The Hereditary Inclusion Body Myopathy Enigma and its Future Therapy

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Summary: Hereditary inclusion body myopathy (HIBM) is a genetic muscle disease due to mutations in the gene encoding the enzyme complex UDP-*N*-acetylglucosamine 2 epimerase–*N*-acetylmannosamine kinase (GNE), which catalyzes the rate-limiting step in sialic acid production. The review describes some of the disease features that may be relevant for further understanding of the metabolic impairment of HIBM and its future therapy. It also addresses the biochemical basis behind the substrate supplementation therapy designed for this condition. **Key Words:** Hereditary inclusion body myopathy, sialic acid, GNE gene, distal myopathy.

INTRODUCTION

Hereditary inclusion body myopathy (HIBM) belongs to a group of muscle disorders characterized by adult-onset muscle weakness (usually distal) with typical histopathological changes.[1] The defective gene associated with this disorder is *GNE*.[2] It encodes a bifunctional enzyme (UDP-*N*-acetylglucosamine 2 epimerase–*N*-acetylmannosamine kinase, or GNE) belonging to the sialic acid synthetic pathway.[3] The disorder has been recognized worldwide, and although it carries several different names (distal myopathy with rimmed vacuoles, or DMRV, and inclusion body myopathy type 2, or IBM2), it has a fairly uniform phenotype associated with numerous recessive mutations (currently more than 50 are known) spread through the two domains of the gene.[1] Given some of its unique features, HIBM seems like a hereditary myopathy with potential therapy (at least theoretically). We will review these features as they are, or may be, relevant to specific therapeutic approaches, but of course this recessive condition may someday become amenable to more general genetic and cell therapies. We have reviewed clinical and pathological aspects of HIBM in greater detail elsewhere.[1]

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DIAGNOSTIC FEATURES OF HIBM

Clinical and histological diagnostic features are as follows.

1) Myopathy starting in the distal muscles of the legs and slowly progressing to involve the proximal musculature, but sparing the quadriceps in the vast majority of patients.
2) Modest elevation of serum creatine kinase.
3) Presence of so-called rimmed vacuoles in the muscle fibers, with minimal nonspecific other changes in fibers histology. Despite the traditional name, the vacuoles are not bound by membrane and present many of the features of autophagic vacuoles.[4]
4) Identification of inclusions (mainly cytoplasmic, but also nuclear) composed of paired helical filament.
5) Necrosis, inflammation, and fibrosis are rarely seen in biopsy samples.
6) Autosomal recessive inheritance.

DO THE CLINICOPATHOLOGICAL FEATURES PROVIDE CLUES TO THERAPY?

We believe that some of the clinical features of HIBM carry clues to understanding its pathophysiology and its potential therapy. The mild rise in creatine kinase indicates

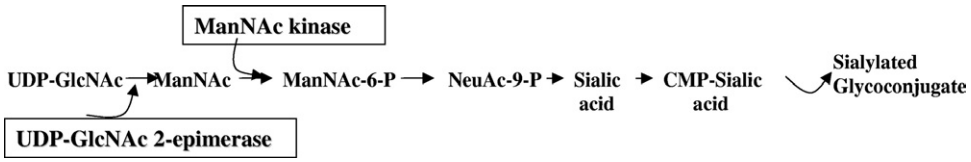

FIG. 1. The biochemical pathway of sialic acid synthesis and its major intermediates.

that there is little active necrosis of muscle fibers, which is supported by the overall histology seen in diagnostic biopsies early in the disease process. This also suggests that muscle cell membrane is functionally intact, preventing major creatine kinase leaks. Thus, the mode of cell death in this myopathy is non-necrotic, unlike the classical muscular dystrophies. Consequently, medications that intervene with muscle fiber necrosis and are tested in muscular dystrophies may not be applicable to HIBM. Likewise, agents that prevent muscle fibrosis will most probably not affect disease progression in HIBM.

