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Selective action of the iminosugar isofagomine, a pharmacological chaperone for mutant forms of acid-β-glucosidase

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ABSTRACT

Gaucher disease is a lysosomal glycolipid storage disorder characterized by defects in acid-β-glucosidase (GlcCerase), the enzyme responsible for the catabolism of glucosylceramide. We recently demonstrated that isofagomine (IFG), an iminosugar that binds to the active site of GlcCerase, enhances the folding, transport and activity of the N370S mutant form of GlcCerase. In this study we compared the effects of IFG on a number of other glucosidases and glucosyltransferases. We report that IFG has little or no inhibitory activity towards intestinal disaccharidase enzymes, ER α-glucosidase II or glucosylceramide synthase at concentrations previously shown to enhance N370S GlcCerase folding and trafficking in Gaucher fibroblasts. Furthermore, treatment of wild type fibroblasts with high doses of IFG did not alter the processing of newly synthesized N-linked oligosaccharides. These findings support further evaluation of IFG as a potential therapeutic agent in the treatment of some forms of Gaucher disease.

1. Introduction

Gaucher disease is caused by mutations in the gene encoding acid-β-glucosidase (GlcCerase), the enzyme that converts the glycolipid glucosylceramide to glucose and ceramide in the lysosome [1,2]. Deficient GlcCerase activity results in the storage of glucosylceramide within macrophages leading to hepatosplenomegaly, anemia, bone lesions and, in some instances, central nervous system involvement [3]. The more severe types of the disease (classified as type 2 or 3) are characterized by central nervous system impairment and early onset. Patients with type 1 Gaucher disease lack neurological symptoms and most commonly present in adulthood.

Intravenous infusions of recombinant human GlcCerase (enzyme replacement therapy, ERT) and inhibition of glucosylceramide biosynthesis (substrate reduction therapy, SRT)

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Abbreviations: GlcCerase, acid-β-glucosidase; IFG, isofagomine; NB-DNJ, N-butyldeoxynojirimycin

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are currently the only available treatment options for Gaucher disease [3]. While effective in managing the visceral symptoms of the disease, enzyme replacement therapy is expensive and cannot address the neurological defects because the replacement enzyme is unable to cross the blood–brain barrier [4]. Treatment with the iminosugar *N*-butyl-deoxynojirimycin (NB-DNJ, Miglustat), an inhibitor of glucosylceramide synthase, has been shown to improve liver and spleen volume and hematological parameters in type 1 Gaucher patients [5]. However, the administration of NB-DNJ is observed to cause several significant side effects including peripheral neuropathy and gastrointestinal complications [6–8]. The gastrointestinal symptoms such as diarrhea most likely arise from the inhibition of the disaccharidase enzymes sucrase and isomaltase [9]. The basis for the development of peripheral neuropathy is not well understood.

The observation that many GlcCerase variants, including the common N370S mutant, exhibit impaired biosynthesis but retain substantial catalytic activity has prompted the use of small molecule inhibitors to facilitate proper folding and trafficking of these mutant enzymes out of the ER with the goal of increasing their activity in lysosomes to a level sufficient to avoid or alleviate disease. Several researchers have demonstrated the ability of iminosugars to increase the activity of mutant forms of GlcCerase [10–15]. We have recently reported that the iminosugar, isofagomine (IFG), enhances N370S GlcCerase activity by approximately three-fold by facilitating its folding and transport out of the ER to lysosomes. IFG also shifted the optimal catalytic pH to the lysosomal pH of 5.2 [10]. In addition, the presence of IFG altered the oligosaccharide processing of GlcCerase in the lysosome, possibly increasing its stability in this compartment.

