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Vitamin b₁₂ supplementation: effects on some biochemical and haematological indices of rats on phenytoin administration

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Abstract

Phenytoin is known to have some toxicological implications. Vitamin B_{12} supplementation during phenytoin administration was investigated to assess the benefits and risks of single vitamin supplementation. This study evaluated the biochemical and haematological effects of vitamin B_{12} on phenytoin toxicity. Twenty-four experimental animals were divided into 3 groups of 8 rats each. The control (group 1) received distilled water as placebo. Groups 2 and 3 were given 5mg/kg body weight of phenytoin for 4 weeks while group 3 in addition to phenytoin received intra-peritoneal administration of 15μg/kg body of vitamin B_{12} twice a week. Biochemical parameters such as AST, ALT, ALP, lipid profile and haematological indices were assayed as indices of toxicity. The result of the study showed that phenytoin administration resulted in anaemia which was ameliorated by vitamin B_{12} co-administration. Phenytoin also increased significantly the leukocyte count upon which B_{12} had no effect. Liver enzymes activities were significantly ($p<0.05$) raised during phenytoin administration and interestingly B_{12} further increased the level of these enzymes. Administration of phenytoin only gave a significant ($p<0.05$) increase in the level of serum Low density lipoprotein cholesterol. Serum cholesterol, TG and HDL-chol were not significantly affected. Although there was no significant change in serum cholesterol, the slight increase was more than 1% which is capable of causing a 3% increase in the risk of coronary heart disease. A significant decrease was also noted when phenytoin was supplemented with B_{12}. We observed that vitamin B_{12} co-administration is beneficial in remitting anaemia and the atherosclerotic risk caused by phenytoin but may enhance hepatotoxicity. By this result we would therefore suggest that the use of vitamin B_{12} alone as supplement during phenytoin administration be discouraged.

Key words: Phenytoin, serum enzymes, lipid profile, vitamin B_{12}, atherosclerosis, hepatotoxicity.

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INTRODUCTION

Vitamin supplementation is known to impart significant benefits in terms of disease prevention and treatments and has been widely accepted as a measure of control of micro nutrient deficiencies. Vitamin B_{12}, generally called cobalamin, is a porphyrin like ring compound with central cobalt atom attached to a nucleotide. Its anti-anaemic function has been known for years. Recent studies have shown that appropriate amount of vitamin B_{12} can protect against dementia[1,2], boost immune function and maintain the nervous system[3]. Also, vitamin B_{12} lowers homocysteine levels and protects against atherosclerosis and other cardiovascular disease[4,5], as well as certain neurological diseases associated with increased homocysteine including Alzheirmer's diseases [6,7]; depression and schizophrenia[8]. Vitamin B_{12} also plays a vital role in maintaining methylation reactions that repair DNA and hence prevents cancer[9,10].

On the other hand, vitamin supplementation during some specific drug therapy in diseases may be deleterious to health and hence defeat the very purpose of its administration[11]. These effects may be due to drug nutrient interactions resulting in either alteration in absorption and metabolism of the vitamin or increased metabolic clearance of the drug hence compromised therapeutic benefit. A number of drugs are known to reduce the absorption of vitamin B_{12} in the gastrointestinal tract. These include proton-pump inhibitors (e. g. omeprazole, lansoprazole) and H_2- receptor antagonists (e. g. Tagamet, pepsid, zanatac). These drugs markedly decrease stomach acid secretion required to release dietary vitamin B_{12} from foods, thus long-term use of proton-pump inhibitors were associated with decreased level of vitamin B_{12} in the blood[12]; however, long-term use of H_2- receptor antagonists did not result in vitamin B_{12} deficiency since the action of the drug is not always prolonged[13]. Metformin decreases vitamin B_{12} absorption by tying up free calcium required for absorption of the IF-B_{12} complex [14]. Other drugs which inhibit vitamin B_{12} absorption are cholestyramine, chloramphenicol, neomycin and colchicines. Nitrous oxide inhibits the two vitamin B_{12} dependent enzymes and hence can produce many of the clinical features of vitamine B_{12} deficiency[15].

Phenytoin is a hydantoin anticonvulsant used widely in the management of generalized and partial seizures[16,17]. It has been shown to alter the bioavailability and metabolism of vitamin B_{12} and folic acid[18,19]. Over 50% of patients on long-term phenytoin therapy demonstrated low serum and red cell levels of vitamin B_{12} and folic acid[20-22]. Several suggestions have been made in attempts to explain the mechanism by which phenytoin alters the metabolism of folates[18,20,23], however, reports on phenytoin-vitamin B_{12} interaction are quite scanty. Since some of the actions of vitamin B_{12} are dependent on folate cofactor, one may suggest that the altered level of B_{12} by phenytoin is related to the phenytoin induced folate deficiency. This argument is based on the observation that administration of folic acid resulted in elevated serum level of vitamin B_{12} in epileptic patients[22].

