BIOCHEMICAL EFFECT OF VITAMIN B6 DEFICIENCY ON BLOOD AND LIVER OF ADULT MALE ALBINO RATS

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Abstract

The result of this study showed that vitamin B₆ deficiency can be corrected using 10mg/100g body weight of vitamin B₆ tablet (pyridoxine) in isoniazid therapy. Isoniazid is a vitamin B₆ antagonist, commonly used in treatment of tuberculosis. Two groups (B and C) of each ten adult albino rats were administered vitamin B₆ antagonist (isoniazid) of 1.5mg/100g animal weight per day for 15 days using oral catheter. Group C were later given vitamin B₆ tablet (pyridoxine) of 10mg/100g body weight for 30 days. A control group (A) of ten rats was set up for a proper experimental evaluation. Analysis at the end of the administration showed that isoniazid in a dose of 1.5mg/100g of body weight significantly modulates (p<0.05) the blood and liver levels of malondialdehyde, lowered plasma levels of liver and blood protein, affected negatively haematological parameters (white blood cell, haemoglobin concentration and blood platelets) of group B compared to the control group (A). Vitamin B₆ tablet correction on group C significantly (p<0.05) affected the analysis compared to group B that was not corrected with vitamin B₆.

Keywords: Vitamin B₆, isoniazid, blood, liver, albino rat

Introduction

Vitamin B6 is a water-soluble vitamin which is unstable in alkali, oxidation reactions and ultraviolet light but stable to heat and acid. It exists in three major chemical forms: pyridoxine, pyridoxal, and pyridoxamine. It exists in three other phosphate forms as: pyridoxal 5’-phosphate (PLP) pyridoxine 5’-phosphate (PNP) and pyridoxamine 5’-
phosphate (PMP). PLP is the most active coenzyme form and most important in human metabolism. It is marketed as pyridoxine hydrochloride in multivitamins and multinutrients products (Hansel et al., 2001). Pyridoxine is found primarily in the liver and muscle. It is utilized in liver in synthesis of PLP, and PLP has been recognized to be vital in transamination and protein metabolism (Martinez et al., 2001, David et al., 2005). Vitamin B6 is essential for red blood cell metabolism. It helps in efficient function of nervous and immune systems (Martinez et al., 2001), glycogen metabolism, nucleic acids and neurotransmitters synthesis; and prevents kidney-stone formation (Pratibha et al., 2000).

The recommended daily allowance (RDA) for vitamin B6 in milligram are 1.3 for men and women under age 50, and 1.7 for men and women over age 50 and 1.9 for pregnant women and 2 for lactating women (Hansen et al., 2001). Clinical signs of vitamin B6 deficiency are rare because of its wide spread in food except in infants. Vitamin B6 deficiency can occur with individuals on poor quality diet. Deficiency of vitamin B6 can result to dermatitis (skin inflammation), glossitis (a sore tongue), depression, confusion, convulsion, anaemia, excretion of oxalate and citrate resulting in kidney stones (Ravin et al., 1987). In adults vitamin B6 deficiency can occur due to malabsorption, alcoholism and drug antagonism (Bor et al., 2003).

The use of drugs (Theophylline for treating asmatich Children; Penicillamine in treating cystinuria, rheumatoid arthritis, and wilson’s disease; L-DOPA for neurological problems treatment; and Oral contraceptive pills for family planning) results in vitamin B6 deficiency which can be reversed by administration of vitamin B6 (Barnard, 1987). The drug Isoniazid (Isonicotinic hydrazine C₆H₇N₃O) used in treatment of tuberculosis. Is a colorless, Odourless crystal (Snider, 1980). It is soluble in ratio of 1:8 in water, 1:5 in alcohol, and slightly soluble in chloroform. Isoniazid is well tolerated at recommended doses. Adverse effects have been reported on patients who are slow activators of isoniazid, and also those whose nutrition is poor. Adverse effect can result in long term treatment with isoniazid. It forms a complex with vitamin B6 and makes it unavailable to be used by the body causing pyridoxine to be excreted in the urine making urinary level of vitamin B6 breakdown products to be above normal (McCatty, 2000). The commonest adverse effects noted on isoniazid is on the blood which include bleeding associated with acquired inhibition of fibrin stablisation and red cells aplasia (Clairborne et al., 1985).

Isoniazid chemotherapy have been reported to experience hepatic injury (Girling, 1998), interstitial nephritis (Collier et al., 1996). Isoniazid adverse effects on the nerves
system include convulsion, encephalopathy, and psychoses (Mckene et al., 1994). Treatment of Isoniazid adverse effect is by administering of pyridoxine hydrochloride for neuritis and other diseases (Snider, 1980).

