Ther Adv Neurol Disord
(2010) 3(2) 127–135
DOI: 10.1177/1756285609359546

A preclinical trial of sialic acid metabolites on distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy, a sugar-deficient myopathy: a review

May Christine V. Malicdan, Satoru Noguchi and Ichizo Nishino

***Abstract*:** Distal myopathy with rimmed vacuoles (DMRV), also called hereditary inclusion body myopathy (hIBM), is a moderately progressive hereditary muscle disorder affecting young adults. DMRV/hIBM is characterized clinically by muscle atrophy and weakness initially involving the distal muscles, and pathologically by the presence of small angular fibers, formation of rimmed vacuoles and deposition of various proteins in the muscle fibers. This disease is known to be caused by mutations in the UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase gene, which encodes the essential enzyme in sialic acid biosynthesis, leading to a reduction of sialic acid levels in the serum and skeletal muscles of affected patients. As it is a metabolic disease, metabolite supplementation is theoretically one of the therapeutic options. In this review, recent animal models for DMRV/hIBM are briefly characterized followed by a focus on the administration of sialic acid metabolites as a reliable therapeutic option to DMRV/hIBM with the following points highlighted: the property of compounds, the pharmacokinetic metabolism *in vivo*, and the therapeutic effects on the DMRV/hIBM mouse model.

***Keywords*:** sialic acid, GNE, muscular dystrophy, amyloid, therapy

Introduction

Distal myopathy with rimmed vacuoles (DMRV) or hereditary inclusion body myopathy (hIBM) is an autosomal recessive debilitating disorder affecting young adults with the age of onset ranging from 15 years to the late 30s [Nonaka *et al*. 2005; Nishino *et al*. 2002], and is due to mutations in the UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (*GNE*) gene [Eisenberg *et al*. 2001]. The disease is characterized clinically by preferential involvement of the tibialis anterior and hamstring muscles and relative sparing of the quadriceps [Nishino *et al*. 2005; Nonaka *et al*. 2005; Argov and Yarom, 1984]. The course of the disease is gradually progressive, whereby patients usually become wheelchair-bound around 12 years after the onset of the disease. Findings in skeletal muscle biopsy include the presence of rimmed vacuoles, which are seen as clusters of autophagic vacuoles on electron microscopy, scattered atrophic fibers, and muscle fiber degeneration.

Despite the identification of the causative gene, the treatment for DMRV/hIBM has remained elusive as the pathomechanism of this disease has not been fully clarified. In addition, the lack of an appropriate model for understanding the disease and evaluating potential treatment options has contributed to the lag in development of a cure. In general, several strategies exist for the treatment of hereditary muscle disorders, such as gene therapy, cell therapy, and a pharmacological approach. Among these options, pharmacological treatment has been the most widely applied strategy to many muscular dystrophies and myopathies, as both the drug and study protocol can be flexibly designed based on the cause, pathogenesis, and symptoms of the disease.

This review focuses on a treatment option based on the notion of decreased sialic acid production in muscle cells owing to mutations in the *GNE* gene. Based on our recent findings on the preclinical trial of sialic acid metabolites to the

Correspondence to:
Ichizo Nishino
Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan
nishino@ncnp.go.jp

May Christine V. Malicdan, Satoru Noguchi
Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan

DMRV/hIBM mouse model, we review the properties of potential compounds taking into account the application *in vivo* of these compounds to mice. Likewise, we also discuss the phenotype of the mouse model and its response to therapy in order to clarify the reliability of sialic acid supplementation for DMRV/hIBM therapy in the future.

Distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy animal models

Several strategies based on genetic technology by manipulating the *GNE* gene have been attempted to generate animal models for DMRV/hIBM. Simple knock-out mice represent embryonic lethality by 9.5 dpc to suggest the importance of sialic acid in early embryogenesis [Schwarzkopf *et al*. 2002]. The same concept of the importance of sialic acid was recently demonstrated in knock-in mice carrying the p.M712T mutation, which is the most common *GNE* mutation among Jewish patients. The p.M712T mice showed a renal phenotype so severe that most homozygous mice could not survive beyond 3 days after birth (P3) [Galeano *et al*. 2007]. This renal phenotype is apparently caused by an anomaly in the morphogenesis of glomerular tissues due to the remarkable reduction in sialylation of podocalyxin, a major sialylated component of podocytes. In the M712T mice that were able to survive beyond P3, however, a phenotype pointing to skeletal muscle weakness or abnormalities in muscle pathology was not found. This result may suggest that the essential requirement of *GNE* activities for a certain level of sialic acid production is different between human and mice; in other words, the need for sialic acid at least during development might be higher in mice as compared with humans.

