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Marked increase of methylumbelliferyl-tetra-*N*-acetylchitotetraoside hydrolase activity in plasma from Gaucher disease patients

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Summary: Methylumbelliferyl-tetra-*N*-acetylchitotetraoside hydrolase activity was increased 53- to 484-fold in plasma from Gaucher disease patients and no activator could be found. High activity was also measured in other lysosomal storage diseases including Krabbe disease, Wolman disease, GM_1-gangliosidosis and to a lesser extent Niemann–Pick disease type B, but the activities were lower than the lowest values in Gaucher patients.

Kinetic properties of the high activity in Gaucher plasma were similar to those of controls. It is not known whether the increased activity represents intrinsic enzyme activity or increased enzyme concentration. It is possible that this enzyme may help in the detection of Gaucher disease or in the assessment of enzyme therapy with *β*-D-glucosidase (Ceredase®).

Gaucher disease, of which three different clinical subtypes exist, is characterized by the accumulation of glucosylceramide (glc-cer) due to a genetic deficiency of lysosomal *β*-D-glucosidase activity (Brady et al 1965). It has long been known that plasma/serum acid phosphatase activity is 2- to 10-fold increased in these patients (Chambers et al 1977; Robinson and Glew 1980). An increase in the activity of enzymes such as lysozyme, angiotensin-converting enzyme (Liebermann and Beutler 1976; Silverstein and Friedland 1977) and other lysosomal enzymes (Öckerman and Kohlin 1969) has also been reported.

On studying 4-methylumbelliferyl-tetra-*N*-acetylchitotetraoside (MU-TACT) hydrolase in plasma (Den Tandt et al 1988, 1993) some seven years ago, we found an occasional Gaucher patient with high activity. Although this was not published at the time, the aim of this report is to further investigate the increase in activity, to determine its specificity and to examine whether it can serve as a marker for Gaucher disease. We also investigated plasma samples from other lysosomal storage diseases. Studies on Gaucher disease have already been reported in studies on chitotriosidase (Hollak et al 1994) — an equivocal name (see below) for the same enzyme since it has no specificity towards tri-*N*-acetyl-glucosamine derivatives — but these authors did not examine possible activators, or the influence of storage materials or other lysosomal storage disorders, and were therefore unable to

comment on the specificity of the increase. Two recent reports (Den Tandt and Van Hoof 1995; Van Diggelen et al 1995) extend the observations to other diseases. We (Overdijk et al 1994) and others (Overdijk and Steijn 1994; Boot et al 1995a; Renkema et al 1995) have gathered direct or indirect evidence that the enzyme is a chitinase, although there is no agreement yet upon its exact molecular weight. The function of MU-TACT hydrolase in plasma is unknown, but it was previously found not to be lysozyme, chitobiase, hexosaminidase, hyaluronidase or neutral endoglucosaminidase (Den Tandt et al 1988, 1993). While this paper was being submitted, an elegant report appeared on the cloning of cDNA encoding chitinase (Boot et al 1995b).

MATERIALS AND METHODS

MU-TACT substrate was obtained from Green Cross Corporation (Osaka, Japan). Glc-cer and concanavalin C (Con A) were purchased from Sigma (St Louis, MO, USA).

Plasma from 220 controls was assayed for MU-TACT hydrolase activity. We also measured the activity of MU-TACT hydrolase and reference enzymes in the following patients: Gaucher disease (14), GM_1-gangliosidosis (6), Niemann-Pick disease type B (1), Farber disease (1), different types of mucopolysaccharidosis (12), mucolipidosis (ML) II and III (4), Tay–Sachs disease (3), Sandhoff disease (1), Fabry disease (2), metachromatic leukodystrophy (3), Wolman disease (1), ceroid lipofuscinosis (1), glycogenosis type II (1), Krabbe disease (1), untreated prostatic carcinoma (5). The samples were stored at −20°C to −35°C. Seven patients with Gaucher disease were described previously (Van Sande et al 1986). MU-TACT hydrolase activity was determined as described (Den Tandt et al 1988) or using a scaled-down micromethod, as follows: 15 μl 0.25 mmol/L MU-TACT, 5 μl 1 mol/L sodium acetate buffer, pH 5.5, and 20 μl acidified plasma (Den Tandt et al 1974) were incubated for 15 and 30 min at 37°C. Gaucher samples were assayed both undiluted and diluted 40-fold and 100-fold, whereas samples from other storage diseases or patients with prostatic carcinoma were assayed undiluted and diluted 40-fold. After incubation, 0.5 mol/L sodium carbonate–bicarbonate, pH 10.7, was added and the fluor-escence was read. Control lysosomal enzymes (β-D-glucuronidase, total hexosaminidase and hexosaminidase A, α-L-fucosidase, acid phosphatase, and β-D-galactosidase) were determined as described (Den Tandt et al 1974; Willems et al 1981; Den Tandt and Scharpé 1991).

