

PAPER ID: PMC3924557

TITLE:

Imino sugar glucosidase inhibitors as broadly active anti-filovirus agents

ABSTRACT:

Ebola virus and Marburg virus are members of the family of Filoviridae and are etiological agents of a deadly hemorrhagic fever disease. The clinical symptoms of Ebola and Marburg hemorrhagic fevers are difficult to distinguish and there are currently no specific antiviral therapies against either of the viruses. Therefore, a drug that is safe and effective against both would be an enormous breakthrough. We and others have shown that the folding of the glycoproteins of many enveloped viruses, including the filoviruses, is far more dependent upon the calnexin pathway of protein folding than are most host glycoproteins. Drugs that inhibit this pathway would be expected to be selectively antiviral. Indeed, as we summarize in this review, imino sugars that are competitive inhibitors of the host endoplasmic reticular α -glucosidases I and II, which are enzymes that process N-glycan on nascent glycoproteins and thereby inhibit calnexin binding to the nascent glycoproteins, have been shown to have antiviral activity against a number of enveloped viruses including filoviruses. In this review, we describe the state of development of imino sugars for use against the filoviruses, and provide an explanation for the basis of their antiviral activity as well as limitations.

INTRODUCTION:

Filoviral hemorrhagic fevers are rare but highly lethal diseases associated with outbreaks in developing countries.^{1,2} The causative agents, Ebola virus and Marburg virus, are considered to be high level biothreat agents by the United States Centers for Disease Control and Prevention and the National Institutes of Health.³ There are no effective vaccines to be used as prophylactics and no effective antiviral interventions to manage the diseases. Because the clinical symptoms of Marburg and Ebola hemorrhagic fevers are difficult to distinguish, a drug that is effective against either would be a first, and one that is effective against both the viruses would be an enormous breakthrough.

Filoviruses are non-segmented negative-strand RNA viruses that produce filamentous enveloped virions.⁴ There are currently four known clinically relevant species of Ebola virus⁴ and a single species of Marburg virus.⁵ Initial virus replication occurs in mononuclear cells and viremia is usually apparent within 2 days after infection.^{6,7,8,9} Death can occur in up to 90% of the infections after 7–10 days of symptoms, usually due to hemorrhagic fevers.⁵

Although the cell receptors for either Ebola virus or Marburg virus have not been fully characterized, the broad cell tropism of the viruses suggests a wide distribution of their receptors. In any regard, the trimeric envelope glycoprotein (GP) spikes of the filoviruses are believed to mediate their entry into host cells via endocytic pathways. Within endo/lysosomal compartments, host endosomal cysteine proteases (cathepsins) cleave the filoviral GP1 protein to generate an entry intermediate comprising an N-terminal GP1 fragment and GP2. Recent work indicates that a cleaved form of Ebola virus GP subsequently interacts with Niemann-Pick C1, an endo/lysosomal cholesterol transporter, to trigger membrane fusion.⁹

As illustrated in Table 1, the pipeline of candidate anti-filovirus therapeutics is limited. However, promising work on inhibition of the virus entry into their host cells with small molecules,²¹ recombinant C type lectins^{13,22} or immuno-adhesion technology,²³ disruption of viral RNA capping²⁴ and directly targeting viral RNA with antisense oligos or siRNAs^{10,11,25,26} have recently been reported.

As Table 1 also indicates, the oligonucleotide-based therapeutics appear to be the farthest along in development, reaching Phase I clinical trials, but challenges remain and the extent to which these approaches will progress is uncertain. Some of these challenges are inherent to the oligonucleotide approach and the lack of efficient cell-specific delivery technologies.²⁷ Although a morpholino modification or a lipid nanoparticle formulation has been used for these approaches in their anti-filovirus applications, specific targeted delivery of oligonucleotide into disease-relevant tissues and cells remains a major issue to address.²⁸

Only a few small molecule antivirals have been shown to have in vivo efficacy against filoviruses. FGI-103, 104, 106 and NSC62914 were discovered using cell-based high-throughput screening, and their antiviral mechanisms are largely unknown. The other two families of small molecules

target host enzymes, the S-adenosyl-L-homocysteine hydrolase and endoplasmic reticulum (ER) α -glucosidases I and II. The ER α -glucosidases are especially interesting because the broad-spectrum antiviral activities of their inhibitors have been extensively demonstrated with a variety of enveloped viruses from many families (Table 1). In addition, more than 1000 US FDA-approved drugs were tested for new antiviral indications.¹⁸ This effort resulted in the discovery that chloroquine could disrupt entry and replication of two or more viruses in vitro and protect mice against Ebola virus challenge in vivo.