Since IFG could potentially inhibit other glucosidases and glucosyltransferases, as observed with NB-DNJ, we tested the effect of IFG on a panel of enzymes involved in oligosaccharide processing and disaccharide hydrolysis. Our results show that, unlike NB-DNJ, IFG does not significantly inhibit the *in vitro* activity of the disaccharidases, sucrase and isomaltase, ER alpha-glucosidase II or glucosylceramide synthase. Furthermore, treatment of wild-type human fibroblasts with high concentrations of IFG did not result in any appreciable changes in asparagine-linked oligosaccharide processing.

2. Materials and methods

2.1. Reagents

Isofagomine (IFG) was obtained from Amicus Therapeutics. NB-DNJ, sucrose, isomaltose, lactose and glucose assay reagent were purchased from Sigma. Ceramide was purchased from Avanti Polar Lipids. [^{3}H] UDP-glucose was from American Radiolabeled Chemicals and [2-^{3}H] mannose was from Perkin-Elmer Life Science Products. Cell culture reagents were purchased from Gibco-BRL (Invitrogen).

2.2. Cell lines and culture

Wild-type primary skin fibroblasts (CRL-1509) were obtained from ATCC, various N370S patient skin fibroblasts (DMN 89.15, DMN 89.45, GC-7 and GC-8) were obtained from Amicus Therapeutics and Caco-2 intestinal epithelial cells were a gift of Dr. William Stenson at Washington University School of Medicine. Cells were maintained at 37 °C in Dulbecco's minimum essential medium supplemented with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin. Cell proliferation was measured using an MTT tetrazolium colorimetric cell proliferation assay kit (Promega).

2.3. Glucosylceramide synthase assay

The effects of IFG and NB-DNJ on glucosylceramide synthase activity were assayed using a modified protocol of Matsuo and colleagues [16]. Ceramides dissolved in chloroform were adsorbed onto silica gel 60 particles (25 μg ceramide per 1 mg silica gel) and dried under nitrogen. The membrane fraction of Caco-2 cells was used as an enzyme source. Each reaction contained 5 mg of ceramide-silica gel suspended in 70 μl reaction buffer (50 mM MOPS pH 6.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 3 mM NAD, 5 mM DTT, 1% CHAPS, 50 μM UDP-glucose and 0.74 MBq of [^{3}H] UDP-glucose). Reactions were initiated by the addition of 30 μl of membranes in reaction buffer and incubated for 60 min at 37 °C. Frequent agitation of the reaction tubes was done to prevent settling of the membranes. Reactions were terminated by the addition of 5 mM EDTA pH 8.0 and unincorporated radioactivity was removed by washing the silica beads several times with PBS prior to scintillation counting.

2.4. Measurement of sucrase, isomaltase and lactase activity

Confluent Caco-2 cells were scraped in cold PBS and pelleted by centrifugation. Cell pellets were gently sonicated in PBS, cleared by low speed centrifugation, and sedimented at 100,000 × *g* for 30 min in a refrigerated Beckman tabletop ultracentrifuge to obtain a membrane/microsome pellet. The membrane preparation was solubilized in ice-cold PBS containing 0.3% NP-40 and 0.4% TX-100. Substrates, sucrose and isomaltose, were prepared in reaction buffer (60 mM sodium citrate buffer, pH 6.0) to a final concentration of 100 mM. Reaction mixtures of 200 μl were prepared containing 10 μl of sedimented membranes and 100 μl substrate (final [substrate] = 50 mM). Reactions were performed at 37 °C for 3–16 h. The samples were briefly boiled to terminate activity, cooled to room temperature and 1 ml of glucose assay reagent (Sigma) was added to the reaction tubes followed by incubation at room temperature for 20 min. Insoluble material was pelleted by centrifugation and the glucose levels were measured by absorbance at 340 nm.

2.5. α-Glucosidase II activity assay

The activity of α-glucosidase II in wild-type fibroblast lysates was measured using *p*-nitrophenyl-α-glucoside as a substrate (3 mM). Various concentrations of IFG and NB-DNJ were added prior to reaction initiation. The reactions were performed for 30 min in reaction buffer (0.1 M sodium phosphate, 1% Triton X-100), 0.5 mM DTT) at pH 6.9 in order to avoid measurement of lysosomal α-glucosidase activity.

