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Research

Variations in the lipid profile of patients with chronic renal failure treated with pyridoxine

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Abstract

Background: Hyperhomocysteinemia and lipid abnormalities are commonly found in patients with chronic renal failure; both are recognized as risk factors for atherosclerosis. The homocysteine-lowering effect of pyridoxine is controversial. This study was performed to determine the effect of a high dose of pyridoxine (300 mg i.v. three times a week) on plasma and red blood cell lipid profile and plasma homocysteine concentration in twelve chronic renal failure patients on regular hemodialysis. Fasting blood samples were taken at the beginning of the study (basal 1), after 30 and 60 days of treatment and 4 months after withdrawal (basal 2).

Results: Pyridoxine supplementation induced a significant decrease in total plasma homocysteine level and also a lowering effect in plasma total cholesterol and triglycerides. These biochemical data increased when the samples were taken at basal 2, reaching the levels obtained at the beginning of the experiment. LDL cholesterol increased whereas HDL cholesterol was reduced during the treatment. In erythrocyte membranes vitamin B6 therapy enhanced the cholesterol/phospholipid ratio as well as the fluorescence anisotropy of diphenyl-hexatriene.

Conclusions: We conclude that high doses of pyridoxine represent an effective strategy to ameliorate both plasma homocysteine levels and lipid profiles in chronic renal failure patients, protecting them from atherosclerosis. Further research using a long-term treatment would be necessary in an attempt to restore the fatty acid pattern and the fluidity of red cell membranes.

Background

Ischemic heart disease and other complications of atherosclerosis are the most common cause of death in patients with chronic renal failure. The pathogenesis of cardiovascular diseases in these patients is of multifactorial origin [1]. Dislipidemia and hyperhomocysteinemia are important factors associated with the early onset of atherosclerosis.

Chronic renal failure is often associated with dyslipoproteinemia, high levels of cholesterol and triglycerides, as well as a decrease in the polyunsaturated fatty acids. Each of these abnormalities has been identified as an independent risk factor for atherosclerosis [2–5]. Some of them persisting and becoming worse during dialysis treatment [6]. On the other hand, an increment of plasma homocysteine concentration is highly prevalent among

patients under hemodialysis [7,8], and it is considered an independent risk factor for atherosclerotic complications of end-stage renal disease [2,9–12].

Hiperhomocysteinemia is closely linked to plasma folate and pyridoxine concentration [9]. Once formed, homocysteine is either remethylated to methionine which requires vitamin B12, folate and riboflavin as a cofactor, cosubstrate, and prosthetic group, respectively, or it undergoes a transsulfuration reaction to form cysteine. The transsulfuration pathway is catalyzed by cystathionine beta synthase which requires pyridoxine (vitamin B_6) as a cofactor. In patients with myocardial infarction, Verhoef and col. [13] demonstrated that the impairment of homocysteine remethylation (dependent on folate and vitamin B12 rather than on vitamin B6) was the predominant cause of high homocysteine levels. An increase in the intermediate products of the transsulfuration pathway was demonstrated in chronic renal disease, including end-stages requiring hemodialysis [14]. In a large cohort of unsupplemented hemodialysis patients it was shown that hyperhomocysteinemia was present in all the patients, though not all of them were deficient in B-complex vitamins [15]. Moreover, hyperhomocysteinemia persists in 75% of dialysis patients despite the routine low dose supplementation with the B-vitamin cofactor/substrates for homocysteine metabolism, and normal or supernormal plasma status of these vitamins [16].

Much of the research on determinants of total homocysteine concentration was focused on folate. It was recently demonstrated that supplementation with high doses of folic acid reduced plasma homocysteine levels in chronic renal failure patients on regular hemodialysis [17,18]. Nevertheless, this therapy induced significant alterations in both plasma and erythrocyte membrane lipid profile which should be taken into account when this therapy is indicated [17].