The composition of the inclusions is unknown, but their small quantity seems to indicate that they are a secondary phenomenon. Autophagic vacuoles are more abundant in HIBM, although it has never been determined if their numbers correlate with disease severity or progression.[4] Autophagy has recently sparked great interest, not only in relation to central nervous system diseases, but also in myology with respect to acid maltase deficiency (Pompe disease). In this lysosomal disorder, the autophagic vacuoles build-up seems to be responsible for the muscle cell damage.[5] The role of autophagy in HIBM remains to be determined; it may be a secondary and even protective mechanism, or a primary destructive process. Thus, future developments of substances that could inhibit autophagy may have relevance to HIBM therapeutics; however, this is beyond the scope of the present review and readers are referred elsewhere.[6]

One of the major enigmas related to HIBM has been the recognition of quadriceps sparing in this disorder,[7] unique among all other myopathies. Even in the bedridden or wheelchair-bound patients, when most leg muscles are paralyzed this major hip muscle retains almost normal power and the typical histology of HIBM was never identified in quadriceps biopsy samples. Understanding what protects this muscle seems to be the secret toward designing therapy; however, work done by us and others has failed to explain this feature. The quadriceps contains similar amounts of GNE as the other muscles, and there is no difference in the metabolic consequences of GNE enzymatic deficiency between this muscle and others affected.[8,9] Whether there are any compensatory metabolic pathways that protect this muscle remains to be elucidated, but we are not aware of such a process, nor of a different embryogenesis for this specific muscle.[10]

An additional clinical observation that may be relevant to therapy is that of two elderly subjects (one now reaching 70 years, belonging to a large Persian Jewish pedigree with several affected members, and the second one from Japan) who are homozygous for *GNE* mutations and yet unaffected. Lack of penetrance is unusual in recessive disorders, and these subjects may possess some other protective mechanism, or one similar to that which preserves the quadriceps.

THE METABOLIC PATHWAY OF GNE AND ITS IMPAIRMENT IN HIBM

Sialic acids are the most abundant terminal monosaccharides on glycoconjugates in eukaryotic cells, comprising a family of more than 50 naturally occurring carboxylated amino sugars with a scaffold of nine carbon atoms.[11] The first two steps of the cytosolic pathway of sialic acid formation are catalyzed by the two distinct functional domains of GNE (FIG. 1). First, the UDP-*N*-acetylglucosamine 2-epimerase domain synthesizes *N*-acetylmannosamine (ManNAc) from UDP-*N*-acetylglucosamine, followed by the ManNAc kinase, which phosphorylates ManNAc to generate ManNAc 6-phosphate.[12,13] GNE is regulated by several different mechanisms, most importantly the feedback inhibition of the epimerase activity by CMP-sialic acid.[14]

Several experimental observations relate to GNE function and sialic acid metabolism in HIBM.

1. Complete absence of GNE is probably incompatible with life. Knock-out inactivation of the gene in mice results in embryonal lethality at day 8.5,[15] and thus GNE is essential for embryonic development. This can explain why homozygosity for two nonsense mutations has not been detected in any HIBM patient.
2. Significant GNE protein deficiency has not been observed in HIBM patients; in fact, GNE protein is expressed at equal levels in HIBM patients and normal control subjects, and no mislocalization of GNE in skeletal muscle could be documented.[9,16]
3. Evaluation of the GNE enzymatic activity showed that the extent of its reduction in lymphocytes, myoblasts, and myotubes from HIBM patients varied between 30% and 60%.[8,17,18]
4. Various analyses of cells from patients[8,17–19] revealed a broad range of bound sialic acid levels in normal control subjects, overlapping with those of affected individuals, indicating that in most HIBM patients reduction in overall sialylation is not a significant feature. It should be noted that in a few cases, a

modest to marked decrease in membrane sialylation did occur, but studies on changes in the α-dystroglycan and neural cell adhesion molecule sialylation, as a proficient marker of glycosylation defects, again show normal results in some HIBM patients and very abnormal findings in others.[8,18–21]

IS METABOLIC TREATMENT POSSIBLE IN HIBM?

When the enzyme GNE was found to be mutated in HIBM, the initial hypothesis was that this disorder would behave like a typical metabolic myopathy, with a lowered metabolic activity that reduces an important synthetic pathway leading to a deficiency in its product. If so, one would try to design an alternative route to supplement the deficient product.