2.6. Lectin affinity chromatography

The preparation and analysis of [2-^{3}H]-mannose labeled glycopeptides from human fibroblasts was done as previously described [17].

2.7. Lysosomal glycosidase activity assays

The lysosomal enzymes acid-β-glucosidase (GlcCerase), β-hexosaminidase, β-glucuronidase, β-galactosidase and β-mannosidase were assayed in cell lysates by using 4-methylumbelliferyl-β-D-glucoside, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide, 4-methylumbelliferyl-β-D-glucuronide, 4-methylumbelliferyl-β-D-galactopyranoside and 4-methylumbelliferyl-β-D-mannoside, respectively, in buffer containing 0.1 M sodium citrate, 0.2 M sodium phosphate, 0.1% Triton X-100, 0.25% sodium taurocholate, pH 5.2. The final substrate concentration was 3 mM for all reactions. Lysosomal α-glucosidase activity was determined by using 3 mM 4-methylumbelliferyl-α-D-glucopyranoside in 50 mM citrate buffer, 0.25% Triton X-100 (pH 4.5). All reactions were quenched by the addition of an equal volume of 0.4 M glycine buffer (pH 10.8).

3. Results

3.1. IFG enhances the activity of N370S GlcCerase

Our previous studies demonstrated the ability of IFG to enhance the activity of N370S GlcCerase by several mechanisms [10]. Since fibroblasts derived from different N370S/N370S Gaucher patients can exhibit variable GlcCerase residual activity [2,10,15], we tested the ability of IFG to enhance enzyme activity from multiple N370S patient fibroblasts. The results are summarized in Table 1. As shown, IFG enhanced GlcCerase activity (2.3–3.0-fold) in all N370S homozygous patient fibroblasts tested. The observation that IFG treatment enhances GlcCerase activity in multiple patients reinforces the potential suitability of this compound as a novel therapeutic agent for some forms of Gaucher disease.

Table 1 – Effect of IFG on GlcCerase activity in different N370S homozygous patient fibroblasts

Cell type	Fold enhancement over untreated fibroblasts
DMN 89.15	3.0 ± 1.0
DMN 89.45	2.5 ± 0.6
GC-7	2.3 ± 0.4
GC-8	3.0 ± 1.2

Patient fibroblasts homozygous for the N370S mutation were treated with 30 μM of IFG for 5 days and GlcCerase activity was measured in fibroblast lysates. The results shown are the average of two to three independent experiments. Standard errors of the mean are noted.

3.2. IFG treatment of human cells does not significantly alter their growth

The potential effects of IFG and NB-DNJ on cell growth following 4 days treatment were evaluated in wild type fibroblasts. Treatment of fibroblasts with 20 mM IFG-tartrate lead to a 20% reduction in cell proliferation compared to untreated cells. The influence of tartrate alone was measured to ascertain any potential contribution of this counterion towards decreased cell proliferation. Treatment of cells with 20 mM tartrate resulted in a 10% reduction in proliferation, suggesting that some of the effects observed following IFG-tartrate treatment might be due to the tartrate ion. Nonetheless, an IC_{50} value of >20 mM in the cell proliferation assay

