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Decrease of plasma taurine in Gaucher disease and its sustained correction during enzyme replacement therapy

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Summary. Gaucher disease is caused by an autosomal-recessive deficiency of glucocerebrosidase. Cells of monocytic/macrophagic origin accumulate glucosylceramide. This leads to hepatosplenomegaly, bone destruction, thrombocytopenia and anemia. Enzyme replacement therapy (ERT) with macrophage-targeted glucocerebrosidase leads to normalization of these parameters. The way of macrophage activation in Gaucher disease is not known. Recently, the osmolytes taurine, betaine and inositol were identified as important regulators of macrophage function in liver. Therefore, the role of plasma taurine in Gaucher disease as a primarily macrophage-derived disease was studied.

Fasting plasma levels were measured from blood samples of healthy control subjects (n = 29, m:f = 11:18, mean age 37 ± 3 years), from untreated Gaucher patients (n = 16, m:f = 7:9, mean age 44 ± 4 years) and those treated for 37 ± 2 months (n = 54, m:f = 19:35, mean age 47 ± 2 years). Amino acid analysis was carried out in a BioChrom amino acid analyzer.

In the untreated patients, plasma taurine was 45 ± 3μM, as compared to the controls with a plasma taurine of 63 ± 4μM ($p < 0.01$). The average increase of plasma taurine during the first year of ERT was 18 ± 8μM (n = 10). Patients treated for an average of 37 months (range 1–9 years of ERT) had a plasma taurine of 65 ± 4μM (n = 54), which was not different from the controls.

It is concluded that Gaucher patients show decreased plasma taurine levels and that therapy of Gaucher disease might correct this. It has to be established, whether decreased taurine availability is a cofactor of the permanent activation of glucosylceramide-storing monocytes/macrophages in this disease.

Keywords: Amino acids – Enzyme replacement therapy – Lysosomal storage disease – Macrophages – Liver disease

Introduction

Gaucher disease is the most frequent lysosomal storage disease. The molecular defect is a genetic deficiency of glucocerebrosidase. Cells of monocytic/macrophagic origin accumulate glucosylceramide, eventually leading to hepatosplenomegaly, hematologic changes and bone destruction. Since 1991, enzyme replacement therapy (ERT) by infusion of macrophage-targeted glucocerebrosidase is available and has been shown to improve the hematologic, visceral and bone changes of type I Gaucher disease (Barton et al., 1991; Pastores et al., 1993; Niederau et al., 1994; Grabowski et al., 1995; Beutler, 1997; Hollak et al., 1997; Niederau et al., 1998). The exact nature of the sustained macrophage activation in this disease is not known (reviewed in Hollak et al., 1997).

Recently, taurine has been identified as an important osmolyte in liver macrophages (Warskulat et al., 1997a,b; vom Dahl et al., 1999). Apart from the role of osmolytes in cell volume homeostasis it has been demonstrated that betaine, taurine and myo-inositol are important modulators of macrophage function at the level of gene expression, release of inflammatory mediators and phagocytotic activity (Warskulat et al., 1996, 1997b; Yancey et al., 1982; Zhang et al., 1995, 1996). Further, a hepatoprotective action of taurine has been described (Waterfield et al., 1993a,b; Wettstein and Häussinger, 1997; vom Dahl et al., 1998). Therefore, the role of plasma taurine in Gaucher disease was studied.

The results show a decrease of plasma taurine in this disease, which is effectively treated and maintained by enzyme replacement therapy.

Methods

Patients

Gaucher patients from all parts of Germany were admitted to the Dusseldorf Gaucher outpatient clinic for routine checks of blood tests, ultrasound and MR radiography every 6–12 months before and during enzyme replacement therapy (ERT). All Gaucher patients had the adult form of the disease (type I). The diagnosis had been confirmed by measurement of glucocerebrosidase activity in blood leucocytes (K. Harzer, Tübingen, Germany [Harzer, 1980]). Treatment was performed by intravenous infusion of either alglucerase (Ceredase®, Genzyme, Boston, MA) or imiglucerase (Cerezyme®, Genzyme, Boston, MA), which was performed on an outpatient basis by the patients' respective local physicians. The dosage was 20, 40 or 60 units of i.v. alglucerase/imiglucerase every other week and was dependent on the severity of the disease. Fasting plasma levels were measured from blood samples of healthy control subjects ($n = 29$, m:f = 11:18, mean age 37 ± 3 years), from untreated Gaucher patients ($n = 16$, m:f = 7:9, mean age 44 ± 5 years) before initiation of ERT and those, who had been treated for more than a year ($n = 54$, m:f = 19:35, mean age 47 ± 2 years).