Our group adopted a different strategy to generate $Gne^{-/-}$h*GNE*D176VTg, a mouse model for DMRV/hIBM. This model harbored a transgene of p.D176V mutated human *GNE* cDNA but is knocked-out of endogenous Gne, creating a scenario in which only mutated GNE proteins are highly expressed and the endogenous *GNE* gene was disrupted [Malicdan *et al*. 2007a,b]. These mice were born at an almost Mendelian rate with a normal appearance (Figure 1(a)). As expected, blood and several organs including skeletal muscle exhibited hyposialylation. With age, these mice reproduced several myopathic phenotypes seen in the muscles of human DMRV/hIBM patients (Figure 1(b)–(d)). After 20 weeks of age, the DMRV/hIBM mice showed physiologic muscle weakness, seen as impaired motor performance of the mouse and reduced force generation of the skeletal muscle [Malicdan *et al*. 2008]. This reduction of the force can be attributed to muscle atrophy, as specific twitch and tetanic forces per cross-section area are maintained at normal values. The reduction in gross size of the skeletal muscle is accompanied by an increase in the number of small angular fibers on muscle cross-sections (Figure 1(c), white arrows). Serum creatine kinase is moderately elevated at this age. After 30 weeks of age, specific force generation in the gastrocnemius and tibialis anterior muscles was notably reduced, while in muscle pathology variation in muscle fiber size was more remarkable, and intracellular deposition of amyloid and other various proteins was noted in the gastrocnemius muscle. After 40 weeks, the muscle force generation increasingly worsened, as reflected by increased twitch/tetanic ratio, which could likely be due to the appearance of the characteristic rimmed vacuole (Figure 1(c), red arrows) and accumulation of autophagic vacuoles [Malicdan *et al*. 2007a,b] that can impair the contractile system of the muscle. With these results, the $GNE^{-/-}$hGNED176VTg mouse is the only existing pathogenic model for DMRV/hIBM at the moment.

Potential compounds for therapy of distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy

DMRV/hIBM is caused by mutations in the *GNE* gene, most of which are missense in the *GNE* gene. *GNE* encodes a critical enzyme, uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase, for the biosynthesis of sialic acid in higher vertebrates including mammals (Figure 2, left panel). This enzyme catalyzes two steps in the sialic acid biosynthesis pathway: the epimerization of UDP-GlcNAc to ManNAc and the phosphorylation of ManNAc, the product of which is the substrate used to make sialic acid. Sialic acid production is determined by a negative feedback effect of the produced sialic acid on this UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE protein) step. The sialic acid product, cytidine monophosphate-neuraminate (CMP-NeuAc), binds the allosteric site of the GNE protein, inhibiting UDP-GlcNAc 2-epimerase activity. In principle, *GNE*