The complication of phenytoin therapy has called for serious concern since the drug is capable of disrupting tissue integrity[24,25]. Anaemia and hepatotoxicity are common findings during phenytoin therapy[17,26]. The heapatotoxicity of phenytoin was accompanied by rashes, fever, lymphadenopathy and eosinophilia, which suggest that the mechanism of toxicity may be a hypersensitive reaction[27, 28]. The phenytoin hypersensitivity syndrome is not fully understood. However, phenytoin metabolism which is mediated by a group of mixed-function oxidases known as cytochrome P_{450} and the intermediate metabolites, known as arene oxides, are important to the immunological responses. The cytotoxic activity of these oxides has been reported and epoxide hydroxylase are responsible for their detoxification[26]. Indivuals that developed phenytoin hypersensitivity syndrome are reported to have lost the ability to detoxify arene oxides and it is believed that family members may have similar inability to metabolize arene oxides, thereby confirming the report in familial cases[29]. Folate is hypothesized to be a cofactor in phenytoin metabolism and may be responsible for the changes in pharmacokinetics of phenytoin usually leading to lower serum

phenytoin concentration and seizure breakthrough in patients taking folate supplementation[20]. The involvement of vitamin B_{12} in the metabolism of phenytoin and a possible modulatory role of vitamin B_{12} on phenytoin toxicity in patients have not been established.

The use of vitamin supplement in both health and diseases; especially in areas, such as Nigeria, where locally sourced daily diets are deficient in essential vitamins; is highly encouraged in view of its health benefits. However, the health risk of individual vitamin used alone and in combination with others in certain disease conditions where drug therapy is instituted is sometimes overlooked. Against this background, we examined the effects of vitamin B_{12} supplement on phenytoin toxicity using experimental rats.

MATERIALS AND METHODS

Animals

Twenty-four growing albino wistar rats weighing 120g to 160g were obtained from the Department of Biochemistry, University of Calabar, Calabar, Nigeria. The animals were kept in a well-ventilated room of standard laboratory condition. They were fed with normal rat formula (Pfizer livestock Co. Ltd. Aba, Nigeria). All the animals were randomly divided into three groups of eight rats each. The control (group 1) received distilled water as placebo. Group 2 and 3 were treated with phenytoin and group 3 in addition to phenytoin received vitamin $B_{12.}$

Administration of phenytoin and vitamin b_{12}

Commercially available phenytoin capsules were obtained form Parke-Davis, Hoofirea. Vitamin B_{12} vials were obtained from Vitabiotic (Nigeria) Ltd. Lagos, Nigeria. 5mg/kg body weight of phenytoin was administered orally to all rats in group 2 and 3; while 15μg/kg of cyanocobalamin (vitamin B_{12}) was given intraperitoneally twice a week to rats in group 3 in addition to phenytoin. The treatment lasted for four weeks.

The animals were sacrificed by suffocation in chloroform fumes and blood collected by cardiac puncture. The blood samples were divided into two. One part was collected into EDTA tubes for haemoglobin, PCV and white blood cell count determinations; while the other part was collected into plain tubes and allowed to clot and retract at room temperature for two hours. Sera were separated by centrifugation at 3000g for 5min using bench top centrifuge (MSE minor England). The sera were collected into sterile plain tubes and stored in refrigerators for analysis. Haemoglobin determinations were performed immediately while all analyses on serum were completed within 24 hours of sample collection.

Heamatocrit Determination

Haematocrit was estimated by using the method of Alexander and Griffiths[30]. Haematocrit tubes were filled by capillary action to the mark with whole blood and bottom end of the tubes were sealed with plasticide and centrifuged for 4 minutes using haematocrit centrifuge. The percentage cell volume was read by sliding the tube along a "critocap" chart until the meniscus of the plasma intersects the 100% line.

Haemoglobin Determination

Cyanmethaemoglobin (Drabkin) method of haemoglobin estimation[31] was employed. 0.02ml of anticoagulated whole blood sample was added to 5ml of Drabkin reagent, mixed and incubated for 5 minutes at room temperature for the colour to develop and the absorbance was read against reagent blank at 540nm using DR 3000 spectrophotometer.

