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Homocysteine, Fibrinogen, and Lipoprotein(a) Levels Are Simultaneously Reduced in Patients With Chronic Renal Failure Treated With Folic Acid, Pyridoxine, and Cyanocobalamin

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Ischemic heart disease and other complications of atherosclerosis are the usual cause of death in patients with chronic renal failure. Important factors associated with early onset of atherosclerosis in these patients are hyperhomocysteinemia, hyperfibrinogenemia, and elevated levels of lipoprotein(a) (Lp(a)). Folic acid (15 mg/d), pyridoxine (150 mg/d), and cyanocobalamin (1 mg/wk) were administered for 4 weeks in 21 patients receiving dialysis, and a simultaneous, statistically significant reduction in the concentration of homocysteine, fibrinogen, and Lp(a) was found. A positive correlation between decreasing homocysteine and fibrinogen levels was also noted. The parameters studied approached presupplementation values 6 months after vitamins were discontinued. The results suggest that vitamin supplementation has a favorable effect on risk factors of atherosclerosis in patients with renal failure and that interactions may exist between homocysteine, fibrinogen, and Lp(a).

IT HAS BEEN OBSERVED that patients with chronic renal failure (CRF) undergoing dialysis have a higher mortality rate due to ischemic heart disease and other complications of atherosclerosis than does the general population.[1] This finding cannot be attributed soley to traditional risk factors of atherosclerosis, such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol or elevated triglyceride levels.[2] In 1996, Bostom et al[3] noted for the first time a significantly higher incidence of hyperhomocysteinemia, hyperfibrinogenemia, and elevated Lp(a) lipoprotein levels in patients with CRF than in healthy controls. The role of homocysteine, fibrinogen, and Lp(a) in the atherosclerotic process has been confirmed in many studies.[4-8] Presumably, hyperhomocysteinemia is the cause of chronic oxidative stress[9] and thus supports vascular inflammation that leads to increases in the concentrations of fibrinogen and Lp(a). Studies in vitro have provided evidence of direct interactions between homocysteine, fibrinogen, and Lp(a).[10] On these grounds, it may concluded that hyperhomocysteinemia, hyperfibrinogenemia, and elevated Lp(a) levels appear to be responsible for early-onset atherosclerosis in patients with renal failure. It follows that pharmacological intervention should be aimed at reducing the concentrations of these risk factors. Unfortunately, no effective methods of reducing these concentrations are known, except for slight reduction of fibrinogen levels by fibrates and nicotinic acid, and of Lp(a) levels by estrogens. However, hyperhomocysteinemia can be controlled in most patients with the aid of B vitamins[11,12] such as folic acid, pyridoxine (B_6), and cyanocobalamin (B_{12}). Taking into account the possible interactions between homocysteine, fibrinogen, and Lp(a) treatment with B vitamins may also be expected to affect the levels of the last two factors.

We therefore aimed the present study at evaluating the influence of combination therapy with folic aid, pyridoxine, and cyanocobalamin on the concentrations of homocysteine, fibrinogen, Lp(a), basic lipid parameters, urea, creatinine, and uric acid in patients with CRF on dialysis therapy.

MATERIALS AND METHODS

The study group included 21 patients (11 male and 10 female) with CRF who were undergoing dialysis at the dialysis unit of the Provincial Hospital in Szczecin. The cause of CRF was chronic glomerulonephritis in 12 patients, chronic pyelonephritis in 7 patients, and polycystic

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kidney disease in 2 patients. The mean age of the patients was 41 ± 9 years (mean ± SD), and the body mass index was 24.5 ± 3.6 kg/m^2. Nine patients had arterial hypertension, but none had diabetes. All patients received calcium carbonate, and some received vitamin D_3 and antihypertensive and cardiac drugs. Doses of all drugs remained unchanged during the study period. Vitamin preparations were not administered.

The patients were placed on a 4-week regimen that included 15 mg folic acid and 150 mg pyridoxine (vitamin B_6) in divided doses daily and 1 mg cyanocobalamin (vitamin B_{12}) weekly. Concentrations of homocysteine, fibrinogen, Lp(a), triglycerides, total cholesterol, HDL and LDL cholesterol, urea, creatinine, and uric acid in serum were measured before and immediately after vitamin supplementation. Measurements were repeated 6 months later in all patients, who consented not to use multivitamin preparations with folic acid, vitamin B_6, or B_{12} during this period. The protocol of the study was approved by the local Committee on Ethics in Medical Studies.