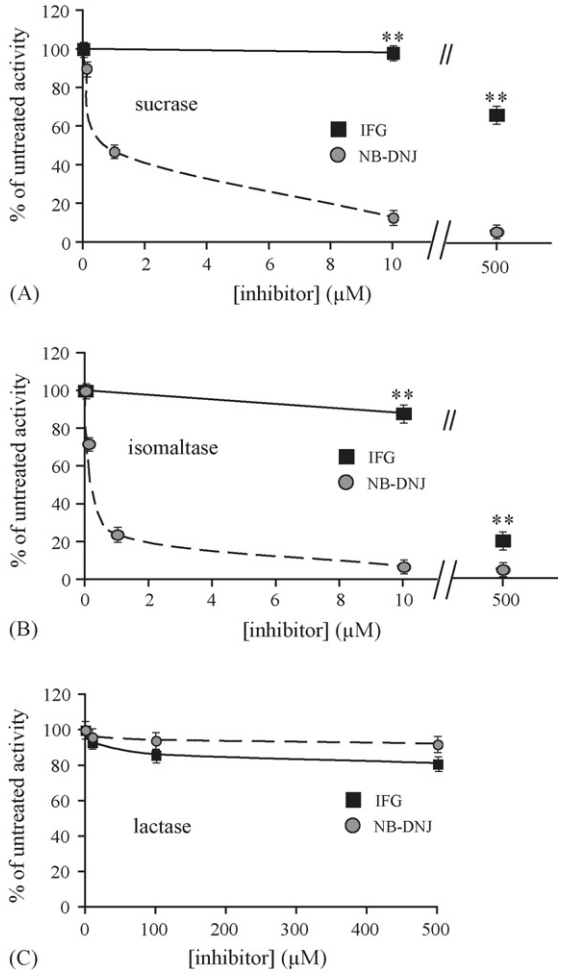

Fig. 1 – The effect of IFG and NB-DNJ on the activity of intestinal disaccharidase enzymes. The activity of sucrase (panel A), isomaltase (panel B) and lactase (panel C) was measured in Caco-2 membrane preparations using the appropriate substrates as described in the Methods section. Various concentrations of IFG or NB-DNJ were added to the reactions prior to initiation. The values are plotted as % of activity in reactions with no inhibitors present and are the average of two independent measurements. Error bars represent standard error of the mean. The double asterisks (**) denote a p value < 0.01 as determined by a one-way ANOVA test.

is six orders of magnitude higher concentration of IFG compared to the amount needed to inhibit GlcCerase in *in vitro* activity assays (5–30 nM) and three orders of magnitude more than required to enhance GlcCerase activity in N370S mutant fibroblasts (30 μM) [10]. No effect on cell growth was observed following 5 days treatment with 5 mM IFG using normal human lymphoblasts. We also observed negligible inhibition of cell growth following treatment of wild-type fibroblasts with millimolar concentrations of NB-DNJ-HCl (data not shown).

3.3. *Effect of IFG on intestinal disaccharidase enzymes*

Previous studies have demonstrated that NB-DNJ is a potent inhibitor of intestinal sucrase/isomaltase (K_i values of 0.26 and 0.37 μM, respectively), but not lactase (K_i value of 4 mM) [9]. Since inhibition of intestinal disaccharidase enzymes by IFG could potentially limit its therapeutic efficacy, we tested whether this iminosugar would inhibit these enzymes *in vitro*. Inhibition curves for IFG and NB-DNJ are shown in Fig. 1. IFG was a much weaker inhibitor of sucrase and isomaltase (panels A and B), with calculated IC_{50} values of >500 μM for sucrase and 100 μM for isomaltase. The calculated IC_{50} values for NB-DNJ in these experiments were 0.43 μM for sucrase and 0.34 μM for isomaltase, consistent with the reported K_i values. In contrast, both IFG and NB-DNJ were poor inhibitors of lactase activity (panel C).

