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Origin of α-mannosidase activity in CSF

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ABSTRACT

The α-mannosidase activity in human frontal gyrus, cerebrospinal fluid and plasma has been analyzed by DEAE-cellulose chromatography to investigate the origin of the α-mannosidase activity in cerebrospinal fluid (CSF). The profile of α-mannosidase isoenzymes obtained in CSF was similar to that in the frontal gyrus but different from that in human plasma. In particular the two characteristic peaks of lysosomal α-mannosidase, A and B, which have a pH-optimum of 4.5 and are found in human tissues, were present in both the frontal gyrus and CSF. In contrast the majority of α-mannosidase activity in human plasma was due to the so called intermediate form, which has a pH-optimum of 5.5. The results suggest that the intermediate form of α-mannosidase in plasma does not cross the blood–brain barrier and that the α-mannosidase activity present in the cerebrospinal fluid is of lysosomal type and of brain origin. Thus the α-mannosidase activity in cerebrospinal fluid might mirror the brain pathological changes linked to neurodegenerative disorders such as Parkinson's disease.

1. Introduction

Lysosomal enzymes have been investigated for their potential role as biomarkers in the diagnosis of neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Dementia with Lewy bodies (DLB) and Fronto-Temporal Dementia (FTD) (Parnetti et al., 2014, 2009; Persichetti et al., 2014a; van Dijk et al., 2013). Increased levels of the lysosomal proteases, cathepsin D and cathepsin B, have been detected in cerebrospinal fluid (CSF) and postmortem brain of AD patients (Cataldo et al., 1997; Schwagerl et al., 1995). Higher activity of the lysosomal β-galactosidase was found in CSF of PD patients than in controls, whereas α-fucosidase activity was decreased in the same patients (van Dijk et al., 2013). Other studies have reported the reduction of β-glucocerebrosidase activity in CSF of patients affected by PD and DLB (Parnetti et al., 2014, 2009) and the increase of β-hexosaminidase activity in CSF of PD patients (McNeill et al., 2014; Persichetti et al., 2014a). A decrease of β-glucocerebrosidase activity and an increase of cathepsin L activity were found in postmortem brains from patients affected by PD (Chiasserini et al., 2015; Li et al., 2011). Moreover, the lysosomal α-D-mannosidase enzyme activity has been already measured by our group in the CSF of DLB, AD and FTD patients and it was found diminished compared to control subjects (Parnetti et al., 2009).

The basis of these changes in lysosomal enzyme activities in CSF in these neurodegenerative disease is not understood.

Lysosomal enzymes consist of a group of more than 50 acid hydrolases involved in the cellular catabolism of macromolecules delivered to lysosomes by endocytosis, phagocytosis and autophagy (Czupalla et al., 2006). Deficiencies in the lysosomal enzymes cause lysosomal storage diseases with an abnormal storage of macromolecular substrates resulting in the perturbation of lysosomal homeostasis (Platt et al., 2012).

Lysosomal enzymes are synthesized in the rough endoplasmic reticulum as inactive precursors and are transported to the Golgi apparatus where they are modified by the acquisition of a mannose-6-phosphate (M6P) recognition marker on one or more N-linked oligosaccharides. Enzymes bearing M6P groups are then recognized in the trans-Golgi by mannose-6-phosphate receptors (M6PR) and trans-

Abbreviations: AD, Alzheimer's disease; BBB, blood–brain barrier; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; ERT, enzyme replacement therapy; EV, extracellular vesicles; FTD, Fronto-Temporal Dementia; LP, lumbar puncture; MAN2B1, lysosomal α-mannosidase; MAN2C1, cytosolic α-mannosidase; M6P, mannose-6-phosphate; M6PR, mannose-6-phosphate receptors; ONDs, other minor neurological disorders; PD, Parkinson's disease

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located via endosomes to the lysosomes where they are activated (Kollmann et al., 2010). Lysosomal enzymes are also secreted from the cell into the extracellular fluid and plasma, and can be internalized by receptor-mediated endocytosis by other cells in the same or different tissues (Begley et al., 2008).

The differences in the lysosomal enzyme activities observed in CSF of AD, DLB, and PD patients compared to controls could result from the altered lysosomal–endosomal system in the brains of these subjects (Parnetti et al., 2014, 2009; Schwagerl et al., 1995; van Dijk et al., 2013). Nevertheless, the proposal that the pattern of lysosomal enzymes in the CSF mirrors the pathological conditions that take place in the brain is only a hypothesis, since the origin of the lysosomal enzymes present in CSF is still unknown. Lysosomal enzymes in the CSF could result directly from the secretory processes that occur in the brain tissue during the transfer of these enzymes from the Golgi apparatus to the lysosomes or they could be secreted from other tissues and reach the central nervous system by the flow of plasma constituents across the blood–brain barrier (BBB) (Begley et al., 2008; Vogler et al., 2005).

The enzyme α-D-mannosidase is very suitable for testing these options because it occurs in multiple forms with different pH-optima and substrate specificities in human tissues and body fluids (Daniel et al., 1994), reflecting its multiple functions in glycoprotein metabolism. In particular there is a distinct non-lysosomal form in plasma. Therefore, comparison of the activity and isoforms of α-D-mannosidase in CSF and plasma, should indicate the contribution of secretion from brain tissues and the flow of plasma constituents across the BBB to the origin of lysosomal enzymes in the CSF.

