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Altered amino acid homeostasis in subjects affected by fibromyalgia

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Abstract

Objectives: To evaluate plasma amino acid (AA) concentrations in patients affected by fibromyalgia (FM) and to study the relationships between their levels and FM clinical parameters.

Design and methods: 20 AAs were assessed in 34 FM patients and in 18 healthy volunteers by means of a modified version of the Waters picotag method.

Results: Significant lower plasma taurine, alanine, tyrosine (Tyr), valine, methionine, phenylalanine and threonine concentrations, and the sum of essential AAs were observed in FM patients vs healthy controls ($P<0.05$). Tyr CAA' ratio and the sum of AAs competing with tryptophan for brain uptake were significantly reduced in FM ($P<0.05$). A significant correlation was found between FM clinical parameters and certain AAs.

Conclusions: Our results suggest probable defects of gut malabsorption of certain AAs in FM patients. Moreover, given the reduced Tyr CAA' ratio in FM patients, a possible impairment of the cathecolaminergic system in the FM syndrome may be suggested.

Keywords: Fibromyalgia; Fatigue; Branched amino acids; Essential amino acids; Tyrosine; Taurine; Muscle energy

Introduction

Fibromyalgia (FM), as defined in the 1990 American College of Rheumatology (ACR) criteria [1] is a chronic, generalized pain condition with characteristic tender points on physical examination, often accompanied by a number of associated symptoms such as fatigue, sleep disturbance, headache, irritable bowel syndrome and mood disorders.

Pathophysiological hypotheses of FM include impairment in the functioning of the hypothalamic–pituitary axis and alterations in neuromodulators and neurotransmitters such as substance P (SP), nerve growth factor (NGF), *N*-methyl-D-aspartate, norepinephrine (NE) and serotonin (5-HT) [2–4]. Substance P and nerve growth factor resulted in an increase in the cerebrospinal fluid (CSF) of patients with primary fibromyalgia but not in fibromyalgia patients with associated painful inflammatory conditions (secondary fibromyalgia) [5–7]. Substance P is a putative modulator of nociception and NGF is the neurotrophic factor that regulates SP synthesis in primary afferent C-fibres, structure thought to transmit pain stimuli. 5-HT and tryptophan concentrations were found to be decreased in serum and CSF of patients with FM [8]. 5-HT is theorized to have a function in stage 4 sleep and pain threshold [9], besides its implication in psychiatric disorders such as depression, anxiety, and obsessive compulsive disorder is often present in fibromyalgic patients.

Considering that tryptophan is the amino acid (AA) precursor of serotonin synthesis and that plasma tryptophan may reflect the status of tryptophan and serotonin in the brain, several authors measured free plasma tryptophan in patients with FM. Moldofsky and Warsh [10] found that free plasma tryptophan is inversely related to morning pain in 8 fibromyalgic patients. Russell et al. [11] reported that the concentrations of serum tryptophan and 9 other amino acids (alanine, histidine, lysine, proline, threonine, serine, taurine and phosphoserine)

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were significantly lower among 20 patients with fibrositis/fibromyalgia syndrome, compared to 20 matched controls. Yunus et al. [12] measuring plasma tryptophan and its transport ratio, together with other twenty-one amino acids, in patients with fibromyalgia found that the transport ratio of tryptophan was significantly decreased in FM patients compared to the control group and the plasma tryptophan level was lower in FM patients than in healthy controls showing a trend towards significance. Also plasma histidine and serine levels were found to be significantly lower in patients with FM than in controls.

Yunus et al. [12] suggested that because tryptophan crosses the blood/brain barrier via a transport mechanism that is shared with other branched chain large neutral amino acids, the transport ratio of these amino acids provides a more meaningful index of their entry into the brain than the plasma concentrations of any one of them alone.

Some authors suggested that a malfunction of energy metabolism may be present in some of the muscle fibres of fibromyalgia patients [13,14]. It is hypothesized that muscle energy depletion could by itself evoke many of the symptoms of fibromyalgia [13,15]. A factor that could contribute to muscle energy depletion is reduced plasma concentrations of the branched chain amino acids (BCAAs), leucine, valine and isoleucine [14]. There is evidence that BCAA supplementation decreases muscle catabolism and has ergogenic values, their infusion displays several CNS-mediated effects including antinociceptive action in healthy subjects but not in FM patients [16].