Total White Blood Cell Count

The estimation of total white blood cell counts was done by visual means using New Improved Neubauer counting chamber[32]. A 1 in 200 dilution of blood was made in Turk's fluid and the counting chamber with its cover glass already in position was immediately filled with the diluted blood using a Pasteur pipette and ensuring that the chamber was filled in one action. The charged chamber was allowed to remain undisturbed for 2 minutes to allow the cells to settle. The cells were them counted using x40 eye piece. Four squares at the corners of the chamber were counted.

Biochemical Determination

Hepatic enzymes of clinical significance and serum lipid profile were determined in

samples spectrophotometrically using kit methods. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were measured using Randox kits. Serum total protein was determined with biurete kit method (Randox U. K.). Total cholesterol, Triglycerides (TG) and HDL – cholesterol were also measured using Randox kits. The absorbances of all the tests were determined using spectrophotometer (HAICH, DR 3000, Germany). LDL – cholesterol was obtained by calculation using appropriate relationship between total cholesterol, TG and HDL – cholesterol[33].

Statistical analysis was carried out using student's t-test. A probability of 0.05 was used as a level of significance.

RESULTS

The effects of vitamin B_{12} supplementation during phenytoin administration in rats were investigated to assess the benefits and risk of single vitamin supplementation. Percentage weight gain, haemoglobin level, haematocrit, total white blood cell count, total serum protein, liver function enzyme activities and serum lipid profile were measured as indices of phenytoin toxicity. Table 1 shows the effects of vitamin B_{12} on haematological indices, weight gain and total serum protein. Weight gain (%) in group 2

(24.46 ± 4.32) and group 3 (18.76 ± 4.03) were significantly (p<0.05) lower than that of control group (37.67 ± 6.89). Haemoglobin (g/dl: 7.55 ± 0.68) and haematocrit (%: 38. 64 ± 4.37) were significantly decreased (p<0.05) by phenytoin treatment; but supplementation vitamin B_{12} (group 3) raised their levels to control values. Total white blood cell count was significantly increase while total serum protein was decreased (p<0.05) in group 2 treated with phenytoin only; these changes were, however, not affected by vitamin B_{12} supplement. The effects of vitamin B_{12} on liver function enzymes activities and lipid profile is shown in table 11. Serum AST and ALP of phenytoin treated rats (group 2: 54.13 ± 4.91 and 151.90 ± 30.33 respectively) increased significantly (p<0.05) when compared with control (37.88 ± 9.39 and 118.61 ± 20.90 respectively); while ALT (26.75 ± 3.41) increased marginally, supplementation with vitamin B_{12} further increased the activities of the three enzymes (AST: 73.17 ± 5.44; ALT: 36.38 ± 6.35; ALP: 192.66± 31.65) when compared with group 2 and with control. Total cholesterol was marginally increased while LDL-cholesterol showed significantly increase (p<0.05) in group 2 rats when compared to control. The levels of TG and HDL – cholesterol were not significantly affected. Vitamin B_{12} supplement was beneficial as it lowers the levels of cholesterol, TG and LDL-cholesterol to normal values.

Table 1: Effect of vitamin b_{12} on haematological indices, weight gain and total protein

GROUPS	TOTAL PROTEIN (g/100ml)	Weight Gain (%)	PCV (%)	Hb (g/100ml)	WBC x10³/ml
CONTROL(gp1)	8.71±0.58	37.67±6.89	48.63±2.62	10.11±0.84	4.60±1.07
PHENYTOIN ONLY (gp 2)	6.71±0.87*	24.64±4.32*	38.64±3.7*	6.85±0.68*	6.14±0.96*
PENYTOIN + VITAMIN B_{12} (gp3)	6.46±0.91*	18.76±4.03**	49.15±2.90*	10.13±0.86	6.44±1.08*

*Values are expressed as mean ± SD, n = 8: *p<0.05, **p<0.01*

Table 2: Effect of vitamin b_{12} on liver enzymes and lipid profile

GROUP	AST (iu/1)	ALT (iu/1)	ALP (iu/1)	TCHOL (mmol/1)	TG (mmol/1)	LDL- Cho (mmol/1)	HDL- Chol (mmol/1)
CONTROL (gp1)	37.88±9.39	23.63±5.26	118.61±20.90	2.12±0.22	0.75±0.22	0.21±0.21	1.57±0.19
PHENYTOIN ONLY (gp2)	54.13±4.91*	26.75±3.41	151.90±30.33*	2.73±0.43	0.92±0.29	0.82±0.23**	1.49±0.13
PHENYTOIN + VITAMIN B_{12} (gp 3)	73.17±5.44**	36.38±6.35*	192.66±31.65**	2.23±0.48	1.06±0.31	0.22±0.18	1.53±0.14

*Values are expressed as mean ± SD, n = 8: *p<0.05, **p<0.01*

The results showed that although vitamin B_{12} supplement is beneficial by remitting anaemia and reducing the atherosclerotic risk in phenytoin treated rats, the hepatotoxic effect of phenytoin may be enhanced.