The metabolism of the sialic acid was well studied before the discovery of its relation to HIBM.[12,13] Some of the early general observations, however, as well as later ones in HIBM patients, made the initial hypothesis difficult to accept. The role of GNE in muscle was thought to be minor, based on its low levels in this tissue compared with that in liver, the main source of sialic acid production and delivery to other tissues. Furthermore, in normal tissue GNE activity exploits only 5% of its potential[22] and, theoretically, this could be augmented in HIBM by compensatory activation.

Such natural compensatory mechanisms could be the existence of the feedback inhibition process acting on the epimerase activity of GNE, which is supposed to enhance sialic acid synthesis when CMP-sialic acid is low, or the presence of additional cellular kinases, such as N acetyl glucosamine kinase, that are capable of using *N*-acetyl mannosamine as a substrate[23] and therefore compensating for a partial defective function of the kinase activity of GNE, at least in HIBM patients carrying the double kinase mutations. Furthermore, reduction of enzymatic activity by 30% to 60% is not expected to impair metabolic pathways in the body to a degree that leads to a disease phenotype (S. DiMauro, personal communication). Most single mutation carriers of classical recessive disorders of enzymatic muscle activity (e.g., McArdle disease and carnitine palmiltoyl transferase deficiency) do have such lowered activity levels without any clinical signs and manifesting carriers are thought to have some additional dominant negative effect.[24] A possible additional effect of the impaired sialic acid pathway is at the heart of metabolic research into the pathophysiology of HIBM.

One should not, however, ignore completely the possibility that some deficient sialylation does occur in HIBM, possibly of only one or very few glycoconjugates, and is amenable to therapy (even if it is not the major metabolic impairment). This possibility is somewhat strengthened by the one existing animal model for HIBM. Recently, a transgenic mouse over-expressing the human *GNE* D176V mutation (which is one of the founder mutations among Japanese patients) was crossed with a heterozygous *Gne* knockout mouse ($Gne^{+/-}$) to obtain $Gne^{-/-}$ transgenic hGNED176V animals.[25] These mice showed hyposialylation in muscle and other organs, and exhibited a reduction in muscle performance from 32 weeks of age and an HIBM-like muscle pathology after 42 weeks of age. Future testing in other myopathic models of GNE defects will have to confirm that this hyposialylation is indeed common to all.

HUMAN THERAPY TRIAL IN HIBM

In a pilot study, the first human trial for therapy of HIBM, excess sialic acid was provided in an attempt to normalize a hyposialylated state. The chosen vehicle was intravenous immunoglobulin preparation. This preparation is in use for various immune-mediated diseases and contains 8 μmol of sialic acid per gram. It was thought that some of it would break off and reach muscle cells.[26] Four patients entered the short-term, open-label trial (only 1 month of therapy), and their response to treatment was monitored by force and functional measurements, subjective reporting, and histology and immunohistochemistry evaluation of muscle samples from the quadriceps. Muscle power composite improved at the end of the study, although functional measurements gave inconsistent results. The muscle immunohistochemistry and immunoblotting for sialylated proteins provided no consistent change. Untoward effects were reported, usually short-lived and in accordance with known side effects of intravenous immunoglobulin (mainly headache and meningeal reaction).

The authors concluded that intravenous immunoglobulin will not be the treatment of choice in HIBM.[26] Their study showed transient improvement in muscle power, but was certainly too short to evaluate repair in such a severe myopathy and placebo effect could not be controlled in an open-label trial. Furthermore, the choice of quadriceps as the source of tissue for outcome measurements is problematic, given that this muscle behaves differently from most other skeletal muscles in HIBM. If the hypothesis of reduced sialylation is correct, the authors suggested searching for another source of sialic acid provider.[26] The metabolic intermediate ManNAc stands as the immediate candidate.

CAN SIALIC ACID OR ITS PRECURSOR ManNAc BE A THERAPEUTIC AGENT IN HIBM?

In principle, both free sialic acid or ManNAc, when provided from the outside, are suitable as generators of CMP-sialic acid to enhance a deficient sialylation state.