Recent advances support the involvement of peripheral and central sensitization disturbances of pain-related processes involving the increased transmission of the excitatory amino acid glutamate. Few studies support the implication of this amino acid in chronic migraine and primary fibromyalgia and demonstrated increased levels of glutamate in the cerebrospinal fluid of affected patients [17]. Larson et al. [8] did not show any variation in the levels of excitatory amino acids (EEAs) in the CSF of FM patients compared to controls, while they found significant correlations between the concentrations of some EEAs (arginine, taurine and glycine) and the tender point index (TPi).

A heterogeneous picture exists in literature about AA levels in FM patients, in light of these results we decided to evaluate plasma AA levels in FM patients and to study the relationships between their levels and age, FM clinical and diagnostic parameters. Because amino acid plasma levels have been found altered in psychiatric disorders [18,19], the presence of psychiatric comorbidity in fibromyalgic patients might represent a confounding factor during the elaboration of results. For this reason we have recruited a population of FM patients with a negative history of psychiatric disorders.

Methods

Subjects

34 patients affected by fibromyalgia (29 F, 5 M), aged 49.56±13.82 years (mean age±S.D.) were enrolled. Patients were recruited and clinically classified at the Division of Rheumatology, University of Pisa (St. Chiara Hospital) according to the 1990 American College of Rheumatology criteria (ACR criteria) [1], which include: pain for more than 3 months from all of the four body quadrants, axial skeletal pain and pain upon digital palpation of at least 11 out of 18 specific bilateral points. Healthy volunteers (17 F, 1 M, 39.35± 12.76 years) were recruited from the Transfusion Centre of the St. Chiara Hospital (Pisa) and they were all routinely monitored blood donors. Exclusionary criteria for normal volunteers were: any of the above ACR criteria for fibromyalgia; use of any medication. Exclusionary criterion for patients was: the presence of a major clinical condition other than fibromyalgia. The patients and controls with recent or past history of psychiatric disorders and pregnant females were excluded from the study. All patients maintained their usual diet or physical activity and they had a drug wash out period of at least 2 weeks before blood sampling.

Written consent was obtained from all subjects after a full explanation of the study.

Evaluation of clinical parameters

Tenderness at tender points was evaluated in each subject using the Fischer dolorimeter [20]. A rheumatologist applied the instrument at a rate of 1 kg/s and the patient was instructed to say when this procedure became painful. The pain threshold was calculated for 18 points, and the tender point (TP) count was determined by the number of tender points that had a threshold of ≤ 4 kg/cm^2. The total fibromyalgic tender point score (right+left) was used in the statistical analysis.

To estimate the impact of fibromyalgia on the quality of life, all the patients received a "Fibromyalgia Impact Questionnaire" consisting of 10 items. The resulting score (FIQ total score), which indicates the impact of the disease on life, ranged from 0 (no impact) to 100 (maximum impact). For each patient an evaluation was also made of fatigue by means of a visual analogic scale (VAS, 0–10). Each patient was asked if they had frequently suffered from unrestful sleep (frequent and/or early awakening as well as inability to fall asleep) [21]. Also the duration of disease (years) was taken into consideration for FM patients.

Blood collection, plasma separation and deproteinization, plasma amino acid identification

Blood sodium–EDTA treated samples were collected and immediately centrifuged at 2600 ×*g* for 15 min. Plasma aliquots were diluted 1:1 with HCl 0.1 N, containing 100 μM internal standard (Iss: beta-alanine, alpha-aminobutyric acid, norleucine). After mixing this plasma dilution, acetonitrile (2.8 vol.) was immediately added to precipitate plasma proteins. Samples were maintained on ice for 15–20 min and centrifuged for 15 min at 12,000 ×*g*, 10 °C. The amino acids were determined by means of a modified version of the Waters picotag method (Waters S.p.a.) using phenylisotiocianate as the derivatizing agent (our unpublished data).