As for many other enveloped viruses,^{29,30,31,32,33,34,35,36} the morphogenesis of filoviruses appears to depend upon ER α -glucosidases mediated processing of envelope glycoproteins. Therefore, the amplification and propagation of filoviruses are sensitive to imino sugar derivatives that inhibit the ER α -glucosidases I and II.³⁷ Due to their unusual antiviral mechanism, imino sugars should be complementary to the other antiviral approaches and have the advantage of being broadly active.

TARGETING HOST FUNCTIONS TO SUPPRESS VIRAL INFECTION:

Medical management of virus infection can, in theory and has in practice, involve targeting either virus or host functions. Targeting virus specified functions such as viral enzymes (DNA or RNA polymerases, proteases, helicases, neuraminidases) has been enormously effective and offered opportunities for great selectivity.^{38,39,40} On the other hand, drugs targeting host functions, upon which the viruses rely, have been approached with more skepticism because of concerns regarding selectivity and toxicity. Table 1 shows a few examples that have been attempted for filoviruses, and discovery of the virus receptor may lead to a few more. The success of the interferon therapy makes the point that it is possible to identify host functions that can serve as targets of broad spectrum antivirals.^{41,42} However, there are, to date, very few examples of small molecule antivirals that target host functions and retain broad activity. It is a short list, including inhibitors of host cellular cyclophilins,^{43,44} inosine 5-monophosphate dehydrogenase,⁴⁵ 3-hydroxy-3-methylglutaryl-coenzyme A reductase,⁴⁶

S-adenosyl-L-homocysteine hydrolase^{14,47} and ER α -glucosidases. The ER α -glucosidase inhibitors are thus in a rare group, having been shown to have selective antiviral activity for multiple enveloped viruses in tissue cultures and, in several cases, in animal models (Table 2). Why viral functions are more sensitive to glucosidase inhibition than are host functions is becoming appreciated and touched on briefly below.

FUNCTION OF ER α -GLUCOSIDASES AND CONSEQUENCES OF INHIBITION:

Briefly, as illustrated in Figure 1, the N-linked glycans of mammalian glycoproteins are 'processed' with the sequential removal of their terminal glucose residues by the ER-resident glucosidases I and II, shortly after becoming glycosylated at specific asparagine residues. Remarkably, cells in culture can tolerate complete shutdown of these ER α -glucosidases.^{74,75,76,77,78} Glucosidase I and II knockout mice have limited life spans.^{79,80,81} People can tolerate long-term (months and years) treatment with glucosidase inhibitors under conditions where there is substantial repression of the ER enzymes. However, there are troubling adverse effects (i.e., gastric distress) which must be taken into consideration.^{36,82}

Glucosidase inhibitors have been approved for the management of type II diabetes, Gaucher's disease. Glucosidase inhibitors had also advanced to phase II human trials for management of hepatitis C virus and human immunodeficiency virus infection.^{82,83} Thus, although mammalian cells and animals tolerate significant, and even total, repression of ER glucosidases, many viruses cannot. Indeed, completion of the life cycle of many enveloped viruses requires functional glucosidases.²⁹ For example, viruses such as the hepadnaviruses, flaviviruses, filoviruses and influenza virus are significantly repressed by imino sugars under conditions where there is no detectable affect upon the host cells, or in the cases of animal experiments, only limited affect upon the host animals^{36,74,84} (Table 2). The sensitivity of these viruses to glucosidase inhibitors is presumably because they possess envelope proteins that must oligomerize, and they have an apparent requirement for glucosidase processing of their glycoproteins presumably to enable time-sensitive proper calnexin mediated folding for their oligomerization.^{35,49,52,58,85}

Taken together, the trend is clear and has not been broken for any of the viruses that have been tested so far: enveloped viruses that bud from intracellular membranes and/or use calnexin dependent pathways are selectively sensitive to glucosidase inhibitors. This has been expanded to viruses from four families causing hemorrhagic fevers (Filoviridae, Bunyaviridae, Arenaviridae and Flaviviridae) (Table 2).