3.4. *Effect of IFG on glucosylceramide synthase*

The ability of NB-DNJ to inhibit glucosylceramide synthase is well established and provides the basis for a substrate reduction approach in the treatment of Gaucher disease [18]. We tested whether IFG could also inhibit glucosylceramide synthase *in vitro* and act as a substrate reducer in addition to a pharmacological chaperone. Caco-2 cell lysates were incubated with various concentrations of IFG and

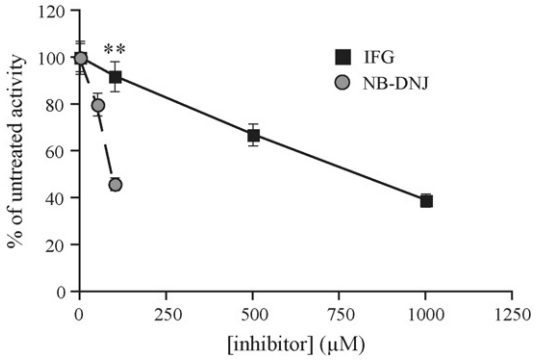

Fig. 2 – The effect of IFG and NB-DNJ on the activity of glucosylceramide synthase. The activity of glucosylceramide synthase in Caco-2 cell membranes was assayed as described in the Methods section. The values are plotted as % of activity in reactions with no inhibitors present and are the average of two independent measurements. Error bars represent standard error of the mean. The double asterisks (**) denote a p value < 0.01 as determined by a one-way ANOVA test.

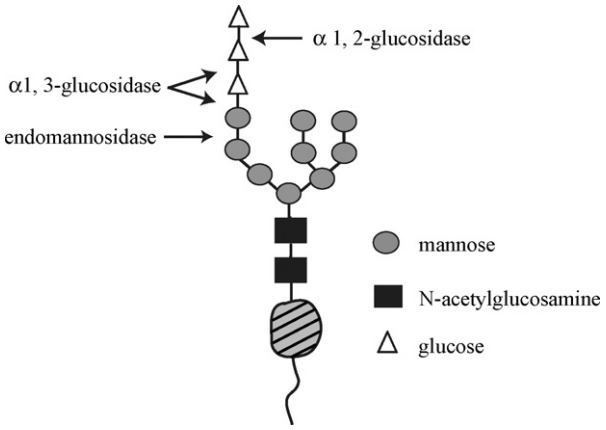

Fig. 3 – The action of various glycosidases on newly-synthesized N-linked oligosaccharides. The structure of the 14-sugar oligosaccharide following transfer from its dolichol carrier to selected asparagines residues and the action of various cellular glycosidases is shown.

glucosylceramide synthase activity was measured using a solid phase substrate assay [16]. As shown in Fig. 2, the presence of 100 μM IFG did not lead to a significant reduction in glucosylceramide synthase activity (<10% inhibition). Moderate inhibition of glucosylceramide synthesis was observed at higher IFG concentrations (1 mM). Comparison of the IC_{50} values for IFG and NB-DNJ reveals that NB-DNJ is at least a 20-fold better inhibitor of glucosylceramide synthase compared to IFG.

3.5. *Effect of IFG on α-glucosidase II activity and processing of N-linked oligosaccharides*

Asparagine-linked glycosylation is initiated in the endoplasmic reticulum by the co-translational transfer of a preformed oligosaccharide ($Glc_3Man_9GlcNAc_2$) from the lipid carrier dolichol to selected asparagines of nascent polypeptides [19]. Three glucose residues (Glcα1,2 → Glcα1,3 → Glcα1,3) are linked to a terminal mannose residue at one of the antennae of the N-glycan structure. Following transfer of the oligosaccharide to the nascent polypeptide, the terminal glucose is removed by α-1,2 glucosidase I whereas the two inner glucoses are removed by α-1,3 glucosidase II (Fig. 3). Failure to trim these glucose residues from high mannose-type N-linked oligosaccharides results in a partial block in their subsequent processing to complex-type glycans [20]. Since NB-DNJ is known to inhibit glucose trimming from the oligosaccharide precursor [21], we tested whether IFG would also inhibit this process.

Wild type fibroblast lysates were assayed in the presence or absence of IFG or NB-DNJ using the color-forming substrate, *p*-nitrophenyl-β-D-glucoside, to assess whether IFG inhibits α-glucosidase II activity *in vitro*. α-1,2 Glucosidase II accounts for the majority of the activity with this substrate when mammalian cell extracts are assayed at neutral pH [22]. Fig. 4 shows that IFG is a weak inhibitor of α-1,2 glucosidase II with an IC_{50} of greater than 200 μM. NB-DNJ is 20-fold more potent as an inhibitor of this glucosidase (IC_{50} of ~10 μM).