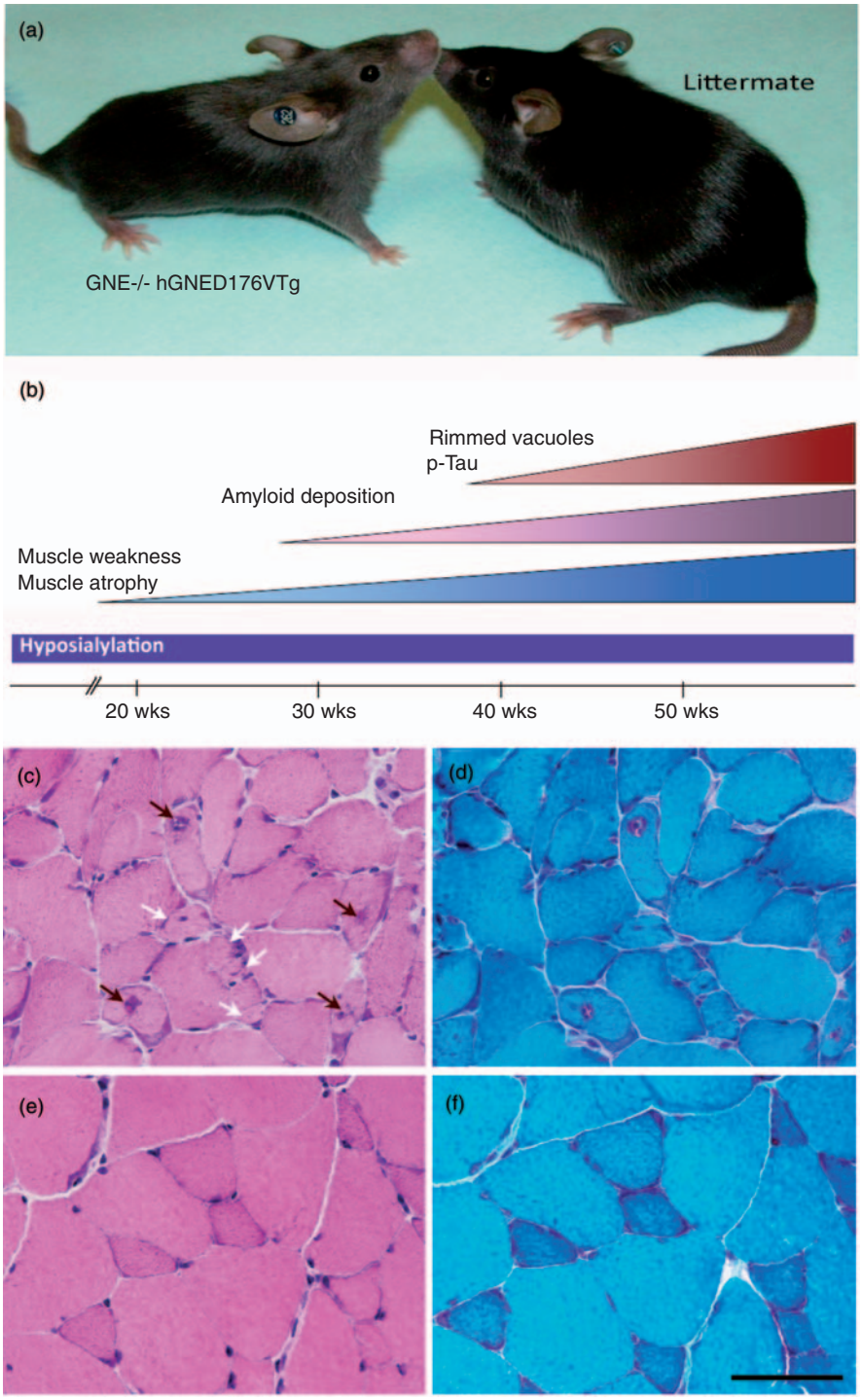

Figure 1. Overall phenotype of the distal myopathy with rimmed vacuoles (DMRV)/hereditary inclusion body myopathy (hIBM) mouse. (a) At birth, DMRV/hIBM mice appear normal, although slightly smaller than control littermates. (b) The onset of changes in muscle pathology are increasingly noted with age. Note that hyposialylation of serum and other organs is observed from birth. Typical fibers with rimmed vacuoles (red arrows) and small atrophic fibers (white arrows) are seen on hematoxylin and eosin (H&E) (c) and modified Gomori trichrome (mGT) (d). (e) H&E and (f) mGT-stained sections of muscle from age-matched control littermates. The characteristic features of DMRV/hIBM in muscle pathology are not seen in littermates. 187 × 295 mm (400 × 400 dpi).