Binding of MU-TACT hydrolase to Con A was done as follows: 50 μl 10-fold diluted plasma was incubated at 23°C for 30 min with 20 μl 1 mol/L sodium phosphate buffer, pH 7, 10 μl 1 mol/L $MgCl_2$, 10 μl 1 mol/L $CaCl_2$, and 50 μl of Con A solution (8 mg/ml in 0.05 mol/L $MgCl_2$ and 0.05 mol/L $CaCl_2$). After centrifugation at 10 000*g* for 30 min, MU-TACT hydrolase and *N*-acetyl-β-D-glucosaminidase activities were determined in the supernatant fraction and the pellet redissolved in the same solution but without Con A.

The influence of glc-cer was tested as follows: glc-cer was dissolved in ethanol followed by the addition of water at 50°C to obtain a 10 mmol/L solution in ethanol 20% (v/v). The final concentration of the lipid was between 0.02 and 2 mmol/L.

Dialysis of plasma was against 0.15 mol/L acetate, pH 5.5 for 20 h at 4°C.

A possible activator of MU-TACT hydrolase activity was assessed by mixing control and Gaucher samples in equal proportions.

For the thermoinactivation experiments, plasma was kept at 37°C, 45°C, and 50°C up

to 24h, sampled at different times, and frozen immediately at −20 to −40°C. MU-TACT hydrolase was determined on all samples simultaneously.

RESULTS

MU-TACT hydrolase activity was similar when measured in plasma from heparinized, citrate or EDTA blood or when serum was used. In a sample frozen for one year (and checked at regular intervals) activities in acidified plasma and serum (Den Tandt et al 1974) were 103% and 96% of the initial activity for normal (non-acidified) plasma and 87% and 97%, respectively, for serum.

The activity of MU-TACT hydrolase in heterozygotes and in patients with Gaucher disease, other lysosomal diseases, patients with prostatic carcinoma, and controls is given in Figure 1. The mean activity of MU-TACT hydrolase was calculated on 220 samples and is somewhat lower than the activity computed on a limited number of samples (Den Tandt et al 1988). The range was between 0.034 and 0.93 nanomoles of substrate hydrolysed per minute per ml. One control sample had very high activity (6 times the mean value) and was not included in our normal range. Some pseudodeficient activities were observed in the controls and patients and will be described separately. The mean activities of the other enzymes in Gaucher plasma compared with controls were as follows: β–D-glucuronidase 128%, total hexosaminidase 253%, hexosaminidase A 208%, α-L-fucosidase 96%, acid phosphatase 200%, and β-D-galactosidase 112% (data not shown).

The addition of glc-cer in different concentrations up to 2mmol/L did not stimulate MU-TACT hydrolase activity. There was also no evidence of a low-molecular-weight activator of MU-TACT activity in Gaucher plasma from dialysis or mixing experiments.

Unlike hexosaminidase activity, MU-TACT hydrolase in Gaucher plasma did not bind to Con A.

MU-TACT hydrolase activity in Gaucher plasma had the same thermal stability as previously reported (Den Tandt et al 1988) in normal plasma, being stable up to 45°C for 24h.

DISCUSSION

Heparinized, citrate, or EDTA plasma or serum can be used for MU-TACT hydrolase measurements since the maximum difference in activity was only 10%. We have already described the characteristics of the enzyme (Den Tandt et al 1993) and recommend acidified plasma because this gives more reproducible results for lysosomal enzyme activities than serum. Samples can be kept frozen for at least one year.

We have previously demonstrated that MU-TACT hydrolase activity in plasma is not due to lysozyme (Den Tandt et al 1988). When we measured lysozyme in one of the patients with Gaucher disease by an immunological method (Brouwer et al 1984) only a 2-fold increase was observed.