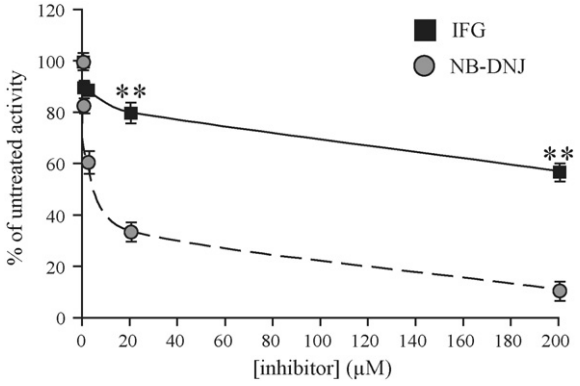

Fig. 4 – Inhibition of α-glucosidase II activity by IFG and NB-DNJ. The activity of α-glucosidase II in wild-type fibroblast lysates in the presence or absence of various concentrations of IFG or NB-DNJ was measured as described in Section 2. The values are plotted as % of activity in reactions with no inhibitors present and are the average of two independent measurements. Error bars represent standard error of the mean. The double asterisks (**) denote a p value < 0.01 as determined by a one-way ANOVA test.

To determine whether IFG alters oligosaccharide processing in cells, wild type fibroblasts were treated with various concentrations of the compound (up to 500 μM) for 24 h and then labeled with [2-^{3}H]-mannose for 1 h to incorporate radioactivity into newly synthesized N-linked oligosaccharides. Following a 4 h chase period to allow oligosaccharide processing to occur, the cells were harvested and glycopeptides prepared by pronase digestion. These glycopeptides were fractionated into three pools by Con A-sepharose chromatography and the relative amount of radioactivity in each pool is shown in Table 2. Con A pools I and II contain primarily processed complex-type N-linked glycans while Con A pool III is enriched in high mannose-type N-linked glycans. Even at concentrations as high as 500 μM, no measurable effect on the distribution of N-glycans was observed indicating that IFG treatment does not lead to any alterations in the processing of N-linked oligosaccharides. By comparison, treatment with NB-DNJ, even as low as 10 μM, resulted in an increase in radioactivity in Con A pool III and a corresponding decrease in pools I and II (Table 2). This increase in the proportion of radioactivity in Con A pool III, due to the inhibition of glucose trimming, is likely underestimated since fibroblasts contain an endomannosidase that can circumvent blocks in glucose trimming and allow processing into complex-type glycans (Fig. 3; [20,23]).

Table 2 – The effect of IFG and NB-DNJ on the processing of N-linked oligosaccharides from wild-type fibroblasts

	Total radioactivity in each pool (%)		
	Con A I	Con A II	Con A III
Untreated	26.1	16.6	57.3
NB-DNJ			
10 μM	23.8	14.6	61.6
100 μM	21.4	15.1	63.5
500 μM	16.5	14.7	68.8
IFG			
100 μM	25.7	16.3	58.0
200 μM	25.6	17.9	56.5
500 μM	24.4	17.8	57.9

Wild-type fibroblasts were treated for 24 h with various concentrations of IFG or NB-DNJ prior to pulse/chase labeling with [2-^{3}H] mannose and glycopeptide preparation. Labeled glycopeptides were fractionated by Con A-sepharose chromatography into three pools representing the different types of N-linked oligosaccharides. A representative analysis is shown. Less than 5% deviation in the pool distribution was observed between independent experiments.

