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Beta-glucosidase 1 (GBA1) is a second bile acid β-glucosidase in addition to β-glucosidase 2 (GBA2). Study in β-glucosidase deficient mice and humans

Klaus Harzer [a], Yotam Blech-Hermoni [b], Ehud Goldin [b], Ursula Felderhoff-Mueser [c], Claudia Igney [d], Ellen Sidransky [b], Yildiz Yildiz [e,*]

[a] Neurometabolic Laboratory, Klinik für Kinder- und Jugendmedizin, University of Tübingen, Tübingen, Germany
[b] Medical Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD, 20892-4405, USA
[c] Kinderklinik, University of Essen, Essen, Germany
[d] Siemens MRT Group, Erlangen, Germany
[e] Department of Internal Medicine I, University Clinic of Bonn, Bonn, Germany

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ABSTRACT

Beta-glucosidase 1 (GBA1; lysosomal glucocerebrosidase) and β-glucosidase 2 (GBA2, non-lysosomal glucocerebrosidase) both have glucosylceramide as a main natural substrate. The enzyme-deficient conditions with glucosylceramide accumulation are Gaucher disease (*GBA−/−* in humans), modelled by the *Gba−/−* mouse, and the syndrome with male infertility in the *Gba2−/−* mouse, respectively. Before the leading role of glucosylceramide was recognised for both deficient conditions, bile acid-3-*O*-β-glucoside (BG), another natural substrate, was viewed as the main substrate of GBA2. Given that GBA2 hydrolyses both BG and glucosylceramide, it was asked whether *vice versa* GBA1 hydrolyses both glucosylceramide and BG. Here we show that GBA1 also hydrolyses BG. We compared the residual BG hydrolysing activities in the *GBA1−/−*, *Gba1−/−* conditions (where GBA2 is the almost only active β-glucosidase) and those in the *Gba2−/−* condition (GBA1 active), with wild-type activities, but we used also the GBA1 inhibitor isofagomine. GBA1 and GBA2 activities had characteristic differences between the studied fibroblast, liver and brain samples. Independently, the hydrolysis of BG by pure recombinant GBA1 was shown. The fact that both GBA1 and GBA2 are glucocerebrosidases as well as bile acid β-glucosidases raises the question, why lysosomal accumulation of glucosylceramide in GBA1 deficiency, and extra-lysosomal accumulation in GBA2 deficiency, are not associated with an accumulation of BG in either condition.

1. Introduction

Following the description of a non-lysosomal glucocerebrosidase [1] in addition to the lysosomal glucocerebrosidase long known to be deficient in Gaucher disease (glucosylceramide lipidosis), several steps were required to clarify the present definition of these two enzymes as β-glucosidase 2 (GBA2; OMIM # 609471) and β-glucosidase 1 (GBA1; OMIM # 230800, 230900, 231000), respectively [2,3]. The identity of the non-lysosomal glucocerebrosidase [1] to an independently described extra-lysosomal enzyme which hydrolysed bile acid–3-*O*-β-glucosides (BG) [4] was not clear. However, this situation changed when this enzyme also named 'microsomal β-glucosidase' or 'bile acid β-glucosidase' [4] was cloned [5]. This was followed by the generation of a 'bile acid β-glucosidase'-deleted mouse model in which extra-lysosomal accumulation of glucosylceramide was shown, and in which this accumulation was attributed to a deficiency in the deleted enzyme, with the recombinant wild-type of this enzyme, then named GBA2, being shown to hydrolyse glucosylceramide in different expression systems [3]. The phenotype of this *Gba2−/−* mouse presenting with male infertility [3] was completely different from that of the earlier known 'Gaucher mouse' (*Gba1−/−*) [6], which had both with visceromegaly and neurological symptoms (as in type 2 of human Gaucher disease, *GBA1−/−*; *GBA1* as a provisional gene symbol instead of the official *GBA1*).

Glucosylceramide is a substrate of both GBA1 and GBA2, and thus the question was raised as to whether BG, another natural metabolically important β-glucoside, is not only a substrate of GBA2 but also of GBA1, i.e., the latter enzyme is a second bile acid β-glucosidase in addition to GBA2. The question was answered by analysing the residual β-glucosidase activities towards BG in tissues and fibroblasts both *in vitro* and *in situ*, in materials from

Abbreviations: BG, bile acid-3-*O*-β-glucoside; GBA1, β-glucosidase 1, lysosomal-glucocerebrosidase; GBA2, β-glucosidase 2, non-lysosomal glucocerebrosidase; LABG, lithocholic acid-3-*O*-β-glucoside.

* Corresponding author at: University Clinic of Bonn, Department of Internal Medicine I, Sigmund-Freud-Str. 25, 53105 Bonn, Germany. Fax: +49 228 287 14698.
E-mail address: yildiz.yildiz@ukb.uni-bonn.de (Y. Yildiz).

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the *GBA1*−/−, *Gba1*−/− and *Gba2*−/− conditions and wild-types, and by degrading BG with pure recombinant GBA1. The existence of two bile acid β-glucosidases raises additional questions regarding why the two substrates, glucosylceramide and BG, differ in their contributions to the biochemical phenotypes in the studied enzyme-deficient conditions, with lysosomal glucosylceramide accumulation in the *GBA1*−/− and *Gba1*−/− conditions, extra-lysosomal glucosylceramide accumulation in the *Gba2*−/− condition [3,7], but no accumulation of BG in either condition according to present knowledge. In particular, bile acid metabolism is essentially normal in *Gba2*−/− [3].

