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Preliminary report: Hyperhomocysteinemia in patients with acute intermittent porphyria

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Abstract

Homocysteine is an intermediate of methionine metabolism, and its elevation in tissues is correlated with an increased risk for vascular diseases. We measured homocysteine in plasma of 24 patients with acute intermittent porphyria (AIP) and long-term high excretion of heme precursors. Fifteen (62.5%) presented hyperhomocysteinemia (total homocysteine in plasma >15 μmol/L). No association was found between hyperhomocysteinemia and either urinary excretion of heme precursors or clinical status. All the patients showed normal levels of vitamin B_{12} and folic acid, but 13 (54%) presented low plasma levels of pyridoxal 5′-phosphate (PLP <15 nmol/L). Cystathionine β-synthase (CBS) catalyzes a major removal pathway of homocysteine and is dependent on both PLP and heme as cofactors. It is hypothesized that, in AIP, CBS reduced hepatic activity resulting from either a low heme status and/or consumptive depletion of PLP due to increased demand by 5-aminolevulinatesynthase hyperactivity can induce hyperhomocysteinemia.

1. Introduction

Acute intermittent porphyria (AIP) is a disorder resulting from a deficiency of hydroxymethylbilane synthase, the third enzyme of the heme biosynthetic pathway [1]. Carriers of mutations within the hydroxymethylbilane synthase gene are at risk of presenting acute neurovisceral attacks that are associated with induction of 5-aminolevulinatesynthase (ALAS) in the liver and overproduction of porphobilinogen (PBG) and aminolevulinic acid (ALA). Some AIP patients with peripheral neuropathy have been reported to present elevated homocysteine in plasma [2]. Homocysteine is an intermediate of methionine metabolism, and its elevated levels are correlated with an increased risk for vascular diseases [3]. Causes of hyperhomocysteinemia include inherited enzymatic defects, dietary deficiencies of vitamin B6, folic acid, or vitamin B12, and renal failure.

We measured homocysteine in plasma in a group of Spanish AIP patients to determine whether this disease is associated with changes in homocysteine metabolism.

2. Results and discussion

We studied 24 Caucasian patients with AIP (20 women and 3 men; 22-54 years old) attending the Hospital Clinic of Barcelona (Spain). All presented a chronic phase of the disease with long-term high excretion of heme precursors and in some cases intermittent clinical exacerbations. All showed a normal renal function. Recurrent crises required regular infusions of heme arginate (Normosang; Orphan Europe) in 4 patients. Approval for the study was obtained from the ethical committee. Urine and blood samples were obtained after overnight fasting following hospital protocols. Blood samples were collected in tubes with EDTA and immediately centrifuged to minimize homocysteine efflux from erythrocytes to plasma. Homocysteine was measured by chemiluminescence immunoassay (ADVIA Centaur; Siemens Healthcare Diagnostics). Analytical methodology and reference limits in fasting condition had been previously set in our laboratory [4].

Conflict of interest: none.

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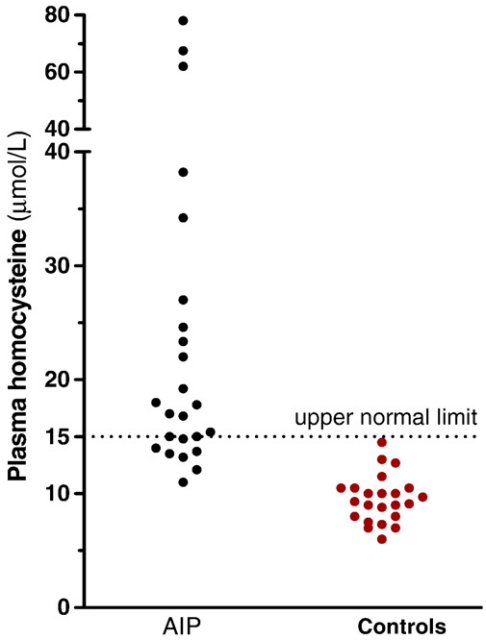

Fig. 1. Acute intermittent porphyria (AIP): plasma total homocysteine concentration (in micromoles per liter) in 24 patients with AIP. The upper normal limit was set at 15 μmol/L according to previous studies and international consensus. All patients presented increased long-term excretion of heme precursors (PBG in urine: range, 10-100 nmol/mmol creatinine; normal limit, <0.8). Controls: plasma homocysteine concentration in 24 healthy controls (age, 20-45 years).

Heme precursors in urine, intraerythrocyte and serum folic acid, and vitamin B_{12} in serum were measured by standard methods. Pyridoxal 5′-phosphate (PLP, vitamin B_6) in plasma was measured by high-performance liquid chromatography [4].

We found 15 patients (62.5%) with hyperhomocysteinemia. Plasma of these patients was reanalyzed by high-performance liquid chromatography (coefficient of variation <4.3%) [4], confirming the high values obtained by immunoassay in all cases. Hyperhomocysteinemia was classified as mild (15-25 μmol/L), intermediate (25-50 μmol/L), and severe (>50 μmol/L) [4]; thus, 3 patients had severe, 3 intermediate, and 9 mild hyperhomocysteinemia (Fig. 1). A second blood analysis performed after 1 to 2 months confirmed the hyperhomocysteinemia in all cases. All patients showed normal levels of vitamin B_{12} in serum (>250 pg/mL) and normal levels of folate in serum and erythrocytes (>3 and >250 ng/mL, respectively). We did not find a correlation between homocysteine in plasma and PBG or ALA in urine. Patients were classified into two groups depending on the occurrence of repeated attacks in the last 4 years; however no association was observed between hyperhomocysteinemia and clinical recurrence.

One patient (woman aged 26 years) received single heme arginate (Heme) infusions every 2 weeks. Homocysteine was measured immediately before and after (4 days) the treatment in 4 consecutive sessions. Plasma homocysteine was reduced by heme infusions, but a recovery was manifest at initiation of the next session: homocysteine (in micromoles per liter): 64 (pre-Heme) → 17 (post-Heme); 64 (pre) → 24 (post); 56 (pre) → 18 (post); 62 (pre) → 17 (post).

Thirteen patients (54%) showed low levels of PLP in plasma (<15 nmol/L). Therefore, all AIP patients were classified as (group A) normal PLP (n = 11) or (group B) low PLP (n = 13). Hyperhomocysteinemia was found in 6 patients of group A and in 9 patients of group B, and no significant association was found between both parameters.

After exclusion of renal failure, dietary deficiencies, or major genetic or hormonal disorders [3], we concluded that hyperhomocysteinemia in these patients was associated with AIP itself. Thus, chronic liver involvement may induce changes in methionine metabolism and partially impair the detoxification of homocysteine. Homocysteine is cleared by two major pathways: (*a*) transmethylation catalyzed by methionine synthase and (*b*) transsulfuration catalyzed by cystathionine β-synthase (CBS). The CBS activity is dependent on both PLP and heme [5], PLP also being a cofactor for ALAS [6]. Thus, CBS-reduced activity in AIP patients could result from either a low heme status in the liver and/or consumptive depletion of PLP due to increased demand by ALAS1 hyperactivity. We found a significant number of patients with low PLP in plasma, confirming other studies on AIP [7]. However, our data regarding a relationship between PLP status and hyperhomocysteinemia are inconclusive. More studies are needed to clarify the origin and clinical significance of hyperhomocysteinemia among AIP patients.

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