To correct hyperhomocysteinemia in dialyzed patients supra physiologic doses of B-vitamins [19], or pharmacologic doses of folic acid and pyridoxine [20] were suggested. The effect of vitamin B6 was missed in many trials because of a much greater effect of folic acid. On the other hand, it was previously observed that oral pyridoxine supplementation had no significant effect on fasting total homocysteine levels in nondialyzed chronic renal failure patients [21] while a lowering effect appeared when it was administered intravenously associated to folic acid [22]. In healthy subjects who are folate and riboflavin replete, low-dose vitamin B6 lowers fasting plasma homocysteine levels [23].

In the present study, we conducted a two-month trial in chronic renal failure patients undergoing regular hemodialysis in order to assess the effect of the intravenous supplementation of high doses of pyridoxine on plasma homocysteine levels as well as its incidence on the lipid profile of plasma and erythrocytes.

Results

Table 1 shows the values for plasma total homocysteine, triglycerides, total cholesterol as well as HDL-cholesterol and LDL-cholesterol levels in the population studied, at the beginning (basal 1), 30 and 60 days after pyridoxine treatment, and four months after withdrawal of treatment (basal 2). Data taken at the beginning of the study showed levels of homocysteine higher than those considered as normal [24]. Pyridoxine administration significantly decreased plasma total homocysteine concentration to normal values, which became high again once the medication was suspended (basal 2).

At the beginning of the study, plasma triglyceride values were higher and HDL-cholesterol slightly lower than those values generally accepted for health controls, while total cholesterol and LDL-cholesterol were found to be within the normal range. Along pyridoxine administration, triglyceride levels as well as total cholesterol and HDL-cholesterol were significantly decreased. LDL-cholesterol showed an increase that became significant after 2 months of treatment. After the suspension of the treatment triglyceride, cholesterol and lipoprotein levels were found to be similar to those obtained at the beginning of the study.

Table 1: Plasma biochemical parameters of chronic renal failure patients treated with pyridoxine

	Days of treatment			
	Basal 1	30	60	Basal 2
Total homocysteine (μmoles/l)	33.2 ± 4.3[a]	22.0 ± 1.5[b]	18.3 ± 1.5[b]	32.5 ± 2.2[a]
Triglyceride (mg/dl)	166.5 ± 5.8[a]	117.0 ± 6.3[b]	109.0 ± 6.5[b]	160.0 ± 6.0[a]
Total cholesterol (mg/dl)	186.3 ± 3.8[a]	170.9 ± 3.7[b]	177.4 ± 5.2[b]	188.5 ± 4.2[a]
HDL-cholesterol (mg/dl)	37.2 ± 1.2[a]	30.2 ± 0.8[b]	33.5 ± 1.1[c]	33.5 ± 0.9[a]
LDL cholesterol (mg/dl)	115.0 ± 2.0[ab]	120.8 ± 3.5[bc]	124.2 ± 2.2[c]	113.1 ± 2.2[a]

Data are the mean ± SEM. Values not bearing the same superscript letter are significantly different at $P < 0.05$.

The fatty acid composition of plasma total lipids obtained in basal samples presented changes in the fatty acid pattern, indicating a mild fatty acid deficiency, frequently observed in chronic renal failure. Thus, saturated acids were increased whereas polyunsaturated acids were below those values reported in health controls [6,25–28]. Pyridoxine administration did not ameliorate this pattern which remained without changes all over the treatment, even after withdrawal (results not shown).

The fatty acid composition of red blood cell membranes is shown in Table 2. The patterns obtained in basal 1 and 2 samples were not different from those obtained from normal subjects [6]. After pyridoxine treatment, a significant increase in palmitoleic and stearic acids as well as a decrease in palmitic acid were observed. No changes were found in polyunsaturated fatty acids belonging to either linoleic or α-linolenic family when compared to the basal 1. Four months after the end of treatment, the pattern showed an enhancement in palmitic, palmitoleic and oleic acids while 20:4 n-6 and 22:4 n-6 acids decreased. Docosapentenoic as well as docosahexenoic acids from n-3 series also diminished significantly.