In plasma from Gaucher patients a marked increase of MU-TACT hydrolase activity was present, the lowest and the highest values being 53 times and 484 times the mean (Figure 1). Of the storage diseases which have been examined, GM_1-gangliosidosis, Wolman disease and Krabbe disease also showed increased activity, the highest being 39-fold in Wolman disease, which is still below the lowest increase (53-fold) in one of the Gaucher patients.

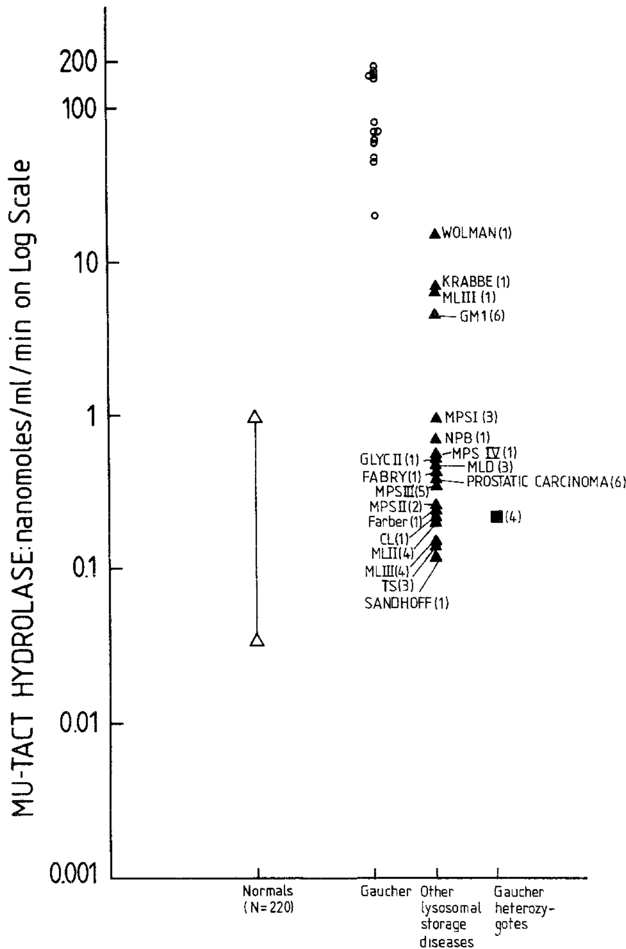

Figure 1 Activity of MU-TACT hydrolase in plasma samples of controls, Gaucher patients and patients with other lysosomal storage diseases and Gaucher heterozygotes. The activity is expressed in nanomoles of substrate hydrolysed per ml of plasma per minute. ML, mucolipidosis; GM_1, GM_1 gangliosidosis; MPS, mucopolysaccharidosis; NPB, Niemann–Pick disease type B; MLD, metachromatic leukodystrophy; CL, ceroid lipofuscinosis; TS, Tay–Sachs disease; GLYC II, Glycogenosis II. Figures in parentheses indicate numbers of patients examined

In one patient with Niemann–Pick disease type B and in the patients with mucopolysaccharidosis I, the activity was within the normal range but high (2 or 3 times the normal mean). We have compared the involvement of histiocytes in the following diseases with the known histiocyte overloading in Gaucher disease (Brady and King 1973a). GM_1-gangliosidosis (Van Hoof 1973) and Wolman disease (Patrick and Lake 1973) have marked histiocytosis. According to Suzuki and Suzuki (1973) 'the globoid cells in Krabbe disease originate from non-neuronal mesodermal cells and they are essentially macrophages'. A lipid-laden histiocyte in Niemann–Pick disease types A and B has been well documented (Brady and King 1973b). It is therefore tempting to speculate that the increase of MU-

TACT hydrolase in Gaucher disease and to a lesser extent in the other storage diseases runs parallel to overloading of histiocytes rich in lysosomes.

In myeloid leukaemia, where high enzyme turnover is evident by increased lysozyme excretion in the urine (Weisner 1984) and where electron micrographs resemble Gaucher cells (Brady and King 1973a), our preliminary data from one patient with acute and another with chronic myeloid leukaemia gave normal plasma values.

As reported previously, patients with MLII and MLIII do not have increased plasma MU-TACT hydrolase activity in contrast to the high activity of many other lysosomal enzymes (Den Tandt et al 1974). However, we have observed one patient with ML III who presented with a 17-fold increased activity on repeated determinations. We cannot explain the finding, unless it is due to genetic heterogeneity or more pronounced lysosomal overloading.