mutations are expected to lead to decreased ManNAc or phosphorylated ManNAc (ManNAc-6P) production, subsequently resulting in the reduction in sialic acid production (Figure 2, middle panel), which was noted in skeletal muscle cells and serum in the DMRV/hIBM patients and model mice [Malicdan *et al*. 2007b; Noguchi *et al*. 2004]. On the other hand, the function of other enzymes involved in sialic acid synthesis is not affected in cells with *GNE* mutations, suggesting that metabolite supplementation may be an effective option in increasing sialic acid levels. In fact, several reports have shown that in cells with deficient *GNE* activity, such as BJA-B K20 and K6 [Keppler *et al*. 1999], Lec3 [Hong and Stanley, 2003] and *GNE* KO ES cells [Schwarzkopf *et al*. 2002], sialic acid levels can be recovered by supplementation either with ManNAc or sialic acid. It is surprising that even in *GNE* KO ES cells, which lack both UDP-GlcNAc 2-epimerase and ManNAc kinase activities, ManNAc treatment had a remarkable effect on the production of polysialic acid antigens on their surface membrane [Schwarzkopf *et al*. 2002]. These previous reports suggest that abundant GlcNAc 6-kinase activity contributes to phosphorylation of ManNAc in these cells [Hinderlich *et al*. 2001], although the pathogenesis of mutations in the ManNAc kinase domain of the GNE protein could not be explained in this concept.

ManNAc and NeuAc

As candidates for drugs for DMRV-hIBM, all metabolites downstream of GNE catalysis may be considered. In general, however, the nucleotide derivatives are thought to be rarely incorporated into cells and the phosphorylated compounds are believed to be dephosphorylated before being incorporated into the cell during their delivery. Theoretically, therefore, only ManNAc and NeuAc can be used for extrinsic administration to augment sialic acid synthesis.

There have been several trials that demonstrated manipulation of cellular sialic acid levels in normal cells and animals by administration of compounds extrinsically. In these trials, it was considered that ManNAc is the preferred molecule to increase the cellular sialic acid level, and that NeuAc may not be efficiently incorporated into cells due to its acidity from the outside, although NeuAc recycles within cells. However, this suggestion was made based on the finding of hyper-sialylation in normal cells and animals [Hirschberg *et al*. 1976]. In contrast, a report showed that NeuAc can be taken up by eukaryotic cells [Oetke *et al*. 2001], supporting its potential use for increasing sialic acid levels intracellularly. Interestingly, ManNAc and NeuAc reportedly use different routes for entry into cells [Bardor *et al*. 2005]. ManNAc gains entry into cells either by diffusion or via a specific transporter. Diffusion is more probable as the cellular incorporation rate is enhanced when the hydrophobicity of ManNAc is increased when modified by O-alkylation. On the other hand, NeuAc is incorporated by micropinocytosis and is subsequently transported from the endosomes to the lysosomes, and finally into the cytosol by the specific transporter, sialin.

The ability of cells to incorporate both ManNAc and NeuAc to possibly a comparable degree is also suggested by our results in DMRV cells, whereby we demonstrated that the addition of ManNAc and NeuAc in the medium of primary cells from DMRV patients recovered sialylation of the cells to a similar level [Noguchi *et al*. 2004]. These results support the notion that both ManNAc and NeuAc, in principle, are equally useful compounds for therapy. However, this does not discount the need to choose carefully the molecule based on the target tissues and their status. For example, targeting the DMRV skeletal muscle for treatment requires the consideration that the muscles are actually atrophic, and that the endocytic pathway itself might be affected.

Another issue that favors the use of either ManNAc or NeuAc as potential compounds for DMRV/hIBM treatment is the source of such compounds. ManNAc and NeuAc are natural monosaccharides produced in the animal body. ManNAc is only present as a free molecule at a trace amount and actually the presence of glycoconjugates that include ManNAc residues or specific glycosyltransferases for ManNAc residues has not been demonstrated, at least among vertebrates. NeuAc is present virtually as a glycoconjugate in glycoproteins and gangliosides, and is almost never a free molecule within cells. While both compounds are naturally occurring, it has been demonstrated that large-scale production for drug synthesis by pharmaceutical companies is possible [Yamaguchi *et al*. 2006].