The amount of different neutral and polar lipids as well as the steady-state fluorescence anysotropy of DPH in erythrocyte membranes, are shown in Table 3. At the beginning of the study, the amount of cholesterol represented about 50% to that of phospholipids. When the patients were treated with pyridoxine, a significant decrease in phospholipids together with an increase in the cholesterol was observed. In consequence, the relationship between cholesterol and phospholipids was markedly incremented. A significant increase in triglyceride and free fatty acids was also observed in red blood cell membranes of treated patients. Patients receiving pyridoxine supplementation for 30 days, showed a significantly increased rotational mobility of DPH. These values were further incremented during the therapeutic treatment, remaining even high after withdrawal.

Table 2: Effect of pyridoxine on the fatty acid composition in erythrocyte membranes

	Days of treatment			
Fatty acid	Basal 1	30	60	Basal 2
16:0	22.0 ± 1.0[a]	19.0 ± 1.3[a]	17.0 ± 1.4[b]	23.8 ± 0.9[a]
16:1	0.3 ± 0.05[a]	1.0 ± 0.2[b]	1.0 ± 0.1[b]	1.3 ± 0.2[b]
18:0	20.4 ± 0.3[a]	22.0 ± 0.5[b]	23.0 ± 0.4[b]	19.3 ± 0.2[c]
18:1	14.7 ± 0.4	14.3 ± 0.5	14.7 ± 1.3	14.2 ± 0.6
18:2 n-6	10.7 ± 0.4	11.0 ± 0.5	10.5 ± 1.0	10.6 ± 0.5
20:3 n-6	1.9 ± 0.1	1.6 ± 0.1	2.1 ± 0.2	1.6 ± 0.1
20:4 n-6	19.4 ± 0.9[a]	19.0 ± 0.7[a]	20.6 ± 0.7[a]	15.7 ± 0.7[b]
22:4 n-6	0.5 ± 0.1[a]	0.4 ± 0.05[a]	0.5 ± 0.1[a]	0.2 ± 0.02[b]
22:5 n-6	4.7 ± 0.5	4.1 ± 0.3	4.7 ± 0.3	3.5 ± 0.2
22:5 n-3	2.6 ± 0.2[a]	2.3 ± 0.2[ab]	2.6 ± 0.2[a]	1.9 ± 0.1[b]
22:6 n-3	4.7 ± 0.3	5.6 ± 0.5	5.0 ± 0.5	4.2 ± 0.3

Results are the means of 12 determinations ± 1 SEM expressed as μg % of total fatty acids. Fatty acids are identified by: number of carbon atoms in the chain is given first, value following the colon represents number of double bonds (zero means saturated fatty acid); number following n- indicates the position of the last double bond counting the double bond from the terminal methyl group. Values not bearing the same superscript letter are significantly different at $P < 0.05$.

Discussion

The present results indicated that the intravenous administration of high doses of vitamin B6 was effective in correcting the hyperhomocysteinemia in the HD patients. However, the homocysteine-lowering effect was mild (55.1%) compared with that of folic acid observed in the same population (31.9%) [17].

The hypertriglyceridemia is the most common plasma lipid abnormality in patients with renal failure, and it has been considered as a risk factor for atherosclerotic vascular disease [2,3]. Triglyceride levels are associated with an increased risk factor even if they are within the range generally considered to be without clinical significance [3]. The reduction of plasma triglyceride levels observed after vitamin B6 treatment indicated an amelioration of this lipid abnormality. In the patients studied total plasma cholesterol levels were within normal values and pyridoxine supplementation had a mild but significant cholesterol-lowering effect (91.3%). Similar observations were reported by Arnadottir et al. when vitamin B6 was orally administered [20]. Contrasting to the reduction of these biochemical results, we showed that HDL-cholesterol decreased and LDL-cholesterol increased after 1 and 2 months of pyridoxine administration. Correspondingly, plasma total cholesterol/HDL-cholesterol as well as LDL-cholesterol/HDL-cholesterol ratios increased during the treatment. This implies an undesirable effect since a risk of coronary disease was attributed primarily to a reduction in total cholesterol/HDL-cholesterol [29].