Homocysteine was determined using frozen (−70°C) samples and high-performance liquid chromatography with a fluorescence detector, according to the method of Araki and Sako[13] and with test kits from Bio-Rad (München, Germany). Fibrinogen was measured according to Clauss, with test kits from bioMerieux (Lyon, France). Lp(a) was measured using electroimmunodiffusion and test kits from ImmunoAg (Vienna, Austria). The concentrations of triglycerides and total cholesterol were determined using enzyme tests, and the content of cholesterol in LDL and HDL fractions was established using precipitation and test kits from Boehringer-Mannheim (Mannheim, Germany).

Statistical Analysis

Results are presented as means ± SD. Statistically significant differences were analyzed using Student's *t* test for paired data. The results obtained for Lp(a) were compared using the nonparameteric Wilcoxon test for paired values. Associations between differences in the concentrations of homocysteine, fibrinogen, and Lp(a) before and after supplementation were evaluated with the correlation coefficient. The level of significance was $P < .05$.

RESULTS AND DISCUSSION

Vitamin supplementation led to significant decreases in the concentrations of homocysteine (33.6%), fibrinogen (18.2%), and Lp(a) (21.0%; Table 1). Total cholesterol, HDL and LDL cholesterol, triglyceride, urea, creatinine, and uric acid concentrations remained unchanged. Six months after vitamins were discontinued, the levels of homocysteine, fibrinogen, and Lp(a) had increased significantly and return to initial values. A positive correlation ($r = 0.638$) between the decreases in concentrations of homocysteine and fibrinogen after treatment was found (Fig 1). No correlation between the decreases in concentrations of homocysteine and Lp(a) or of Lp(a) and fibrinogen was seen.

We have reproduced the observations of Bostom et al[3] by finding homocysteine levels almost twice in excess of 14 μmol/l, the upper normal limit, in all patients with CRF. This was accompanied by elevated levels of fibrinogen and Lp(a). Lipid parameters, with the exception of HDL cholesterol levels, were within normal limits, again in agreement with the observations of Bostom et al.

Numerous reports show that attempts to reduce the levels of fibrinogen and Lp(a) have been unsuccessful.[14,15] On the other hand, hyperhomocysteinemia can be controlled with B vitamins, which are cofactors in the metabolism of this amino acid.[11,12,16] A recent prospective study by Rimm et al[17] in 80,000 women showed a strong inverse relationship between serum concentrations of folic acid and vitamin B_6 on one hand and ischemic heart disease on the other. Serum concentrations of folic acid and vitamin B_6 in patients undergoing dialysis are low.[18] However, administration of folic acid may be risky when the level of vitamin B_{12} in serum is insufficient.[19] Therefore, cyanocobalamin should also be used for the management of hyperhomocysteinemia in patients undergoing dialysis whenever its serum level cannot be established. In most countries, patients undergoing dialysis routinely receive supplementation with water-soluble vitamins.[20] Often this supplementation does not protect against hyperhomocysteinemia, possibly because the usual daily doses of folic acid (1 mg), vitamin B_6 (10 mg), and B_{12} (12 μg) are too low.[20] On the other hand, it remains to be established whether long-term supplementation with high doses of B vitamins, often for the lifetime of the patient, is safe. Taking these considerations into account, we used combination therapy with folic acid vitamin B_6 and B_{12} administered in high doses for a relatively short period. It appears from the literature that this regimen is in most cases effective and well tolerated by patients. The doses we used were similar to those reported by Bostom et al[12] as was the approximate 30% reduction in homocysteine levels in our patients who had not previously

Table 1. Effects of 4 Weeks' Supplementation of Folic Acid, Pyridoxine, and Cyanocobalamin on Homocysteine, Fibrinogen, and Lp(a) in 21 Dialysis Patients

Variable	Before Treatment	After Treatment	*P*	6 Months Later*	*P*
Homocysteine (μmol/L)†	36.6 ± 11.6	24.3 ± 7.2	<.0001	31.4 ± 9.8	<.0001
Fibrinogen (g/L)	4.03 ± 0.70	3.30 ± 0.60	<.0001	3.76 ± 0.82	<.006
Lp(a) (mg/dL)	31.0 ± 17.3	24.5 ± 13.4	<.0006‡	30.1 ± 24.5	<.013‡
Total cholesterol (mg/dL)	176.4 ± 36.2	173.5 ± 35.6	NS	168.4 ± 38.2	NS
Triglycerides (mg/dL)	151.6 ± 62.8	137.8 ± 52.0	NS	145.7 ± 54.2	NS
LDL cholesterol (mg/dL)	116.5 ± 37.2	117.5 ± 35.8	NS	109.7 ± 42.1	NS
HDL cholesterol (mg/dL)	38.33 ± 7.38	39.28 ± 7.44	NS	37.66 ± 7.76	NS
Creatinine (mg/dL)	10.7 ± 3.7	11.4 ± 2.8	NS	11.7 ± 3.1	NS
Urea (mg/dL)	161.3 ± 51.2	157.0 ± 42.3	NS	167.0 ± 45.5	NS
Uric acid (mg/dL)	7.5 ± 3.2	7.2 ± 3.9	NS	6.9 ± 4.2	NS

* Six months after termination of treatment.