Lysosomal α-mannosidase activity (EC 3.2.1.24) with an acidic pH optimum is ubiquitous in human tissues where it occurs as two major forms, A and B, that can be separated by ion-exchange chromatography on DEAE-cellulose (Cheng et al., 1986). The A and B forms are the product of a single gene (MAN2B1), as demonstrated in the lysosomal storage disease α-mannosidosis (MIM 248500) in which both A and B are lacking (Beccari et al., 1999). In addition to the lysosomal α-mannosidase, a cytosolic α-mannosidase (MAN2C1) is expressed in human liver (Phillips et al., 1976), rat liver (Bischoff and Kornfeld, 1986; Grard et al., 1994; Shoup and Touster, 1976) and mouse tissues (Costanzi et al., 2006; Paciotti et al., 2014) and has an optimum pH of 6.5. Finally in human serum and plasma the predominant form is α-mannosidase with an intermediate pH-optimum of 5.5 (Hirani and Winchester, 1979; Hultberg et al., 1976). The relationship of the intermediate form to the other α-mannosidases and its physiological role are not understood and it is probably encoded by a different gene. The activity of the different α-mannosidases can be reliably determined in tissues, plasma and CSF using fluorogenic artificial substrates in specific assays (Persichetti et al., 2014b) and the multiple forms of the enzyme can be separated by ion-exchange chromatography.

The aim of this study is to determine the type and origin of α-mannosidase activity in the CSF by comparing the α-mannosidase activity and isoenzyme composition in CSF with those in human plasma and frontal gyrus, a representative brain tissue.

2. Materials and methods

2.1. Ethics statements

Written informed consent was obtained from all the subjects included in this study. The procedures for CSF and plasma samples were approved by the Ethical Committee of the Umbria Region, Italy, whereas the procedures of the Netherlands Brain Bank (Amsterdam, The Netherlands) were approved by the Institutional Review Board and Medical Ethical Board (METC) from the VU University Medical Center (VUmc), Amsterdam.

2.2. CSF and plasma samples

CSF and venous blood were collected from five subjects affected by other minor neurological disorders (ONDs) requiring lumbar puncture (LP) for diagnostic purpose (epileptic seizures, transient global amnesia, mononeuropathy or postural instability). LP were performed between 8.00 and 10.00 a.m. The CSF samples (10 ml) were collected in sterile propylene tubes and centrifuged for 10 min at 2000 × *g* before being used (Persichetti et al., 2014b). One milliliter of the sample was dialyzed overnight against 10 mM sodium phosphate buffer, pH 6.0, centrifuged for 10 min at 16,000 × *g* and then used for the anion-exchange chromatography. Venous blood was collected at the same visit to the outpatient clinic in heparinized tubes and the plasma was separated on the same day by centrifugation at 2000 × *g* for 15 min. One milliliter of plasma was dialyzed overnight against 10 mM sodium phosphate buffer, pH 6.0, centrifuged for 10 min at 16,000 × *g* and then used for the anion-exchange chromatography.

2.3. Brain tissue samples

Five brain tissue samples of the superior frontal gyrus (F2) were obtained from the Netherlands Brain Bank, from non-neurological donors, without a history of cognitive impairment or movement disorder. Neuropathological assessment was performed according to standardized neuropathological diagnostic procedures and confirmed an absence of significant neuropathology. 100 mg of postmortem human frontal gyrus were homogenized in 50 mM sodium phosphate, 150 mM sodium chloride, pH 7.0 buffer, 20% (w/v), using an ULTRA-TURRAX homogenizer. 0.1% NP-40 detergent was added and the lysate was sonicated for 30 s on ice at 20 W. The sample was kept on ice for 30 min and centrifuged for 10 min at 16,000 × *g*. The supernatant was dialyzed overnight against 10 mM sodium phosphate buffer, pH 6.0 and centrifuged for 10 min at 16,000 × *g*. The supernatant was used for the anion-exchange chromatography.

2.4. Ion-exchange chromatography on DEAE-cellulose

Anion-exchange chromatography was performed as previously described using DEAE-cellulose (Whatman DE-52; Beccari et al., 2000). The dialyzed sample was loaded onto a column of DEAE-cellulose (1 ml total volume) equilibrated with 10 mM sodium phosphate buffer, pH 6.0. Unretained proteins were eluted with the column buffer, then a linear gradient of NaCl (0–0.5 M in 50 ml of column buffer) was applied. Finally the column was eluted with 1.0 M NaCl in the same buffer. Proteins were detected in the eluate by monitoring the absorption at 280 nm using a spectrophotometer (Cary UV, model 50B10; Varian, Palo Alto, California, USA). Fractions (0.5 ml) were collected at a flow rate of 0.5 ml/min. Samples (20 µl) of each fraction were assayed for α-mannosidase activity at pH 4.5 and 5.5.

2.5. Enzyme assay

The enzymatic activity of α-mannosidase was assayed in CSF, plasma and tissue supernatants using the fluorogenic substrate, 4-methylumbelliferyl-α-D-mannopyranoside as previously described (Beccari et al., 1997; Chiasserini et al., 2015; Persichetti et al., 2014b). α-Mannosidase activities measured at pH 4.5 and 5.5 were defined as lysosomal and intermediate activities, respectively.

Each sample was incubated with 40 µl of the fluorogenic substrate (3 mM) at 37 °C for the appropriate time. The reaction was stopped by adding ice-cold 0.2 M glycine–NaOH buffer, pH 10.4, to a final volume of 0.3 ml. The fluorescence of the liberated 4-methylumbelliferone was measured on a BMG Labtech FLUOstar OPTIMA fluorometer (excitation, 360 nm; emission, 446 nm).

One unit (U) of enzyme activity was defined as the amount of enzyme that hydrolyses 1 nmol of substrate/min at 37 °C. Protein