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Aberrant tryptophan transport in cultured fibroblast from patients with Male Idiopathic Osteoporosis: An in vitro study

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ABSTRACT

It has been demonstrated, that long-term chronic tryptophan deficiency, results in decreased serotonin synthesis, which may lead to low bone mass and low bone formation. Findings from studies in male patients with idiopathic osteoporosis suggested a decreased transport of tryptophan in erythrocytes of osteoporotic patients, indicating that serotonin system defects may be involved in the etiology of low bone mass. Tryptophan is the precursor of serotonin, and a disturbed transport of tryptophan is implicated in altered serotonin synthesis. However, no study has investigated the tryptophan transport kinetics in MIO patients. The aim of this study is to investigate the kinetic parameters of tryptophan transport in fibroblasts derived from MIO patients compared to age and sex matched controls.

Fibroblast cells were cultured from skin biopsies obtained from **14** patients diagnosed with Male Idiopathic Osteoporosis and from **13** healthy age-sex matched controls, without a diagnosis of osteoporosis. Transport of the amino acid tryptophan across the cell membrane was measured by the cluster tray method. The kinetic parameters, maximal transport capacity (V_{max}) and affinity constant (K_m) were determined by using the Lineweaver-Burke plot equation.

The results of this study have shown a significantly lower mean value for V_{max} ($p = 0.0138$) and lower K_m mean value ($p = 0.0009$) of tryptophan transport in fibroblasts of MIO patients compared to the control group. A lower V_{max} implied a decreased tryptophan transport availability in MIO patients.

In conclusion, reduced cellular tryptophan availability in MIO patients might result in reduced brain serotonin synthesis and its endogenous levels in peripheral tissues, and this may contribute to low bone mass/formation. The findings of the present study could contribute to the etiology of idiopathic osteoporosis and for the development of novel approaches for diagnosis, treatment and management strategies of MIO.

1. Introduction

Osteoporosis in young and middle-aged men, in the absence of secondary causes, is sometimes called “idiopathic osteoporosis”. The etiology is heterogeneous but several studies, including our own, have shown signs of osteoblast dysfunction and bone histomorphometry variables indicating low bone formation (Chavassieux and Meunier, 2001; Pernow et al., 2009; Pernow et al., 2006). Hormonal dysfunctions including mutations or polymorphisms in the aromatase, the estradiol receptors or IGF-1 system have been suggested as possible pathophysiological pathways (Khosla et al., 1998; Orwoll, 1998; Gennari and Bilezikian, 2013; Bilezikian, 1999).

We have previously reported that Male Idiopathic Osteoporosis (MIO) patients have decreased erythrocyte tryptophan levels, compared to healthy controls. We found a positive correlation between the bone histomorphometric parameters (wall thickness, trabecular thickness and mineral apposition rate) and the erythrocyte tryptophan levels in the MIO patients. There was no difference in plasma tryptophan levels indicating a difference in tryptophan transport over the cell membrane (Pernow et al., 2010). Tryptophan is an essential amino acid that belongs to the group of large neutral amino acids (LNAA) and it is the precursor of serotonin (5-hydroxytryptamine, 5-HT). Serotonin is

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synthesized in a two-step pathway from L-tryptophan by the rate limiting enzyme tryptophanhydroxylase (Tph). Inhibition of Tph2 results in decreased production of serotonin in the brainstem. A lower central serotonin level increases the sympathetic outflow and an increased activation of osteoblast beta adrenergic receptors results in lower bone formation (Niedzwiedzki and Filipowska, 2015). It has been demonstrated in an animal model, that chronic tryptophan deficiency results in decreased serotonin synthesis, which can lead to low bone mass and low bone formation (Sibilia et al., 2009). The brain serotonin production is dependent on the amount of tryptophan transported over the blood brain barrier (BBB). Thus, a lower tryptophan transport capacity could result in lower bone formation. The rate of serotonin synthesis depends on affinity of tryptophan to the transporter protein and the transport of tryptophan across the plasma membranes. In the human fibroblast cell model, tryptophan transport has been functionally characterized (Vumma et al., 2011). Tryptophan is mainly through LAT1 isoform of system-L through the fibroblast cell membranes at different concentrations ranges (Vumma et al., 2011). Skin derived fibroblast cells have been used as an in-vitro cell model in many studies to examine the transport of amino acids across cell membranes of patients with schizophrenia, bipolar disorder and autism (Hagenfeldt et al., 1987; Flyckt et al., 2001; Persson et al., 2009; Johansson et al., 2011). The aim of the present study was to investigate the kinetic parameters of tryptophan transport in fibroblasts obtained from men with idiopathic osteoporosis and from healthy controls.