3.6. *Effect of IFG on the activity of other lysosomal glycosidases*

To assess whether IFG inhibits the activity of other lysosomal glycosidases, *in vitro* assays were performed using wild type fibroblast detergent lysates. IFG was a very weak inhibitor of lysosomal acid α-glucosidase, the enzyme responsible for lysosomal glycogen breakdown (IC_{50} value of >200 μM). By contrast, NB-DNJ has been shown to strongly inhibit acid α-glucosidase with an IC_{50} value at least 100-times lower compared to IFG (2 μM). Less than 10% inhibition of the lysosomal hydrolases β-galactosidase, β-N-acetylhexosaminidase, β-mannosidase and β-glucuronidase was observed at 500 μM IFG. The negligible inhibition of these enzymes by IFG is consistent with a previous report [13].

4. Discussion

Enzyme replacement therapy and substrate reduction therapy represent the current available options for the clinical management of Gaucher disease. While both treatments are effective in improving the hematologic and visceral symptoms in patients with mild, non-neuronopathic forms of the disease, their efficacy may be limited by their inability to cross the blood–brain barrier (ERT) or adverse side effects (SRT). Recent studies have demonstrated the ability of sugar analog inhibitors to increase the activity of mutant lysosomal enzymes in patient fibroblasts by acting as pharmacological chaperones [10–15]. As active site inhibitors, these compounds bind to the mutant enzymes and stabilize them in the ER, thereby preventing their ER-associated degradation and facilitating their folding and transport out of this compartment to lysosomes. We have recently reported that the iminosugar IFG increases GlcCerase activity via multiple mechanisms in N370S Gaucher fibroblasts [10]. Besides protecting newly synthesized N370S GlcCerase from ER-associated degradation, IFG treatment also improves the catalytic properties of mutant enzyme. In order to be successful as a therapeutic agent, IFG should selectively increase GlcCerase activity without inhibiting other cellular enzymes that are responsible for the hydrolysis of glucose-containing disaccharides (lactase and sucrase–isomaltase) and oligosaccharides (N-linked oligosaccharides and glyco-

Table 3 – Summary of the inhibitory effects of IFG and NB-DNJ on various glucose-binding enzymes

Enzyme	IC_{50} values		Reference
	IFG	NB-DNJ	
Acid β-glucosidase	30 nM	>500 μM	[10,33]
Lactase	>1 mM	>1 mM[a]	Present study, [9]
Sucrase/isomaltase	>100 μM	<1 μM[a]	Present study, [9]
Glucosylceramide synthase	~1 mM	10 μM	Present study, [33]
α-Glucosidase II	200 μM	5–10 μM	Present study, [33]
Glycogen phosphorylase	0.7 μM	>1 mM	[28,32]
α1,6-Glucosidase	n.d.	4.5 μM	[32]
Acid α-glucosidase	>200 μM	2 μM	Present study, [32]

n.d.: not determined.
[a] K_i values.

gen). The data presented in this study show that IFG has significantly less inhibitory activity toward a number of glucose-metabolizing enzymes compared to NB-DNJ, as summarized in Table 3.

The sucrase–isomaltase enzyme complex is an enterocyte-specific, brush-border membrane disaccharidase that is required for the hydrolysis of dietary sucrose and some starches. Since only monosaccharides are absorbed, sucrase/isomaltase deficiency via genetic mutations or pharmacological inhibition with sugar analogs (e.g. NB-DNJ) leads to increased luminal disaccharides and subsequent osmotic diarrhea [9,24,25]. In contrast to NB-DNJ, we show that IFG is only a weak inhibitor of these enzymes and is therefore unlikely to cause gastrointestinal problems at clinically relevant doses (Fig. 1). Sierks and colleagues showed that IFG inhibits yeast isomaltase, with a K_i of 7.2 μM, a value somewhat lower than the 100 μM IC_{50} value we determined for the human enzyme [26]. The failure of IFG and NB-DNJ to inhibit lactase is consistent with previous work showing that galactose analogs such as NB-DGJ but not glucose analogs are good inhibitors of this enzyme [9].