Another important factor in coronary heart disease is the level of PUFA [30,31]. Fatty acid abnormalities have been found in patients with different types of atherosclerosis [32] and myocardial infarction [33]. The fatty acid pattern observed in chronic renal failure patients is indicative of an essential fatty acid deficiency. Polyunsaturated fatty acids decreased whereas saturated fatty acids increased in plasma and red blood cell membranes, altering the fluidity in the latter [6,25–28]. During pyridoxine administration the pattern of either plasma or erythrocytes polyunsaturated fatty acids remained invariable. The decrease observed in arachidonic, 22:4 n-6 and 22:5 n-3 acids in red cell membranes after withdrawal was attributed to the gradual deterioration of the fatty acid pattern observed in these patients [6].

Table 3: Pyridoxine effect on the lipid composition (mol %) and fluorescence anisotropy (rs) of DPH in erythrocyte membranes

	Days of treatment			
	Basal 1	30	60	Basal 2
Phospholipids	65.8 ± 0.9[a]	57.3 ± 0.5[b]	57.8 ± 0.8[b]	58.0 ± 1.3[b]
Cholesterol	33.2 ± 0.9[a]	38.2 ± 0.6[b]	37.5 ± 0.8[b]	39.1 ± 1.3[b]
Triglyceride	0.4 ± 0.03[a]	2.0 ± 0.1[b]	2.1 ± 0.1[b]	1.0 ± 0.2[c]
Free fatty acids	0.6 ± 0.01[a]	2.5 ± 0.1[b]	2.6 ± 0.1[b]	1.9 ± 0.2[c]
Cholesterol/phospholipids	0.50	0.67	0.65	0.67
r_s	0.235 ± 0.001[a]	0.249 ± 0.002[b]	0.249 ± 0.002[b]	0.253 ± 0.002[a]

Data are the mean ± SEM. Values not bearing the same superscript letter are significantly different at $P < 0.05$.

On the other hand, the relative percentage of cholesterol increased and phospholipids decreased significantly, in the red cell membranes after pyridoxine treatment. The structural order of lipids in membranes is inversely related to the molar cholesterol/phospholipid ratio [34], and it is essentially determined by cholesterol content and the degree of saturation of the phospholipid acyl chains [35]. We consider that the enhancement of cholesterol/phospholipid ratio obtained from erythrocyte membranes was the main caused for the decline of fluidity in the bilayer, since polyunsaturated fatty acids remained invariable, and the enhancement of free fatty acids of total lipids represented only 1–3% of the bilayer components.

Comparing the biochemical profiles obtained after the administration of pyridoxine to those reported when the same patients were treated with folic acid [17], we conclude that vitamin-B6 was highly effective in decreasing triglyceride levels in spite of a mild depression produced in total homocysteine levels. Moreover, pyridoxine produced a moderate increment (5%) in LDL-cholesterol versus the enhancement observed after folic acid administration (35%). The lipid pattern of erythrocyte membranes was similar in the samples obtained from the patients under either pyridoxine or folic acid treatment.

Conclusions

Based on these results, we consider that i.v. pyridoxine therapy appears to be an effective and appropriate strategy to ameliorate plasma homocysteine, triglycerides and total cholesterol levels in patients with chronic renal failure, who are reliably known to be at high risk of atherosclerosis. However, it was insufficient to restore polyunsaturated fatty acid pattern and erythrocyte lipid profile. Regarding that it was evident that correction of hyperhomocysteinemia with folic acid in patients on dialysis, produced an increase in the serum concentration of unsaturated fatty acids [17], we considered that further research using vitamin B6 for a long period of time would be necessary in an attempt to restore the fatty acid pattern and to improve the rigidity of the red cell membranes.