The Paper is aimed

Show that vitamin B6 (pyridoxine) tablet at (1.5mg/100g weight) is used to correct vitamin B6 deficiency. Also, that Isoniazid is a Vitamin B6 antagonist. Tuberculosis is one of the opportunistic diseases of HIV/AIDS, and isoniazid being one of the effective drugs for the treatment may be administered with vitamin B6 tablet (pyridoxine) at 10mg/100g body weight to avoid vitamin B6 deficiency diseases on patients.

Materials And Methods

Animals and samples collection

Thirty adult male albino rats were obtained from the Experimental Animal Centre, College of Medicine, University of Lagos. The animals weighed between 200-300g, and were kept ten animals to a cage under twelve hour light and twelve hour dark cycle daily. The rats chows were obtained from the Animal Centre, University of Lagos. Vitamin B6 (Pyridoxine) and Isoniazids tablets were purchased from Pharmacy Department, Lagos University Teaching Hospital, Lagos. The enzyme analysis kit: Randox glutamate-oxaloacetic transaminase kit and all other reagents were of analytical grade.

Animal Treatment

The animals were divided into groups (A, B, and C), fed with rat chows throughout the study period ad libetum. Feed and water intake per day were noted. The animals were weighed daily. Animals in group B and C were treated with drug Isoniazid 1.5mg / 100g animal weight per day for 15 days. Group C was treated with pyridoxine at the dosage of I0mg /100g animal weight for 30 days. The Group A rats were fed with only Rat chows and water throughout the study period. At the end of administration of isoniazied groups B and A were sacrificed, and group C was sacrificed after administration of vitamin B6 tablets. The blood and Liver were collected in lithium hyperinized and sterile bottles and centrifuged at 1500g for 20 minutes to obtain blood plasma and blood serum respectively.
Haematological Analysis

Haemoglobin (Hb) count

The method described by Dacie et al. (1996), was used. Oxy-haemoglobin was released by addition of 5ml distilled water to a specimen of blood (0.04ML) and its stability ensured by adding dilute ammonia (99%). The oxy-haemoglobin was estimated using a Spectrophotometer at 545nm. A blank was prepared by placing 5ml of distilled water into a test tube with 99% ammonia hydroxide. The percentage of haemoglobin in gm/dl was read on a conversion chart.

White blood cell (WBC) count

Method described by Nauber was used. The red cell membrane was lysed with glacial acetic acid solution (2%) which converts the haemoglobin to haematin. The Gentian violet stains the WBC for identification. The blood sample was diluted in 20 v/v with 2% glacial acetic acid tinged with gentian violet in a white blood cell micropipette. The samples were mixed, and loaded into Nauber counting chamber. The number of white blood cells was counted in each of the four large corners of ruled area. The sum of cells in 4 corner squares = N, Dilution Factor = 20, Lowest objective of microscope used = 10, white cell count in one (cu.mm) of blood = N*20*10=N*50/cumm of blood.

Blood platelets

Method of Nauber was used. Fragments of cytoplasm from giant multinucleated cell (Megakaryocyte). The determination is useful as part of a series of test for coagulation and hemorrhagic disorders. The blood sample was diluted with 1 in 100 diluting fluid (3g of sodium citrate and 1ml of formaldehyde in 100ml of distilled water). The sample were mixed thoroughly and filled in a nauber counting chamber and kept for 10minutes to allow cells to settle. The cells were counted in 5 out of 25 squares of the centre small square = N

The blood platelet = N * 1000 cells

Assay for blood plasma and Liver tissue for protein Analysis

The blood plasma was obtained from centrifuged blood sample and the supernatant collected for blood protein analysis the liver samples were homogenized and centrifuged, the supernatant was collected for liver protein analysis. Estimation of Total plasma and Liver protein was carried out using spectrophotometric method of Biuret, Bradford and erythrosine-B (Gomall et. al., 1949; Bradford 1976; Soedjak (1994). Standard was prepared and placed
in different test tubes to a particular volume with distilled water. The same volume of unknown protein was placed in another test tube. Equal volume of Biuret reagent was added to each of the tube mixed, warmed at 37°C for 10 minutes, cooled after absorbance was read at 540nm against a blank. The concentration of the unknown protein was determined using standard curve.