DISCUSSION

In this study, growth retardation, increased risk of cardiovascular diseases, anaemia, and hepatocellular toxicity were observed in young albino wistar rats treated with therapeutic dose of phenytoin. A significant reduction in percentage weight gain was associated with phenytoin treatment and even in combination with vitamin B_{12}. This finding that vitamin B_{12} supplement could not cause significant weight gain in rats receiving phenytoin treatment suggests the importance of the presence of other vitamins including folic acid for proper growth of the animals. Reports have shown that phenytoin administration causes alterations in the metabolism of vitamin B_{12} and folic acid[20,23] which are essential in DNA synthesis and cell proliferation.

The significant difference in the value of total serum protein, observed in this study, indicates that liver cell integrity may have been affected by the treatment. Since hepatocytes constitute a major source of serum protein, decreased protein level may therefore result from the effect of the drug on liver cells resulting in decreased protein synthesis. The phenytoin-induced anaemia was remitted by vitamin B_{12} treatment as evident by raised haemoglobin and PCV levels in group 3 rats. The activities of AST, ALT and ALP were increased by phenytoin treatment in rats. These findings agree with previous report that long-term therapy with phenytoin caused increased AST, ALT, ALP and enlargement of the liver in epileptic patients[34]. The increased activities of liver function enzymes in this study indicate possible hepatocellular damage. Also the decrease in total serum protein and the increased circulation white blood cell further points to impaired liver function and cellular inflammation.

Interestingly, vitamin B_{12} supplementation during phenyton treatment resulted in further increases in the activities of liver functions enzymes; AST, ALT and ALP, but the levels of serum protein and circulating white blood cells in these rats were not significantly different from those without vitamin B_{12} supplement. These findings showed that vitamin B_{12} supplement does not remit the hepatotoxicity of phenytoin but may rather enhance it and that vitamin B_{12} may not have a significant role in the metabolic clearance of phenytoin. Phenytoin is reported to lower the serum and red cell levels of vitamin B_{12} and folate[21,35]. Phenytoin-induced folate depletion in rat liver has also been reported[23]. Thus, the mechanism of phenytoin hepatoxicity may, in addition to phenytoin hypersensivity syndrome [17,27], be related to the drug-induced alteration in folic acid-vitamin B_{12} interactions. The conversion of coblamin to methylcobalamin requires 5-methyltetrahydrofolates. Methylcobalamin is an important coenzyme of vitamin B_{12} which participates in the convertion of homocysteine to methionine. In addition to increased folate requirement for metabolic clearance of phenytoin and phenytoin-induced folate malabsorption, the conversion of cobalmin to methylcobalamin may constitute an additional constrain to the availability of folate for other cellular functions. This may be the reason for enhanced phenytion hepatotoxicity by vitamin B_{12} co-administration without additional source of folic acid.

Studies on serum lipid profile showed only marginal increases in total cholesterol and a significant increase in LDL cholesterol in rats treated with phenytoin without vitamin B_{12} supplement. These findings are in line with report of Luoma et al[36] who demonstrated increases in serum cholesterol and triglyceride levels in healthy volunteers and epileptic patients treated with phenytoin. Our studies have shown that phenytoin-induced increases in total cholesterol, LDL-cholesterol and hence the atherosclerotic risk could be reduced by administration vitamin B_{12}. The effect of vitamin B_{12} supplementation on lipid kinetic during phenytoin treatment may not be unconnected with the action of 5-deoxyadenosylcobalamin, a coenzyme of L-methylmalonyl–CoA mustase which catalyze the conversion of L-methylmalonyl-CoA to succinyl-CoA, an important reaction in energy production from fats and proteins.

In conclusion, we observed that vitamin B_{12} co-administration during phenytoin therapy remits anaemia and the atherosclerotic risk caused by phenytoin but may enhance its hepatotoxicity. Therefore we suggest that the use of only vitamin B_{12} as a monovitamin supplement during phenytoin administration be discouraged.

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