Amino acid analysis

Amino acid analysis was performed with a BioChrom 20 (Pharmacia, Freiburg, Germany). Plasma samples were deproteinized by mixing 100 μl of plasma with 100 μl of

10% sulphosalicylic acid solution in 0.5 M lithium citrate buffer. After cooling at 4°C for 30 min, the mixture was centrifuged for 5 min at 16.000 rpm. The volume of sample loaded on the ion-exchange column was 40 μl of the deproteinized supernatant. For amino acid analysis a standard stepwise elution by 5 lithium citrate buffers was used through a column using 8 μm cation exchange resin (Blom and Huijmans, 1992). The amino acids were detected with ninhydrin reagent through a reaction coil set at 135°C. An external standard (Sigma, Munich, Germany) was used for calibration. Data analysis was performed by EZChrome software (Scientific Software, San Ramon, CA).

Statistics

Data were expressed as means ± S.E.M. Statistics were performed by Student's t-test. A probability value $p < 0.05$ was considered to indicate a significant difference.

Results

Before initiation of enzyme replacement therapy, Gaucher patients with the adult form of the disease showed significantly decreased plasma levels of taurine, methionine, tyrosine and histidine, whereas the levels of ornithine were slightly increased (Table 1, Fig. 1). The patient groups were comparable with respect to gender (m:f = 11:18 in the control group vs. m:f = 7:9 in the Gaucher group). The mean age was 37 ± 3 years in the control group and 44 ± 4 years in the naive (untreated) Gaucher group. The results of plasma amino acid analysis in the control group correspond well to those published for adults from this area (Brenner et al., 1985).

In 10 patients with adult-type Gaucher disease, enzyme replacement therapy was installed. The mean dosage was 40–60 U alglucerase (7 patients) or imiglucerase (3 patients) intravenously every other week. The patients were controlled for their plasma taurine after a mean of 14 ± 4 months. After this time period, plasma taurine had increased in all but one patient (Fig. 2). On average, plasma taurine had increased from 45 ± 3 μM to

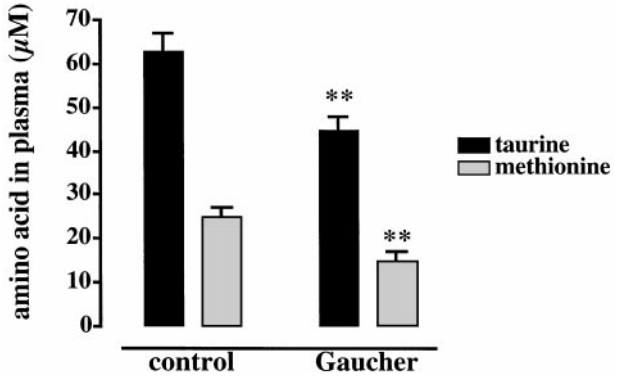

Fig. 1. Plasma taurine and methionine levels in healthy controls and untreated Gaucher patients. Plasma amino acids were determined in healthy subjects (n = 29) and Gaucher patients before initiation of therapy (n = 16). Data are given as means ± S.E.M.

Table 1. Plasma levels of different amino acids and urea in healthy controls and patients with Gaucher disease type I

	Control	Gaucher
taurine	63 ± 4	45 ± 3**
urea	5370 ± 348	5220 ± 347
aspartic acid	10 ± 1	11 ± 2
threonine	129 ± 7	106 ± 10
serine	104 ± 5	107 ± 7
glutamic acid	56 ± 8	51 ± 8
glutamine	414 ± 29	381 ± 45
proline	245 ± 20	286 ± 36
glycine	219 ± 14	234 ± 19
alanine	368 ± 20	314 ± 28
citrulline	28 ± 2	28 ± 5
valine	226 ± 11	191 ± 15
cystine	42 ± 3	34 ± 4
methionine	25 ± 2	15 ± 2**
isoleucine	62 ± 3	54 ± 3
leucine	116 ± 6	103 ± 6
tyrosine	71 ± 4	55 ± 5*
phenylalanine	57 ± 3	59 ± 3
ornithine	58 ± 4	88 ± 7**
lysine	167 ± 7	166 ± 9
histidine	88 ± 4	71 ± 3**
arginine	87 ± 7	68 ± 7

Plasma samples from 29 fasted healthy volunteers and 16 naive (untreated) Gaucher patients with the adult form of the disease (type I) were taken, deproteinized and analyzed by conventional amino acid chromatography. Data were given in μmol/L as means ± S.E.M. Significance was calculated by means of Student's *t* test: *, **: significant versus respective control, *, $P < 0.05$, **, $P < 0.01$.

63 ± 4μM in these 10 patients. The mean increase was 18 ± 8μM (range −1–74μM). To exclude an experimental bias in the naive Gaucher group, the plasma taurine levels were determined in Gaucher patients who had been on therapy for more than one year. After an average treatment time of 37 ± 2 months, the plasma taurine levels in 54 Gaucher patients with ERT (48 on alglucerase, 6 on imiglucerase) was 65 ± 4μM (Fig. 3), which was not different from the control levels in healthy individuals (Table 1). Surprisingly, the levels of tyrosine (60 ± 3μM), methionine (16 ± 1μM) and histidine (73 ± 2μM) did not significantly change during therapy (Table 1). Likewise, ornithine levels remained elevated in spite of this long treatment period (75 ± 5μM).

