The Origins of Glucosylsphingosine in Gaucher Disease

John J. Flanagan, Brian Ranes, Nastry Brignol, Rick Hamler, Sean W. Clark
Amicus Therapeutics Inc, 1 Cedar Brook Drive, Cranbury, NJ 08512

Abstract

Glucosylsphingosine (GlcSph), a lysoglycosphingolipid and a substrate of acid-β-glucosidase (glucocerebrosidase or GCase), accumulates in cells and tissues of human Gaucher disease patients and mouse models that exhibit reduced GCase activity. The accumulation of GlcSph and the more abundant GCase substrate, glucosylceramide (GlcCer), is implicated in the visceral and neuronal pathologies observed in Gaucher disease through mechanisms that remain uncertain. Further, the mechanism for the generation of GlcSph, whether by de novo synthesis, deacylation of GlcCer, or a combination of both, remains unclear. Here we report that cells lacking acid ceramidase activity accumulate significantly less GlcSph when GCase activity is inhibited. To examine the role of deacylation in the production of GlcSph, we treated wild-type and Farber disease fibroblasts, which are deficient in acid ceramidase activity, with the irreversible GCase inhibitor conduritol-B-epoxide (CBE). Inhibition of GCase elevates both GlcCer and GlcSph levels in wild-type fibroblasts. In contrast, CBE-inhibition of GCase in Farber cells resulted in an accumulation of GlcCer, but not GlcSph (less than 10% than seen in wild-type cells). In addition, we reduced acid ceramidase expression in HEK293T cells via siRNA, resulting in decreased acid ceramidase mRNA levels (>90%). Following knock-down in the presence of CBE, GlcSph accumulation was reduced by more than 50%. These results are consistent with the hypothesis that acid ceramidase is an important enzyme in the generation of GlcSph when GCase activity is compromised. Further work is in progress to elucidate whether the location of GlcCer accumulation or the activities of other ceramidases affect the accumulation of GlcSph.

Glucosylsphingosine – a pathological lipid in Gaucher disease

Isolation and characterization of glucosylsphingosine from Gaucher's spleen

J Lipid Res. 1974 Sep;15(5):484-90.

Accumulation of Glucosylceramide and Glucosylsphingosine (Psychosine) in Cerebrum and Cerebellum in Infantile and Juvenile Gaucher Disease

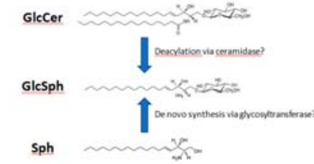
J Neurochem. 1982 Sep;39(3):709-18.

Elevated plasma glucosylsphingosine in Gaucher disease: relation to phenotype, storage cell markers, and therapeutic response

Blood. 2011 Oct 20;118(16):e118-27.

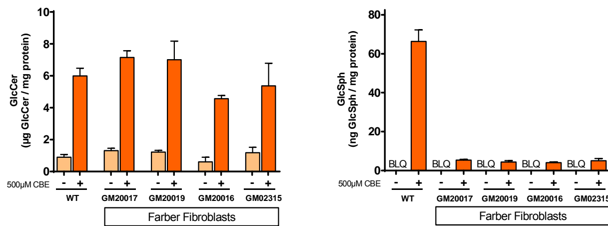
For nearly forty years, the accumulation of GlcCer and GlcSph has been demonstrated in affected tissues of Gaucher patients – both in the viscera and CNS. Despite its lower abundance when compared to GlcCer, GlcSph has been shown to be cytotoxic to cells at very low concentrations. Moreover, clinical evidence supports that elevated levels of GlcSph play a role in Gaucher disease pathology.

Potential pathways for glucosylsphingosine formation



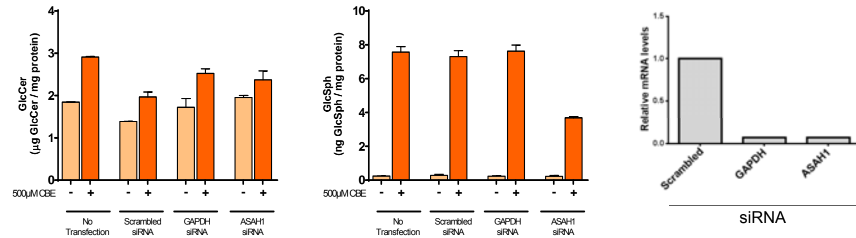
The synthesis of the lysoglycosphingolipid, GlcSph, is not well established. One potential pathway for GlcSph formation is the direct deacylation of GlcCer via a cellular ceramidase. Another possible mechanism for GlcSph synthesis is the addition of a glucose moiety onto sphingosine by a glycosyltransferase. These pathways may not be mutually exclusive as a combination of both routes may contribute to the formation of GlcSph.

