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Metabolic Disorders of Vitamin B_6 in Chronic Kidney Disease Patients

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Abstract

Background. Vitamin B_6 (VB_6) is a water soluble vitamin which is important for the normal function of multiple organ systems. It is metabolized to the active molecule pyridoxal-5-phosphate (PLP).
Purpose of the study was to investigate the metabolic disorders of VB_6 in patients (pts) with various chronic kidney diseases (CKD).
Methods. Erythrocyte (Er) VB_6 was investigated in 15 pts suffering from chronic glomerulonephritis and nephrotic syndrome (CG NS), GFR stage 1; urinary excretion (UE) of VB_6 was investigated in 15 healthy subjects during maximal water diuresis, in 12 pts with CKD, GFR stage 3 - 4, during the diet with restriction (2g/day) or high intake (15g/day) of NaCl and in other group of 15 CKD pts, GFR stage 3-4, after i.v. administration of 20 mg furosemide. The effect of 50 mg pyridoxine (P)/day on several parameters of cellular immunity in haemodialysis (HD) pts was investigated. In addition Er VB_6 was investigated in 16 HD pts during erythropoietin (EPO) treatment. Vitamin B_6 in plasma and urine was determined by radioenzymatic assay, and Er VB_6 was determined by means of an indirect method and it was expressed as the effect of PLP in per cents. Plasma oxalic acid was determined by spectrophotometric method using oxalate oxidase which is free from vitamin C interference.
Results. In pts with CG NS plasma VB_6 (15.5 ± 3.8 nmol/L) and Er VB_6 (effect of PLP: 42.1±7.5%) were significantly decreased ($p<0.01$), and plasma oxalic acid was significantly elevated (9.8±2.3 μmol/L), ($p<0.01$). Six months supplementation by 50 mg P/day led to the effect of PLP in the normal range (16.3±1.4%) in CG NS pts. Maximal water diuresis in healthy subjects and i.v. administration of 20 mg furosemide in CKD pts led to the significant increase of UE of VB_6 . No change of UE of VB_6 during the diet with high intake of NaCl (15g NaCl/day) in CKD pts was found. Urinary excretion of VB_6 depends on water diuresis. Three-month use of daily 50 mg P led to a significant improvement of some parameters of cellular immunity in HD pts. The effect of PLP in HD pts treated by EPO increased from 19.0±1.8 to 30.4±2.4%, ($p<0.01$). After three months supplementation of 20 mg P/day, the effect of PLP was in the normal range.
Conclusion: We recommend administering 5-50mg P/ day for prevention of VB_6 deficiency in CKD pts.

Keywords: chronic kidney disease; haemodialysis; chronic glomerulunephritis and nephrotic syndrome; vitamin B_6

Introduction

Vitamin B_6 is a water soluble vitamin, which is important for the normal function of multiple organ systems. It is metabolized to the active molecule PLP, which serves as the coenzyme for more than 100 enzymes; PLP influences protein and lipid metabolism, metabolism of several aminoacids and formation of antibodies [1-3]. Besides VB_6 catalyzes transformation of glyoxalate to glycine. That is the salvage metabolic way to prevent oxalate formation [4]. Causes of VB_6 deficiency in CKD pts are: decrease amount in diet, altered phosphorylation of pyridoxal on PLP, increased degradation of PLP or absorption in tissues, VB_6 loss through dialysis membranes and other [5,6].
Supplementation of VB_6 in HD pts range from 0-20 mg/ day. Increased dose of VB_6 (30-300 mg/day) was administered to increase plasma PLP concentration and to combat impaired cellular immunity [6-8].
The purpose of the study was to investigate plasma and Er VB_6 in pts suffered from CG NS, to investigate UE of VB_6 during maximal water diuresis in healthy subjects, during sodium diuresis and after i.v. administration of 20 mg furosemide in pts with CKD, GFR stage 3-4. Besides the aim was to investigate the influence of high dose 50 mg P/day on the impaired cellular immunity and the influence of EPO treatment on Er VB_6 in HD pts.

Patients and methods

In the first part of the study we investigated a group of 15 CG NS pts, GFR stage 1, (10 men and 5 women, mean age 37.5±5 years) before and after 6 months administration of 50 mg P/day. In the second part of the study UE of VB_6 during maximal water diuresis was determined in 15 healthy subjects and during sodium diuresis in 12 CKD pts, GFR stage 3-4, and after i.v. administration of 20 mg furosemide in 15 CKD pts, GFR stage 3-