ManNAc, however, seems to be a better choice for the following reasons.

1. The incorporation of these compounds into the cell is via different mechanisms. Sialic acid is taken via an endocytotic process and released to the cytosol via the lysosomal pathway; in contrast, ManNAc is transported through the plasma membrane by diffusion.[27] Although it is not clear which mechanism is operational in muscle cell, that of ManNAc seems to be less complicated.
2. Sialic acids can be degraded in cells by various aldolases (specific or nonspecific) into ManNAc and pyruvate; thus, one should use ManNAc for better control of its supplementation.
3. Sialic acid is a strong acidic sugar, which may affect gastric function.
4. Currently, the price of sialic acid production is about 10 times higher than that of ManNAc, and if this ratio stays constant, then economical consideration should also be applied.

ManNAc is the sugar intermediate generated by the epimerase reaction, which is the rate limiting step in the sialic acid biosynthesis (FIG. 1). The idea behind its use is that supplementation of ManNAc to this pathway will normalize intracellular CMP-sialic acid level and consequently improve the hyposialylation of affected glycoconjugates. The CMP-sialic acid production via this supplement is not controlled by the biofeedback inhibition that takes place at the epimerase step, and consequently the level of this nucleotide sugar may increase over the normal physiological level, thus relieving the chronic hyposialylation state that exists in the HIBM-affected muscle cells. It is also postulated that the excess intermediate will compensate for the reduced overall activity of the pathway, especially if the epimerase domain is mutated (as occurs in one of the Japanese homozygous founder mutations and in other patients with double mutations in this domain), but also if other *GNE* mutations decrease the affinity of ManNAc kinase to its substrate. In those patients with only GNE kinase mutations, it is supposed that other kinases take over and convert ManNAc to ManNAc-6-phosphate, which can then be further metabolized to sialic acid. Because of their kinetic features, these kinases need high ManNAc concentrations, such as can be reached only by exogenous supplementation. There are, of course, some assumptions in this theoretical model which are not yet proven, especially those related to the compensatory activation of other kinases and the overall sialylation deficiency.

Some support to the hypothesis that GNE defects lead to hyposialylation that can be corrected by ManNAc comes from the observation in another animal model of GNE defect. This knock-in model,[28] using the most common homozygous mutation in the GNE kinase domain (M712T, the mutation in the Middle Eastern cluster of HIBM[2,29]), did not produce a muscle disease. Instead, the animals died at day 3 after birth from what appears to be a unique renal abnormality, probably due to hyposialylation of some essential renal membrane protein. This is a rather surprising result, in contrast with the first model of HIBM.[25] When pregnant mice were fed with ManNAc before giving birth and also during nursing, some pups survived longer, probably having milder renal abnormalities (but no muscle disease phenotype—perhaps because they had not reached sufficient age).

A formal trial of ManNAc in humans has not yet been approved by regulatory authorities. The substance is currently not produced by a pharmaceutical company and no safety studies were done. Nonetheless, we are aware of a few patients who buy the material from a nonpharmaceutical company and use it without medical supervision (in doses up to several grams per day). Obviously, under such conditions efficacy and safety cannot be assessed. Because the material is not protected by patent, the chances of it being developed into a testable drug seem small. We hope that future testing in myopathic animal models will produce enough interest to progress with this potential medication.

OTHER MECHANISMS OF HIBM PATHOPHYSIOLOGY

Because it seems that necrosis is not the mode of cell death in HIBM, and hyposialylation may not be the only explanation of the muscle disease process, other mechanisms may be operational. We believe that the mode of muscle cell loss is apoptotic death.[30] Some other functions of GNE may turn out to be important in the pathophysiology of the disease, such as the presence of GNE in the nucleus,[16] or its possible interactions with other proteins, such as α-actinin-1,[31] which may provide new directions for our choice of potential treatments.

CONCLUSION

Hereditary inclusion body myopathy is unique among myopathies, with some metabolic impairment that could be potentially treated before corrective gene and cell therapies are shown to be effective in myology. We hope that regulatory issues currently preventing the formal testing of ManNAc in humans will be resolved and that supervised trials can begin.

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