Glucosylsphingosine levels are reduced in ASAH1-deficient fibroblasts



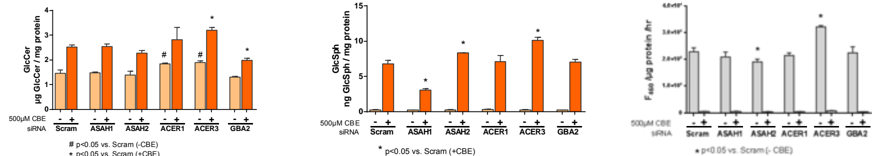
Wild-type and ASAH1-deficient (Farber disease) fibroblasts were incubated with or without 0.5 mM conduritol-B-epoxide (CBE) for five days. Cells were washed and sonicated prior to LC/MS determination of GlcCer and GlcSph levels. GlcCer and GlcSph levels were normalized to total protein content. **Results**: Cells incubated with CBE had increased levels of GlcCer, and no detectable GCase activity (not shown). In the absence of CBE, wild-type and Farber cells had levels of GlcSph that were below the limit of quantitation (BLQ). In wild-type cells incubated with CBE, GlcSph levels increased substantially. However, in all of the ASAH1-deficient cell lines incubated with CBE, a considerable reduction (>90%) of GlcSph was seen when compared to wild-type cells incubated with CBE. Columns represent mean + SD.

Knockdown of ASAH1 reduces glucosylsphingosine levels in HEK293T cells



HEK293T cells were transfected with the indicated siRNA oligos and incubated with or without 0.5 mM CBE for 72 hours. Cells were washed and sonicated prior to measurement of mRNA levels (*right panel*) and determination of lipid levels by LC/MS (*left, middle panels*). GlcCer and GlcSph levels were normalized to total protein content. Relative mRNA levels of GAPDH and ASAH1 are expressed as fold-change when compared to cells that were given a scrambled siRNA control. **Results**: Cells incubated with CBE had increased levels of GlcCer. In the absence of CBE, cells had very low levels of GlcSph, but GlcSph levels increased significantly when cultured in the presence of CBE. However, cells incubated with ASAH1 siRNA showed a significant decrease (~50%) in GlcSph formation. No decrease in GlcSph levels were seen with scrambled or GAPDH siRNAs. Both GAPDH and ASAH1 mRNA levels were reduced by >90% when respective siRNA oligos were transfected into the cells. For lipid levels, columns represent mean + SD

Role of other ceramidases in glucosylsphingosine formation



HEK293T cells were transfected with siRNA oligos directed towards various ceramidases (ASAH1, ASAH2, ACER1, ACER2, ACER3). Twenty-four hours after transfection, the cells were incubated with or without 0.5 mM CBE, and cultured for an additional 48 hours. Cells were washed and sonicated prior to GCase activity measurements (*right panel*) and LC/MS determination of lipid levels (*left, middle panels*). GlcCer and GlcSph levels were normalized to total protein content. **Results**: Cells incubated with CBE had increased levels of GlcCer. In the absence of CBE, cells had very low levels of GlcSph, but GlcSph levels increased significantly with CBE. However, cells incubated with siRNA against ASAH1 showed a significant decrease in GlcSph formation. Also, significantly greater levels of GlcSph were generated in cells incubated with siRNA directed towards ASAH2 and ACER3, observations that require further examination. Columns represent mean + SD. Statistical analyses were carried out using one-way ANOVA (p<0.05). Knockdown of ASAH1 and ACER3 mRNA levels was 80% and 30%, respectively. Various qPCR primer pairs for ASAH2 and ACER1 were tested for validation, but failed to meet amplification efficiency parameters or the dissociation curves indicated multiple products. Ceramidase activity assays are currently being developed to test for residual activity after siRNA transfection.

Summary and Conclusions

- Accumulation of the sphingolipid GlcCer is a hallmark of Gaucher pathology
- The contribution of a related, toxic sphingolipid GlcSph (lyso-GlcCer) to the Gaucher disease process has gained recent attention
- Our experiments suggest that glucosylsphingosine is derived primarily from GlcCer by the activity of the lysosomal enzyme acid ceramidase – although we cannot exclude the possibility that some low level *de novo* synthesis occurs, or that other ceramidases can act on GlcCer
- Our studies suggest that decreased GCase activity increases glucosylsphingosine by a dual mechanism: (1) the elevation of GlcCer leads to greater production of GlcSph via acid ceramidase, and (2) the loss of GCase activity reduces the rate of GlcSph degradation

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