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4. In the third part of the study was investigated the influence of 50 mg P/day on some parameters of cellular immunity in 30 HD pts (13 men and 17 women, mean age 41±7 years) during 3 months. The influence of EPO treatment on Er VB_6 in 16 HD patients (mean age 43±6 years) (group B) was investigated during 15 months and they were compared with the group A of 14 HD pts (mean age 40±5 year) without EPO treatment. Group B of HD patients was supplemented with oral P (5mg/day) for 12 months and with 20 mg/day for the next 3 months. Vitamin B_6 in plasma and urine was determined by radioenzymatic assay using Bühlmann Laboratories AG kit „Vitamin B_6 REA". Reference range of VB_6 in plasma was 25-130 nmol/L. Erythrocyte vitamin B_6 was determined by means of indirect method, i.e. by assessing the activity of erythrocyte aspartate aminotransferase with and without addition of coenzyme PLP in vitro.The effect of PLP on the activity of Er enzyme displayed an indirect relationship with the concentration of Er VB_6 and it was expressed in per cents (9). Reference range of PLP was 0-20%. The parameters of cellular immunity were determined by rosetting assay (10). Plasma and urinary oxalic acid were determined by spectrophotometric method using oxalate oxidase, which is free from vitamin C interference (11). Normal range of plasma oxalic acid was 2.0 - 5.5 µmol/L. Statistical analysis of obtained results was performed using non-parametric Mann Whitney U test.

Results

Table 1. Plasma Vitamin B_6 and effect of PLP and plasma oxalic acid in patients vith chronic glomerulonephritis and nephritic syndrome (CG NS), GFR stage 1

Group of patients	n	Plasma vitamin B_6 (nmol/L)	Effect of PLP (%) (Erythrocyte vitamin B_6)	Plasma oxalic acid (µmol/L)
CG NS	15	15.5±3.8**	42.1±7.5**	9.8±2.3**
Control group	30	65.7±25.3	15.4±1.2	3.8±1.7

**p<0.001 versus control group values

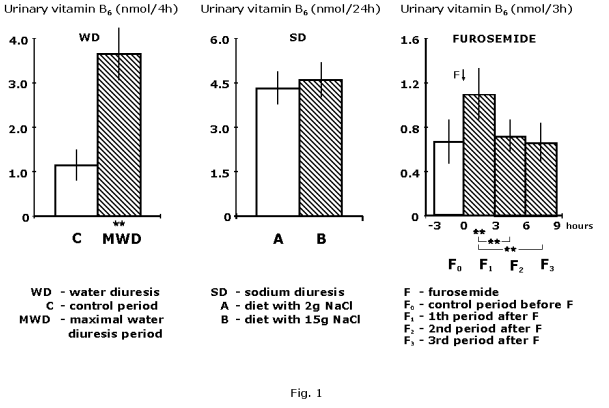

Fig. 1. Urinary excretion of vitamin B_6 after maximal water diuresis in healthy subjects, after sodium diuresis and after i.v. administration of furosemide in CKD patients, GFR stage 3-4

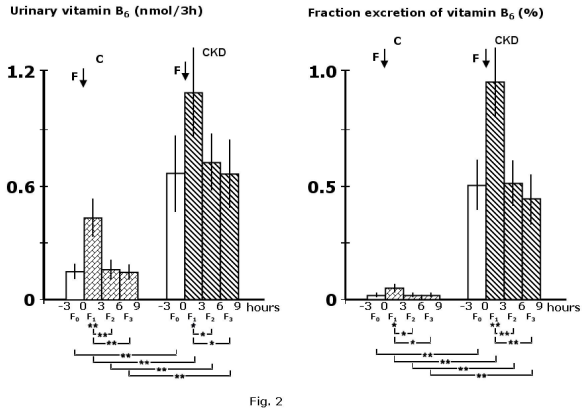

Fig. 2. Urinary excretion and fraction excretion of vitamin B_6 after i.v. administration of furosemide in control group (C) and in CKD patients, GFR stage 3-4

In pts with CG NS, GFR stage 1, plasma VB_6 and Er VB_6 were significantly decreased ($p<0.01$), and plasma oxalic acid was significantly elevated ($p<0.01$), (Table 1). Six months supplementation by 50 mg P/day led to the effect of PLP in the normal range (16.3±1.4%) in CG NS pts. Maximal water diuresis in healthy subjects and i.v. administration of 20 mg furosemide in CKD pts, GFR stage 3-4, led to a significant increase of UE of VB_6. During sodium diuresis UE of VB_6 did not change in CKD pts, GFR stage 3 – 4 (Figure 1). Urinary excretion and fraction excretion of VB_6 after i.v. administration of furosemide were significantly elevated in CKD pts, GFR stage 3-4, in comparisom with a control group in all urinary collection periods (Figure 2).

Tree-month use of daily 50 mg P led to a significant improvement of some parameters of cellular immunity (Table 2). Erythrocyte VB_6 was in the normal range in group A pts without EPO treatment. It was also normal in group B before EPO treatment. During EPO treatment, the PLP effect increased significantly indicating a significant decrease in Er VB_6. This was evident by the end of the 6th and 12th month of the study. Supplementation with P (20 mg/day) during the last three month of the study led to significant increase in Er VB_6 and became normal by the end of the 15th month of the study (Table 3).