CURRENT USES AND LIMITATIONS OF GLUCOSIDASE INHIBITORS:

A wide variety of structurally diverse compounds, from both nature and synthesis, have been identified as glucosidase inhibitors.^{86,87} Some of them have been successfully used to treat human diseases.^{82,83} For example, inhibition of intestinal glucosidases digesting carbohydrates is therapeutic for the management of type II diabetes.⁸⁸ The imino sugar N-butyl-deoxynorjirimycin (NBDNJ) is currently approved for the treatment of Gaucher's disease, with patients taking near gram amounts a day for many years.^{89,90} However, the molecular target of the imino sugar in Gaucher's disease is a glucocerebroside transferase, which is highly sensitive to NBDNJ, and not ER α -glucosidases. Nevertheless, these successes demonstrate the principle of tolerability of this category of drugs, as well as their practical therapeutic benefits. The benefits of imino sugar glucosidase inhibitors in the treatment of viral infections have been less clear. The currently available and human-tested glucosidase inhibitors are all limited by side effects and poor pharmacokinetic properties that make them less appealing for controlling chronic diseases. For example, although glucosidase inhibitor cellgosivir met the viral reduction milestone for the treatment of chronic hepatitis C in human studies, the drug was not advanced presumably because of gastritis and, frankly, the emergence of more potent direct acting antivirals. Thus, a consistent problem has been maintaining serum concentrations of compounds that are antiviral without causing the intestinal upsets, due to the inhibition of intestinal glucosidases.^{30,91}

ANTIVIRAL ACTIVITY OF IMINO SUGARS AGAINST HEMORRHAGIC FEVER VIRUSES:

There are now several reports showing that orally administered imino sugar derivatives inhibited dengue virus^{59,60,61,62} and Japanese encephalitis virus ⁶⁷ in mice, as well as woodchuck hepatitis virus in woodchucks.³⁰ Although the reduction of viremia level was limited, the results were encouraging. We note, however, that reduction of viremia by even 1–2 logs has been anticipated to significantly improve clinical outcomes by reducing the severity of disease and increasing survival rates.^{39,92} Along these lines, we recently reported imino sugar compounds that significantly reduced mortality in mouse model of lethal derivatives inhibited dengue virus infection.⁶³ Taken together, these results make it reasonable to consider finding broadly active glucosidase inhibitors reaching therapeutic levels in people for multiple viral indications. However, as indicated, one major limitation for developing imino sugar antivirals has been the lack of potency and/or poor pharmacokinetic properties, which lead to difficulty in maintaining therapeutic drug concentrations in vivo. We have improved the antiviral potency of imino sugars from the platform (NBDNJ) by more than 500-fold (Figure 2). For example, based upon the encouraging in vivo efficacy results achieved with Dengue mouse models, we have synthesized imino sugar glucosidase inhibitors, represented by tert butyl urea (u) DNJ (19029), with submicromolar antiviral activity against four families of hemorrhagic fever viruses in cultured cells.³⁷ In addition, significant in vivo efficacy in Ebola and Marburg virus infected animals has also been achieved (70%–80% protection).

MOLECULAR BASIS OF THE ANTIVIRAL SELECTIVITY OF GLUCOSIDASE INHIBITORS:

Imino sugars, such as those with DNJ head groups, are glucose mimetics competitive inhibitors of the N-glycan processing enzymes glucosidase I and II (Figure 2). As stated and shown in Figure 1, all N-linked glycans, following transfer to acceptor asparagine amino acids on nascent glycoproteins, are 'trimmed' in the ER by a series of sequentially active glycoprocessing enzymes. ER α -glucosidases I and II are the first enzymes to function in this pathway.^{77,78} Specifically, all nascent N-linked glycoproteins contain three terminal glucose residues at the distal termini of their N-glycans following transfer of the oligosaccharide to the protein by way of the enzyme oligosaccharyltransferase. Immediately following this transfer, the terminal glucoses are removed, sequentially, by the action of ER resident enzymes glucosidases I and then II. The ER chaperons, calnexin and calreticulin, recognize monoglucosylated N-glycans and then 'fold' the nascent glycoprotein after which it is further processed by mannosidases and transferred to the Golgi where it is further modified into its characteristic complex carbohydrate structures. Underglucosylated polypeptides that have not been folded and have not transferred to the Golgi may get second chances following reglucosylation and the possibility of calnexin and calreticulin interactions by the enzyme UDP-glucose glycoprotein: glucosyltransferase. However, ultimately, misfolded or unfolded polypeptides are sent to the proteasomes where they are degraded. Thus, glucosidase inhibitors prevent the interaction of nascent N-glycoproteins with calnexin and calreticulin and cause their misfolding and degradation. Misfolded or unfolded polypeptides may be sent to the proteasomes for degradation. It has been known for more than 20 years that many