2. Materials and methods

2.1. Patients

The phenotypes and genotypes of the Gaucher patients studied are listed in Table 1. Informed consent was provided under a clinical protocol approved by the Institute Review Board. We included patients with low residual glucocerebrosidase activity, in particular the Gaucher type 2 patient G1 [8].

2.2. Materials

The following reagents were procured from commercial sources: isofagomine D-tartrate (No. I816010, Toronto Research Chemicals, Toronto, Canada), sodium taurocholate (No. 86340 Sigma–Aldrich, Taufkirchen, Germany), and glucosyl-(stearoyl-1-[^{14}C])ceramide with a specific radioactivity of 2035 dps/nmol (No. ARC-1331, Biotrend, Köln, Germany). Lithocholic acid β-3-*O*-[U-^{14}C]glucoside with a specific radioactivity of 629 dps/nmol was a gift from F. Dallacker, Department of Organic Chemistry, Technical University of Aachen, Germany. Pure recombinant GBA1 was pooled from small traces of one of the industrial preparations for Gaucher enzyme replacement therapy that remained in the ampoules that had been therapeutically used strictly as prescribed. These ampoules, otherwise handled as waste, were a generous gift from a Gaucher patient who was responding well to therapy.

2.3. Methods

2.3.1. Crude tissue preparation

Frozen human and murine liver and brain samples (from stocks stored at −70 °C) were homogenised in 10 volumes (w/v) of water, put through three cycles of freezing and thawing, and centrifuged at 500*g* (3 min) to remove non-homogenisable material. The supernatant, i.e., uniform homogenate was used in the enzyme assays. Solubilisation of the enzymes was by the chemicals present in the assays.

2.3.2. Preparation of cultured fibroblasts

Murine or human primary (up to 10th passage) fibroblasts were cultured to early confluency, and then placed in fresh RPMI medium containing 10% calf serum. After 24-h incubation they were washed with saline and given a final wash with water (10 s). The cell layer was homogenised (briefly sonicated) in 0.4 ml water in the culture flask, and the homogenate was used for the enzyme assays. This method led to similar specific activities as those in homogenates of trypsin-harvested cells.

2.3.3. Protein determinations

Were performed using the Lowry technique; bovine serum albumin was the standard.

2.3.4. In vitro enzyme assays

2.3.4.1. Bile acid β-glucosidase. The assay comprised 30–250 μg protein in 70 μl total volume with 80 μM lithocholic acid β-[U-^{14}C]glucoside and 0.15 M sodium acetate, pH 5.5. No $MnCl_2$, EDTA or dithioerythritol [4] were added, because they had no effect on the lithocholic acid β-glucosidase activity in these preparations. After 90-min incubation at 37 °C, the reaction was stopped by the addition of 70 μl methanol. One third of the mixture was applied to a silica gel TLC plate (No. 1.05721, Merck, Darmstadt, Germany). The solvent mixture for chromatography contained chloroform, methanol and water (14:6:1 by volumes). The chromatogram was radioscanned, and the radioactivity peaks corresponding to lithocholic acid β-glucoside and glucose were identified. Enzymatically released glucose was evaluated quantitatively using commercial hard- and software (LB2821, Berthold, Wildbad, Germany). The shift in radioactivity from lithocholic acid β-glucoside to glucose was a measure of bile acid β-glucosidase activity. For assays using the GBA1 inhibitor *isofagomine* (Fig. 1B), a 10 min pre-incubation was performed at 37 °C prior to the addition of the β-glucoside substrate with and without 4.5 (fibroblasts, 2.9) μM isofagomine. For pure *recombinant GBA1* (Table 3), the above assay was modified by increasing the concentration of lithocholic acid β-[U-^{14}C]glucoside up to 175 μM, adding 0.8% (w/v) taurocholate, using 12.5 μg enzyme, and reducing the incubation period to 5 min.

2.3.4.2. Glucosylceramide β-glucosidase (glucocerebrosidase). The assay contained 30–250 μg protein in 70 μl total volume plus 6 μM glucosyl-(stearoyl-1-[^{14}C])ceramide), 0.08 M sodium acetate, pH 5.5, 0.02% triton X-100, 0.4% (w/v) taurocholate and 0.35% heat-inactivated bovine serum albumin. The assay was incubated for 120 min at 37 °C, and the reaction was stopped by the addition of 70 μl methanol. Half of this mixture was applied directly to a silica gel TLC plate (No. 1.05721, Merck). Chromatography was performed using a solvent mixture of chloroform, methanol and acetic acid (47:1:2 by volumes). The chromatogram was radioscanned to quantify the radioactive peaks of glucosylceramide, enzymatically released ceramide and free fatty acid. The shift in radioactivity from glucosylceramide to the breakdown products was a measure of glucocerebrosidase activity. For pure recombinant GBA1 (Table 3), the above assay was modified by increasing the concentration of the glucosylceramide substrate from 6 (radioactive substrate) to 175 μM by adding unlabelled, native glucosylceramide isolated from Gaucher spleen, increasing the taurocholate concentration to 0.8% (w/v), using 12.5 μg enzyme, and reducing the incubation period to 5 min.

Table 1
Different Gaucher disease patients with phenotype and genotype.

No.	Gaucher disease (GD) phenotype	Age at examination/death (years)	*GBA1* genotype	Reference
G1	GD type 2, liver failure, arthrogryposis	Neonatal death	c.1515_1516ins AGTGAGGGCAAT/R120Q	[8]
G2	GD type 2, severe neurological involvement	0.9/0.9	G202R/G202R	
G3	GD type 2	Neonatal death	D409H; H255Q/recNciI	[18]
G4	GD type 3, oculomotor abnormality	1.9/alive	L444P/L444P	
G5	GD type 2, severe neurological involvement	1.2/1.8	Not done	