Discussion

The results indicate a decreased plasma taurine in untreated Gaucher patients and its rapid correction during enzyme replacement therapy.

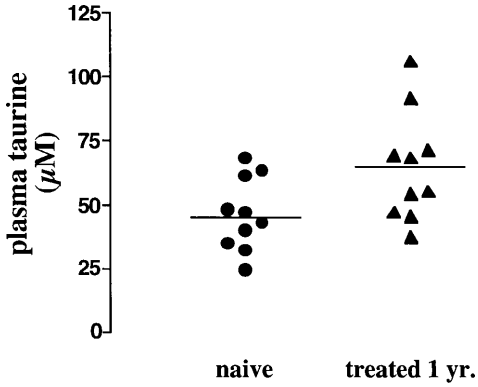

Fig. 2. Individual development of plasma taurine levels during a one-year period of enzyme replacement therapy. Individual data with the respective means are shown from 10 patients with enzyme replacement therapy (ERT) for an average 14-month period. The average increase of plasma taurine within a year of ERT was 18 ± 8μmol/L (n = 10, range −1–74)

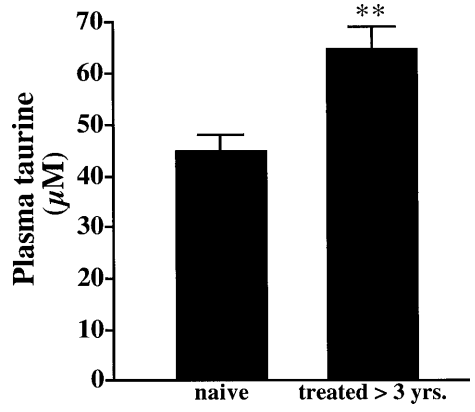

Fig. 3. Normalization of plasma taurine in Gaucher patients during long-term enzyme replacement therapy. Plasma taurine levels were compared between naive Gaucher patients (n = 16) and those who had received therapy for 37 ± 2 months (range 1–9 years of therapy, mean dosage 45 U/kg body wt. every other week i.v.). Data are given as means ± S.E.M.

A normal plasma taurine is maintained during longer treatment periods in this disease.

The rapid increase of plasma taurine corresponds to the effects of enzyme replacement therapy on other blood and plasma abnormalities, e.g. anemia, thrombocytopenia and the increase of serum tartrate-resistant acid phosphatase, angiotensin-converting enzyme (ACE), ferritin and chitotriosidase (reviewed in Hollak and Aerts, 1997). The improvement of these blood parameters during enzyme replacement therapy is usually fastest within the first year of therapy and then declines to steadier rates of improvement (Zimran

et al., 1995; Grabowski et al., 1995; Beutler, 1997; Niederau et al., 1998; Barton et al., 1990).

It might be speculated that the increased plasma availability of taurine after installation of therapy might improve delivery of this compound to peripheral tissues, in which it could exert its anti-inflammatory and cytoprotective actions on cells of macrophagic/monocytic origin (Warskulat et al., 1996, 1997b; Zhang et al., 1995; vom Dahl et al., 1998; Denkert et al., 1998).

Nevertheless, the exact reasons for the diminished plasma levels of taurine in this disease remain unclear. If the high amount of macrophages in this disease contributes essentially to plasma taurine levels, it might be hypothesized that the high intracellular burden of undegraded glucosylceramide represents a volume challenge, which eventually leads to an intracellular depletion of taurine. Macrophages have been shown to release interleukin-1 upon addition of glucocerebroside (Gery et al., 1981) and increased levels of tumor necrosis factor-α, interleukin-6 and interleukin-10 are present in this disease (Michelakakis et al., 1986; Allen et al., 1997). Therefore, cytokine-dependent down-regulation of TAUT-1, the taurine transporter, whose activity in liver non-parenchymal cells is controlled by osmotic stress and osmolytes (Warskulat et al., 1997a,b, 1998), is conceivable.

Plasma taurine reflects a balance between high intracellular tissue levels of this osmolyte (in the millimolar range), an extracellular compartment with a comparably low concentration (plasma), taurine conjugation to bile salts, which is quantitatively negligible, and its biliary and urinary excretion (Huxtable, 1992). Therefore, the exact nature of the decrease of plasma taurine levels in this disease is speculative and has to be further characterized in *in vitro* studies. Nevertheless, untreated Gaucher disease resembles a sustained inflammatory state (Hollak et al., 1997; Hollak and Aerts, 1997) and the increased availability of taurine in this disease during therapy might participate in the slow deactivation of the lipid-burden macrophages.

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