**Estimation of lipid peroxidation (LPO)**

Lipid peroxidation in blood plasma and liver were estimated spectrophotometrically using Thiobarbituric acid-reactive substances (TBARS) method as described by (Varshney and kale 1990), and is expressed in terms of malondialdehyde (MDA) per-mg-protein. This method is based on the reaction between 2-Thiobarbituric acid (TBA) and malondialdehyde (MDA), an end product of lipid peroxides during lipid peroxidation. On heating in acidic pH, the product forms a pink complex which absorbs maximally at 532nm and fluoresces at 533nm. It is readily extractable into organic solvents such as but-1-ol. This test is often calibrated using MDA as the standard and thus, the results are expressed as the amount of free MDA produced.

**Assay for Liver Enzymes: Serum-Glutamate- Oxaloacetate- Transaminase (SGOT).**

Wroblenski, 1959, reported that serum transaminase activity increase in most disorders that produce hepatic dysfunction. Elevated transaminase values are among the first Laboratory abnormalities detected in the precultured phase of hypertitis.

SGOT analysis was carried out using Randox enzyme kit as described by Wroblenski, (1959).Glutamate – Oxaloacetate transaminase is measured by monitoring the concentration of Oxaloacetic hydrazone formed with 2,4-dinitrophenylhydrazine.

**RESULTS AND DISCUSSION**

Pathological observation on the day of sacrifice on the Animal Livers in Group B and C appeared reddish to dark red. There were fat – like deposits on the Liver of Group B and slight fat deposits on group C. the Liver of Group B appears enlarged compare to C, but C is more enlarged than A.

In fig 6, the absorbance reading showed that Group B has the highest value of 0.11 with concentration of 39.8iu/l, while group C 0.08 with concentration of 28iu/l, group A has 0.045 with concentration of 14.5iu/l. It shows that the vitamin B6 deficiency impaired the values of all the parameters in group B and may have affected the amino acids metabolism.
because vitamin B6 was deficient. Vitamin B6 is a cofactor in transamination reaction of amino acid metabolism (Martinez et al 2001, David et al 2005). Two transaminases GOT and GPT, catalyzes the transfer of a keto acid group forming an amino acid group. Thus the decrease level of transaminase enzyme SGOT in group B can be due to extensive tissue destruction which may have caused enlargement of the liver of group B.

In fig 1, the blood platelet count (per μl) of group A (165,000) was on the normal range of (150,000-400,000 per μl). While the blood of group B was (32,470) is far below the normal range and group C was (135,125) is below normal range but higher than that of Group B. The result is the mean value of the groups. In fig 2, Group B has a very low Haemoglobin concentration (g/100ml) of 7.2 far below normal, while Group C has a low Hb concentration of 10.3, and Group A has a normal Hb concentration of 15. In fig 3, White blood cell count (/cumm of blood) of Group A was (7975) and C was (5988) both are within the normal range of white blood cell count (3,500-10,000/cumm of blood). While that of Group B was (3148) and is below normal WBC range.

In fig 4, the mean plasma protein concentration of the groups (mg/dl) showed remarkable significant differences. Group A mean plasma protein concentration was 6.9, while, Group B was 4.3 Group C 5.8. The mean protein concentration of the Animal Livers for Group A was 6.5, Group B 3.9, and Group C was 5.5. The abnormalities in the blood is suspected to be as a result of the decrease in protein levels in the tissue which is the main component of the plasma which acts as a medium, carrying its constituents to specialized organs of the body. As blood passes through the intestinal circulation, nutrients are absorbed into the plasma and carried to the Liver and other tissues. As it passes through the lower kidneys, metabolic waste products are filtered off into the Urine. The majority of the cells suspended in the plasma are the erythrocytes which contain a high concentration of haemoglobin – the oxygen carrying pigment which imparts the red colour on to the blood. The leucocytes are very much fewer in number but several different forms of them exist, each having different but complementary roles associated with the physiological defence mechanism. The platelets are small cells which are intimately concerned with the blood – clothing process. Thus all blood parameters on Group B and C may have been impaired as a result of the protein level of liver and plasma compare to the blood parameters of Group A which is significantly different from that of other groups.
Fig 1: Blood platelet count

Fig 2: Haemoglobin concentration (g/100ml)
Fig 3: White blood cell count/Cumm of blood

Fig 4: Protein concentration in plasma (blue) and liver (red) mg/dl
Fig 5: Estimated lipid peroxidation value in serum (blue) and liver (red) mol/mg protein.

Fig 6: Estimated serum glutamate oxaloacetate transaminase iµ/l (SGOT)
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