Table 2. Effect of PLP and parameters of sellular immunity in haemodialysis patients (n=30) with oral administration of pyridoxine 6 mg/day and after 3-month administration of pyridoxine 50 mg/day

Parameter	Control group	Haemodialysis before treatment	Patients after treatment
1. Effect of PLP (%)	14.54±1.45	15.62±1.42	10.40±1.35
2. Percentage of T lymphocyte ERFC	62.30±8.10	29.10±3.10 **	53.00±4.50 b
3. Absolute number of T lymphocyte ERFC	1417±390	421±81 **	1288±22 b
4. Percentage of T lymphocyte A-ERFC	34.1±13.0	14.6±4.3 **	26.3±8.2 a
5. Absolute number of T lymphocyte A-ERFC	859±319	197±28 **	508±105 b

ERFC-E rosette-forming cells, A-ERFC-active E rosette-forming cells
**p<0.01 versus control group values; [a]p<0.05, [b]p<0.05 before versus sfter treatment

Plasma VB_6 level in HD pts treated by EPO and supplemented by P (20mg/day) was 92.2±31 nmol/L. The mean concentration of plasma oxalic acid in HD pts was 40.3±9.8 μmol/L. Besides the following relationships were found: direct relationship between plasma oxalic acid and effect of PLP (Er VB_6), (r=0.564, p<0.05) in CG NS pts, GFR stage 1, and indirect relationship between plasma oxalic acid and plasma VB_6 (r=-0.832, p< 0.01) in HD pts were found. Except of these findings indirect relationship between effect of PLP and plasma VB_6 in HD pts (r=-0.845, p<0.01) was found.

Table 3. Haematocrit, serum iron and effect of PLP in haemodialysis patients during 15 months of EPO treatment

Group	Month	Supplementation with Pyridoxine (mg/day)	Supplementation with Ferrum-Lek (mgx3/week)	Haematocrit (%)	Serum iron (μmol/L)	Effect of PLP (%) (Erytrocyte vitamin B_6)
A (n=14)	0	5	none	27±1	13.9±1.7	17.7±2.1
	6	5	none	28±1	13.5±1.2	16.4±2.7
	12	5	none	27±1	14.0±1.1	16.8±3.9
B (n=16)	0	5	none	26±1	21.2±3.4	19.0±1.8
	6	5	50-100	36±2**	23.3±2.4	29.4±1.8**
	12	5	50-100	35±1**	25.1±3.2	30.4±2.4**
	15	20	50-100	35±2**	24.8±2.5	16.4±1.9[a]
Normal values				38-48	10-26	0-20

*p≤0.05, **p≤0.01 versus initial values, [a]p<0.01 versus 12th and 15th month
A-group of patients without erythropoietin treatment; B-group of patients treated by erythropoietin

Discussion

Deficiency of plasma and Er VB_6 was found in every CG NS pts, GFR stage 1. This finding was very important from diagnostic and therapeutic point of view. There are a number of possible causes of VB_6 deficiency in the nephrotic syndrome. These include: 1. binding of VB_6 to albumin, which is a significant component of urinary proteins; 2. increase degradation of VB_6. Deficiency of VB_6 may lead to decreased formation of antibodies, decreased synthesis of IgG, disorders of lymphocyte and suppressor cell function, and trombembolic complication [12,13]. Supplementation of VB_6 is necessary before the beginning of the treatment CG NS pts, especially before the corticoid and immuno-supressive treatment [14]. Deficiency of plasma and Er VB_6 was one of the causes of hyperoxalaemia in these pts. Urinary excretion of VB_6 depended on water diuresis. Furosemide increased diuresis in residual nephrons, but in this condition furosemide can participate in the origin of VB_6 deficiency [15]. Three-month supplementation of 50 mg P/day led to the significant improvement of electrophoretic mobility of peripheral blood lymphocytes and some other parameters of cellular immunity [6,16, 17]. This supplementation treatment is also suitable for HD patients with recurrent skin and other infection caused by deficiency of cellular immunity [6,7]. Vitamin B_6 is required as cofactor in the rate-limiting first step in heme synthesis, i.e., the formation of δ-aminolevulinic acid. In addition, VB_6 may have a role in the mitochondrial reaction incorporating iron into protoporphyrin which is the final step in heme synthesis [18]. In addition results suggest that VB_6 consumed much more with the increased synthesis of haemoglobin during EPO therapy in HD pts resulting in the fall of Er VB_6. This fall was prevented when P supplementation was increased from 5 to 20 mg/day [19].

Conclusion

In conclusion, according to obtained results we re-

commended to administer 5-50mg P/day for prevention of VB_6 deficiency in CKD pts.

Conflict of interest statement. None declared.

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