Subjects and methods

Patients

Twelve uremic patients (both sexes: 5 men, 7 women, aged 47.9 ± 15.5) undergoing maintenance hemodialysis were studied. The underlying renal diseases were adult polycystic kidney disease (2 cases); hypertensive nephrosclerosis (3 cases); obstructive uropathy (3 cases) and unknown diagnosis (4 cases). Their biochemical and dialysis situation (mean ± standard deviation) before the study were: body mass index 21.6 ± 3.1; dialysis time 41 ± 31.7 months; serum creatinine 8.4 ± 3.3 mg %; hematocrit 33.2 ± 5.8%; ktv 1.38 ± 0.37; intraerythrocytic folic acid 1177.3 ± 187.8 ng/ml; serum B12 vitamin 489.2 ± 270.5 pg/ml; serum albumin 4.07 ± 0.35 g %. To prevent folate and pyridoxine deficiency, all patients received an intravenous (i.v.) supplementation of folic acid (10 mg) and pyridoxine (300 mg) postdialysis, for three months before starting this study. Dialysis was carried out with bicarbonate dialysate, volumetric machines (Gambro AK90), and polysulphone capillary filters 1.8 m2 low flux (Fresenius F8). At the end of each session, all patients received an i.v. supplementation of 300 mg pyridoxine three times a week for 60 days. Samples were taken at the fasting state (12 h) and immediately before the hemodialysis session at the beginning of the study (basal 1), after 30 and 60 days of treatment, and four months after withdrawal (basal 2). The study was approved by the HIGA San Martín hospital's ethical committee.

Isolation of erythrocyte membranes

Blood was collected in test tubes containing an anticoagulant ethylenediaminetetraacetic acid (EDTA) solution (Wiener Lab., Rosario, Argentina). Whole blood was centrifuged, the plasma was immediately separated, and the packed red blood cells were washed four times at 4°C with buffered solution containing NaCl (140 mM), KCl (5 mM), NaHSO4 (1 mM) and Tris buffer (10 mM, pH 7.4). After agitation they were kept at 4°C for 10 min, and centrifuged at 16,000 g for 15 min. This procedure was done twice, leaving a substantially hemoglobin-free pellet of erythrocyte membranes, which was resuspended in a

small amount of supernatant and stored at -70°C until assayed.

Chemical determinations

Plasma cholesterol and triglycerides were determined using commercially-available enzymatic methods (Wiener Lab., Rosario, Argentina). High-density-lipoprotein (HDL) cholesterol was also analyzed enzymatically after precipitation of very-low-density and low-density lipoproteins (LDL) with magnesium-dextran (Wiener Lab., Rosario, Argentina). Plasma total homocysteine concentration was determined using an enzyme immunoassay method (Axis Biochemical, Oslo, Norway).

Lipid extraction and analysis

Lipids from plasma and erythrocyte membranes were extracted with chloroform-methanol (2:1 v/v). An aliquot from the organic phase was methylated and analyzed using a Hewlett-Packard Model 5840-A gas-liquid chromatograph equipped with a flame-ionization detector. Another aliquot from the organic phase was separated to determine phospholipid and neutral lipid content through a flame ionization detector (FID) of an Iatroscan apparatus model TH 10. Lipids were separated on previously activated chromarods type S-III under a double-development system. The first mobile phase was hexane-benzene (70:30 v/v) whereas the second one was benzene-chloroform-formic acid (70:25:2 v/v/v). Lipid species were quantified by comparison with known amounts of pure standards run under the same conditions. The signals from the FID were registered on a Hewlett-Packard model HP-3396 A integrator.

Fluorescence anisotropy measurements

Steady-state fluorescence anisotropy (rs) was measured in erythrocyte membranes in a SLM 4800 C spectrofluorometer as previously described by Garda et al. [36]. The probe used was 1,6-diphenyl-1,3,5 hexatriene (DPH). Excitation wavelength was 360 nm and the emitted light was passed through a sharp cut-off filter (Schott KV 389). Light scattering of blanks represented less than 5% and fluorescence values were corrected accordingly. The phospholipid:probe ratio was maintained at more than 200:1 (mol:mol) in order to minimize possible probe-probe interactions.

Statistical analyses

Results were tested statistically using either the Student t-test compared to the respective control or the one-way analyses of variance (ANOVA) as appropriate.

Authors'contributions

AMG and LAT participated in the study design, carried out the treatment of the patients in hemodialysis as well as their clinical evaluation and obtained the blood samples. GD participated in the design of the study, developed the lipid methodology and wrote the most of the manuscript. All authors have read and approved the final version of the manuscript.

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