† Normal homocysteine level <14 μmol/L.

‡ Wilcoxon test for paired values.

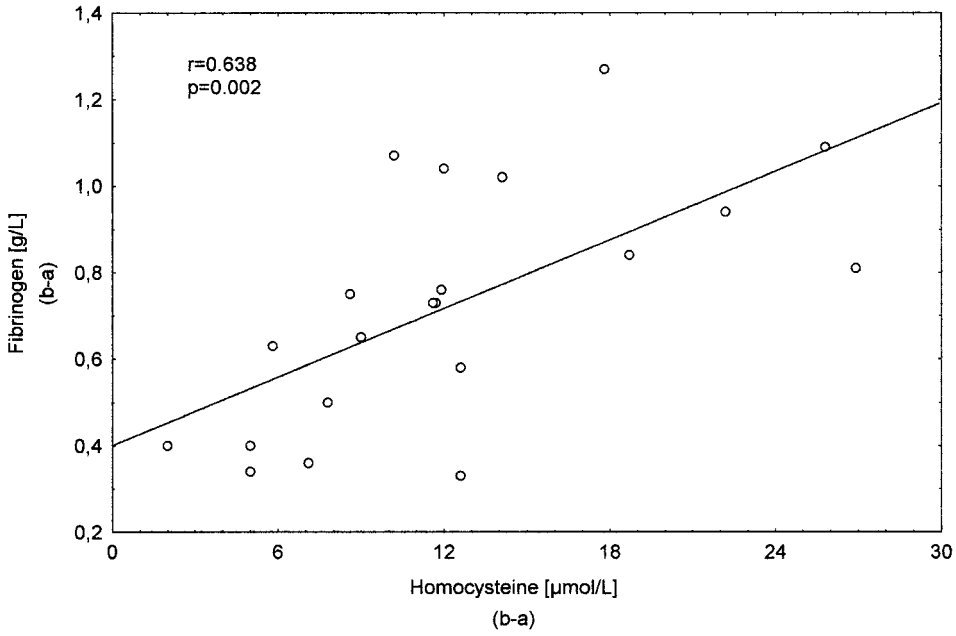

Fig 1. Correlation between decreases in concentrations of homocysteine and fibrinogen after treatment with vitamins. (b, before treatment; a, after treatment).

received routine vitamin supplementation. We also noted a reduction in the levels of fibrinogen and Lp(a), which were not included in the study of Bostom.

The simultaneous decreases in concentrations of homocysteine, fibrinogen, and Lp(a) and the positive correlation between homocysteine and fibrinogen make it interesting to search for interrelations between these factors. Harper et al[10] have observed in an in vitro study that homocysteine, even at a low concentration of 8 μmol/L, could modify the structure of Lp(a) by exposing lysine-binding domains in the apo(a) molecule.[10] In consequence, the binding between such Lp(a) and fibrinogen was greatly increased. However, *N*-acetylcysteine, used by Wiklund et al,[21] reduced concentrations of homocysteine (by 45%) without affecting Lp(a) levels. The mechanism of action of this compound relies on the disruption of bonds between homocysteine and proteins in Lp(a).

Another explanation for interactions between homocysteine, fibrinogen, and Lp(a) is offered by the theory of oxidative stress.[9] Homocysteine, like other amino acids with thiol groups, undergoes autooxidation in serum with production of superoxide radicals and hydrogen peroxide. The so-called oxidative stress that develops is the cause of chronic vascular inflammation and one of the mechanisms through which homocysteine promotes atherogenesis. Studies in CRF patients show disequilibrium between free radical–generating systems and the production of antioxidants.[22] Because oxidative stress associated with renal failure accelerates atherosclerosis and exaggerates the anemia of kidney disease, hyperhomocysteinemia in these patients should be viewed not only as the probably cause of vascular damage but also as an aggravating factor in respect to the status and prognosis of renal failure.[23] Therefore, we believe that folic acid and vitamins B_6 and B_{12}, by reducing the concentration of homocysteine, have inhibited the inflammatory process in vessels, thereby improving functional parameters in renal failure.

Our present results call for a prospective study in a large group of patients to evaluate the influence of vitamin therapy on the concentration of some biochemical risk factors of atherosclerosis in view of the anticipated reduction in morbidity and mortality due to cardiovascular disease. In fact, this suggestion is concordant with the recent opinion of American Heart Association on homocysteine.[24]

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