NB-DNJ has been used to inhibit glucosylceramide synthase and thus reduce substrate accumulation in Gaucher patients. We tested IFG on this enzyme to determine whether this iminosugar would also inhibit glucosylceramide synthase. In contrast to the effect of NB-DNJ on glucosylceramide synthase, we found that IFG is a weak inhibitor inhibition of this enzyme in the *in vitro* assays, reinforcing the selectivity of the drug. At the present time, it is unclear whether long-term inhibition of glucosylceramide synthase will have any adverse consequences in human patients. Since IFG does not inhibit this enzyme, we predict that chronic administration of IFG would not affect glycolipid biosynthesis.

Several enzymes that bind to glucose are also present in the endoplasmic reticulum including two glucosidases, ER α-glucosidase I and ER α-glucosidase II, involved in the initial processing of N-linked oligosaccharides. Alterations in glucose trimming on newly synthesized glycoproteins can influence the ability of these proteins to fold properly and to be monitored by the ER quality control system [27]. Chronic inhibition of protein folding and quality control in the ER could subsequently affect the function of several different proteins and may account for some of the side effects observed with prolonged treatment with sugar analogs. Our results demonstrate that IFG does not significantly inhibit α-glucosidase II activity *in vitro* or in cultured cells and therefore minimizes these concerns.

IFG has been reported to inhibit glycogen phosphorylase in a dose-dependent manner with IC_{50} values ranging from 0.7 μM in liver and muscle homogenates [28] to 1–3.3 μM in brain and astrocyte homogenates [29]. Waagepetersen and coworkers found that high concentrations of IFG (400 μM) prevented norepinephrine-induced glycogen degradation in intact astrocytes while a lesser concentration of the compound (5 μM) had only a small effect. High concentrations of IFG (400 μM) also caused an accumulation of glycogen in neocortical astrocytes and cultured mouse optic nerves [30,31]. In hepatocytes, IFG inhibited both basal and glucagon-induced glucose production at much lower concentrations (IC_{50} values of 3 and 2 μM, respectively) [28]. The reason for this difference in sensitivity is not clear. Our studies with human fibroblasts expressing the N370S mutant form of GlcCerase showed that incubation with 30 μM IFG resulted in optimal enhancement of enzyme activity [10]. Since this is higher than required to inhibit glycogen phosphorylase in hepatocytes, it will be important to determine the effect of this concentration of IFG on glycogen metabolism in rodents.

NB-DNJ and NN-DNJ have also been reported to inhibit glycogen breakdown *in vitro* and in mice, but in these instances the mechanism does not involve inhibition of glycogen phosphorylase [32]. Rather, low micromolar concentrations of these compounds inhibit acid α-glucosidase, the enzyme that mediates the breakdown of glycogen in lysosomes, and the glycogen debranching enzyme, α-1,6-glucosidase. Treatment of mice with these agents resulted in the accumulation of glycogen in liver and muscle. The effect of NB-DNJ was transient, but glycogen accumulation persisted for months when NN-DNJ was administered. Further, the NN-DNJ effect was seen at a 10-fold lower dose (250 mg/(kg day)) than required for the NB-DNJ effect. The difference in the effects of these two iminosugars was attributed to the increased hydrophobicity of NN-DNJ that leads to increased in vivo uptake and tissue retention of the compound [32]. It should be noted that NN-DNJ, like IFG, has been shown to increase the activity of GlcCerase in some Gaucher patient fibroblasts [15]. These findings reinforce the need to examine the effect of IFG on glycogen metabolism in rodents in future studies.

In summary, IFG appears to act in a selective fashion to increase the activity of GlcCerase. It does not significantly inhibit the activity of several other glucose-binding enzymes,

several of which are inhibited by NB-DNJ (Table 3). This selective action of IFG warrants its further evaluation as a potential therapeutic agent in the treatment of some forms of Gaucher disease.

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