2. Materials and methods

2.1. Study subjects

Fourteen men with idiopathic osteoporosis were recruited from the Department of Endocrinology, Karolinska University Hospital. The median age was 56 years (range 39–68). Osteoporosis was defined as 2.5 SD below the mean Bone Mineral Density (BMD) of young healthy men at the lumbar spine or hip. There was a history of osteoporotic fractures in 10 patients; 4 had vertebral fractures, one had hip fracture and 5 had other peripheral fractures. The reason for the finding of osteoporosis in the 4 patients without fractures was familial disposition. Eight patients had a family history of osteoporosis. None of the patients had secondary osteoporosis, i.e. endocrine disorders, malabsorption syndromes, alcoholism or medications known to interfere with bone metabolism. Three patients with osteoporosis had been included in the previous study on amino acids in Male Idiopathic Osteoporosis (Pernow et al., 2010). Osteoporosis was diagnosed at admittance to the hospital, in some cases several years prior to the present study. The T- and *Z*-score at the time of diagnosis are given in the clinical presentation of the patients as well as previous or ongoing medical treatment for osteoporosis. For patients BMD at lumbar spine and femoral neck were measured with Hologic QDR 4500 DXA (Hologic, Waltham, MA, USA) and Lunar DPX-L or Lunar Prodigy (Lunar, Madison, WI, USA). The data are listed as T-score; standard deviation (SD) from the mean of normal young adult males according to a reference population provided by the manufacturer (Lunar: USA reference population v101, Hologic: NHANES) and Dual Energy X-Ray Absorptiometry, Lunar Prodigy Advance PA + 130198.

Fibroblast cell lines derived from the skin biopsies of patients. One biopsy was lost in preparation. The presented data is from 14 patients with MIO (Table 1). The excluded patient did not differ in clinical presentation from the other MIO patients.

Fourteen healthy age-matched male volunteers were recruited from the university staff, with a median age of 56 (range 42–66) served as controls (Table 1). Interviews revealed no significant previous or present illness and they took no medication. One of age-matched controls (ID-code: FFP-O-20) showed to have osteoporosis and has been excluded from the study. The presented data is from 13 healthy age-matched male controls (Table 1).

Table 1
Characteristics and bone mineral density of male osteoporotic patients and age-matched control men.

	MIO (n = 14)	Control (n = 13)	p-Value
Age at study	56 ± 11	55 ± 8	ns
Age at diagnose	48 ± 9	na	
Body weight (kg)	78 ± 7	84 ± 17	ns
Body height (cm)	180 ± 6	180 ± 3	ns
BMI (kg/cm2)	24 ± 2	26 ± 5	ns
Family history of osteoporosis	8 yes 5 no 1 na	None	
Fractures	10 yes 4 no	1 yes 12 no	
BMD lumbar spine			
Tscore, mean ± SD	− 3,0 ± 0,7 (n = 13)	0,6 ± 1.5	p < 0,001
Median (range)	− 3,3 (− 4.4 to − 1.9)	0,2 (− 1.5 to 2.7)	
Zscore, mean ± SD	− 2.7 ± 0,7 (n = 13)	0,7 ± 1,2	p < 0,001
Median (range)	− 2.8 (− 3.9 to − 1.8)	0,4 (− 1.8 to 2,4)	
BMD femoral neck			
Tscore, mean ± SD	− 2,4 ± 1,1 (n = 13)	− 0.6 ± 1,1	p < 0,001
Median (range)	− 2.6 (− 4.1 to 0,0)	− 1.3 (− 1.6 to 1,3)	
Zscore, mean ± SD	− 1.7 ± 1,1(n = 13)	0,04 ± 0,9	p < 0,001
Median (range)	− 2.0 (− 3.5 to 0,9)	0,0 (− 1.5 to 1,6)	
Treatment for osteoporosis	10 yes (bisphosphonates) 4 no	No	

Notes: variables are shown as mean ± S.D. Abbreviation: (n =), number of subjects, na: not available; ns: not significant; S.D.: standard deviation; BMI: body mass index; BMD: Bone mass density. Z-score = (observed BMD - mean BMD for age- and sex-matched healthy subjects)/SD for age- and sex-matched healthy subjects. T-score: BMD in a patient compared to young normal controls. Osteoporosis is defined as 2.5 SD below the mean BMD of young healthy men at the lumbar spine or hip.

2.2. Materials

All growth media used for cell culture, antibiotics and fetal bovine serum (FBS) were obtained from Gibco Invitrogen (Sweden). Tissue culture flasks and multi-well plates were from Costar Europe Ltd., Costar NY. [3H] L-tryptophan with a specific activity of 20 Ci/mmol was purchased from Larodan Fine Chemicals AB (Malmö, Sweden). D-Glucose was purchased from Ambresco (Ohio, USA) and phosphate buffered saline (PBS) was obtained from the National Veterinary Institute (SVA) (Uppsala, Sweden). Scintillation cocktail (Optiphase, Hisafe 3) and liquid scintillation counter (Winspectral1414) were from Perkin Elmer Life Sciences (USA). Scintillation vials were purchased from Sarstedt AB (Sweden). 96-well plates used for protein determination were purchased from Nunc (Roskilde, Denmark). The tryptophan solutions were made in PBS and the pH was maintained between 7.35 and 7.40.

2.3. Cell culture

Fibroblast cells were cultured in plastic tissue culture flasks containing minimal essential medium (MEM) supplemented with 10% FBS, L-glutamine (2 mM/l), penicillin (100 mg/ml), streptomycin (100 mg/ml) and Amino-Max™. Cells were maintained in a humidified atmosphere of 5% CO_2 in air at 37 °C. Confluent cells harvested and seeded in to 2 cm^2 multi-well plates and grown till confluent for approximately 5 days were used for carrying out tryptophan transport assays. Cell lines between 4th and 15th passages (number of splitting) were used in the experiments.