. Clinical pharmacology and medical treatment. In: Laidlow J, Richens A, Chadwick D, editors. *A textbook of epilepsy*. 4th ed. London: Livingstone Company; 1993. p. 495-559.
19. Berg MJ, Ficham RW, Ebert BE, Schottelius DD. Phenytoin pharmacokinetics: before and after folic acid administration. *Epilepsia* 1992; 33:712-20
20. Hacroft D. Diagnostic enzymology: In: James AM, editor. *Analytical chemistry by open learning*. 1st ed. New York: John Willy & Son; 1987. p. 156-210
21. Stroev EA, Makaraova VG. *Laboratory manual in biochemistry*. Moscow: Mir, Publishers;1989. p.162-4.
22. Akpanabiatu MI, Igiri O, Eyong EO, Eteng MU. Biochemical and histological effects of *Eleophorbia drupifera* leaf extract in Wistar albino rats. *Pharmaceut Biol* 2003; 41(2):96-9.