Glucosylsphingosine (GlcSph), a lyso-glycosphingolipid 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 unclear. Further, the mechanism for the generation of GlcSph, whether by de novo synthesis, deaclylation 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 deaclylation in the production of GlcSph, we treated wild-type and Farber disease fibroblasts, which are deficient in acid ceramidase, 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.

Wild-type and ASAH1-deficient (Farber disease) fibroblasts were incubated with or without 0.5 mM CBE for five days. Cells were washed and sonicated prior to 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 (BLO). 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.

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.