Since plasma acid phosphatase activity is generally increased in patients with Gaucher disease (Chambers et al 1977; Robinson and Glew 1980) and in prostatic carcinoma (Robinson and Glew 1980), we have also determined MU-TACT hydrolase in the plasma of five patients with prostatic carcinoma, but no hyperactivity was detected.

Besides the increased activity of plasma acid phosphatase in Gaucher disease, there is also a well-known hyperactivity of angiotensin-converting enzyme (Liebermann and Beutler 1976; Silverstein and Friedland 1977; Van Sande et al 1986) and certain glycosidases (Öckerman and Kohlin 1969). We have confirmed these observations, since many glycosidases in Gaucher plasma have increased activity, the highest being hexosaminidase (2.5 times normal). Acid phosphatase was not as active as might be expected; it was increased only 2-fold as found by Hollak et al (1994). However, with MU-phosphate as substrate, other acid phosphatases beside the lysosomal one are measured..

Although enzymes in plasma do not reflect leakage from the leukocytes, we have determined leukocyte MU-TACT hydrolase activity in two patients with Gaucher disease and found normal values. Also, no increase of MU-TACT hydrolase was found in four obligate heterozygotes, indicating that one normal allele is sufficient to obtain normal plasma MU-TACT hydrolase activity in Gaucher heterozygotes (Figure 1).

We have checked whether increased enzyme activity could be due to enzyme activation using dialysis, addition of glc-cer to a control sample, and mixing experiments. Dialysis of plasma from Gaucher patients had no appreciable effect, refuting the hypothesis of a small-molecular-weight activator. For the addition of glc-cer, we assumed that the normal plasma concentration was about 0.01 mmol/L and mimicked the amount in Gaucher disease with concentrations between 0.02 and 2 mmol/L, but MU-TACT hydrolase activity was not increased in control plasma under these conditions. Upon mixing control and Gaucher plasma, the theoretical intermediate value was found. From the results of these experiments it can be concluded that it is unlikely that the increased MU-TACT hydrolase activity in the Gaucher samples is due to an enzyme activator.

Finally, the thermoinactivation profile of the Gaucher plasma enzyme and its non-binding to Con A is similar to controls, precluding gross structural differences making the enzyme more or less prone to inactivation.

Whether plasma MU-TACT hydrolase activity determinations could be used as a single test for detection or screening of Gaucher disease is uncertain, but high activities should carry a high degree of suspicion. However, we do not know whether enzyme activities are

also raised in presymptomatic or very mild cases or in newborns with the disease. Also, it has not been proved that all Gaucher patients present the same plasma enzyme increase. Pseudodeficiency (almost zero activity) in control and Gaucher samples has been observed by us. We have not included these data here since they probably belong to a separate group and cannot be considered as belonging to the same cohort of data. From our very limited experience it would therefore appear that Gaucher patients have either increased activity or are pseudodeficient. However, we think that the level of increased MU-TACT hydrolase activity in Gaucher disease runs parallel to the severity of the disease.

Preliminary results indicate that ethyleneglycolchitin inhibits MU-TACT hydrolase and that a dye-labelled (Remazol Brilliant Violet) chitin-hydrolysing enzyme is also significantly increased in Gaucher plasma to the same order of magnitude as MU-TACT hydrolase. This suggests that human plasma MU-TACT hydrolase is similar to or related to chitinase present in serum of various animals (Lundblad et al 1974). Our data thus confirm other studies (Overdijk et al 1994; Overdijk and Steijn 1994; Boot et al 1995a,b; Renkema et al 1995). Finally, to introduce some conformity, we suggest the name chitinase or chitosidase be adopted for this enzyme regardless of whether fluorogenic or coloured derivatives of di-, tri-, tetra-, and higher *N*-acetylglucosamines or other dye-labelled, modified or radiochemical chitin substrate has been used, provided that activity of lysozyme is non-contributing. This may not always be the case with all the substrates indicated above.

Editors note: The observations in this paper are similar to those reported recently by Yufeng Guo et al (1995). Elevated plasma chitotriosidase activity in various lysosomal storage disorders. *J Inher Metab Dis* **18**: 717–722. Chitosidase or chitinase is the name suggested by these authors.

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