viruses are sensitive to glucosidase inhibition, and the sensitivity of Sindbis virus, influenza virus and fowl plague virus envelope proteins was reported in the 1980s.³³

Viruses that depend upon calnexin and calreticulin would thus be expected to be the most sensitive to glucosidase inhibition. And we developed a 'biogenesis' theory that viruses that bud from the endoplasmic reticulum would be sensitive to glucosidase inhibitors. This was not to say that viruses that did not bud from the ER would not be sensitive to glucosidase inhibitors, and indeed the infectivity of retroviruses which bud from the plasma membrane is apparently greatly affected by glucosidase inhibitors.⁹³ We would now offer a refinement of the 'biogenesis' theory, in light of what is now known, to claim that viruses that depend upon the calnexin/calreticulin type pathway for morphogenesis will be sensitive to glucosidase inhibition.

These predictions, for the most part, seem to be supported by experimental results. Most strikingly is the example of hepatitis B virus and bovine viral diarrhoea virus which are both sensitive to glucosidase inhibition, and are two completely different viruses.^{8,94,95,96} Hepatitis B virus has a DNA genome and is a human pararetrovirus that primarily infects hepatocytes. Bovine viral diarrhoea virus is a flavivirus with an RNA genome and grows primarily in non-liver cells. Both, however, acquire their envelopes by budding into the endoplasmic reticulum.^{8,94,95,96} Both, we now know, also depend upon calnexin for the maturation of specific viral glycoproteins.^{97,98,99,100} As Table 2 shows, both are also greatly inhibited by glucosidase inhibitors at concentrations that do not apparently affect cell viability.

ADDITIONAL BIOLOGICAL ACTIVITY OF IMINO SUGARS:

Although imino sugars have been developed as host glucosidase inhibitors to disrupt viral glycoprotein folding and consequentially, virion morphogenesis, the compounds have additional biological activities due to either on-target suppression of ER α -glucosidases or off-target interference of other cellular components.¹⁰¹ For example, on-target inhibition of ER glucosidase results in viral glycoprotein degradation has been shown to enhance major histocompatibility complex I presentation of epitopes derived from viral envelope glycoproteins. Moreover, changes in the glycan structures associated with viral glycoproteins may also alter their interaction with C-type lectins and consequentially, inhibit pro-inflammatory cytokine production.⁶² In addition, some imino sugars may even have off target activities, repressing the virus by mechanisms that do not involve inhibition of ER glucosidases.⁵⁹ Off-target activities are not unusual in compound development, and in the case of extra biological effects upon antigen presentation and cytokine function, could even be beneficial. They may also provide interesting research leads. However, it is essential that such activities be recognized and be part of the decision to either progress or disqualify a compound, and if compounds with off-target activity are advanced, these activities must be monitored.

CONCLUSIONS:

The imino sugars have, in the past, been proposed primarily for use in chronic infections such as human immunodeficiency virus and chronic viral hepatitis.^{30,82,83} However, they may be best suited to treat acute viral infections, such as filoviral hemorrhagic fevers which involve weeks of therapy, as opposed to chronic infections which may require years of use. This would certainly avoid the complications that sometimes appear with longer-term use. Moreover, the new generation of compounds being brought forward should be designed to avoid or reduce intestinal distress. Finally, used for life-threatening infections such as the hemorrhagic fevers or severe influenza, either alone or in combinations with other management regimes, the glucosidase inhibitors could provide a powerful option in the treatment tool kit.