Route of administration of sialic acid metabolites

Previous reports have suggested that extrinsically administered sialic acid is rapidly excreted

to urine. Uptake, metabolism and excretion of orally and intravenously administered radioisotope-labeled NeuAc and sialyllactose were examined in mice and rats. In 20-day-old mice, 90% of orally administered NeuAc was absorbed from the intestine at 4 h [Nöule and Schauer, 1981] but between 60 and 90% of NeuAc was excreted in the urine within 6 h. Only small amount of NeuAc (less than 6%) was incorporated into tissues, and was subsequently metabolized to ManNAc and pyruvate. These data imply that the small amount of sialic acid in food cannot be directly used for the synthesis of glycoconjugates for the purpose of increasing the levels of sialic acid in the tissues. In intravenous injection of NeuAc into rats, 90% was excreted in the urine within 10 min. Oral administration of sialyllactose resulted in the longer retention of sialic acid in tissues, however almost all were excreted within 24 h in which a half amount was metabolized to NeuAc. Sialyllactose injected intravenously into rats was rapidly excreted similarly to NeuAc.

Continuous administration of NeuAc orally and intraperitoneally for 8 days was examined in 14-day-old rat pups and resulted in a more remarkable incorporation of NeuAc into brain gangliosides and glycoproteins [Carlson and House, 1986]. This experiment suggested that orally administered NeuAc might be more significantly incorporated depending on the timing in animal development, and if the same dose was administered in several aliquots, then exogenous NeuAc might be more efficiently utilized as a substrate for sialylation of membrane gangliosides and glycoproteins. Further, when compared with the study carried out in older mammals, these experiments suggest that older animals did not show significant incorporation of sialic acid, at least after acute dosing. These likewise imply the need for a frequent and prolonged administration of metabolites in order to increase the sialic acid in the tissues.

We also analyzed the pharmacokinetics of NeuAc and ManNAc in blood and urine after intragastric and intraperitoneal administration in adult mice [Malicdan *et al.* 2009]. After a single intraperitoneal injection of NeuAc, the sialic acid level in the blood is notably increased within minutes, but 90% is found in the urine within 5–30 min and almost all is excreted within 4 h. After giving a single dose by an intragastric route, the levels in the blood are half as compared with the intraperitoneal route, but the excretion rate is slower as 70% is found in the urine within 30–60 min. Although after 120 min, the NeuAc levels in blood by both routes returned to a constant level, administration by an intragastric route resulted in a higher level of NeuAc even in the constant state. A similar pattern of excretion was seen after a single dose of ManNAc was given. These results suggest again that NeuAc and ManNAc are rapidly excreted and the intragastric route may be more advantageous in increasing the blood levels of sialic acid. For the treatment of DMRV/hIBM mice, we used the intragastric route by adding the compounds to drinking water (1-day dose of compounds dissolved in water, the amount of which was adjusted to what the mouse can drink within the day). By this method, the daily dose was divided into small aliquots and the frequency of drug intake is actually increased as mice drink 11.13 ± 1.28 times a day [Ritskes-Hoitinga *et al.* 2004].

Treatment of distal myopathy with rimmed vacuoles/hereditary inclusion body myopathy mice

In designing a protocol for preclinical therapeutic trials, several factors are considered, including the property of the drug and the progression of diseases. In addition, the purpose of the treatment can either be aimed at prevention, arrest of disease progression, or cure from the diseased condition. The purpose of treatment is relevant in diseases that have a defined onset and progression, thus the goal of therapy would largely be affected by the starting time of the treatment. As DMRV/hIBM is a disease affecting young adults, maximum efficacy of treatment with sialic acid and its metabolites may be expected in preventing the onset of disease. In our recent paper, we examined the effect of sialic acid compounds in preventing the development of a myopathic phenotype in the DMRV/hIBM mouse model [Malicdan *et al.* 2009].

The study protocol that we used involved administering ManNAc to the mice from a preclinical age (5–6 weeks) continuously until the mice reached the age when all myopathic symptoms are found (54–57 weeks). ManNAc was added to drinking water and given in three doses: 20 mg (low dose), 200 mg (medium dose), and 2000 mg (high dose)/kg body weight of mice in a day. During the treatment period, survival rate was remarkably improved as compared with control-treated mice at all three doses. At the

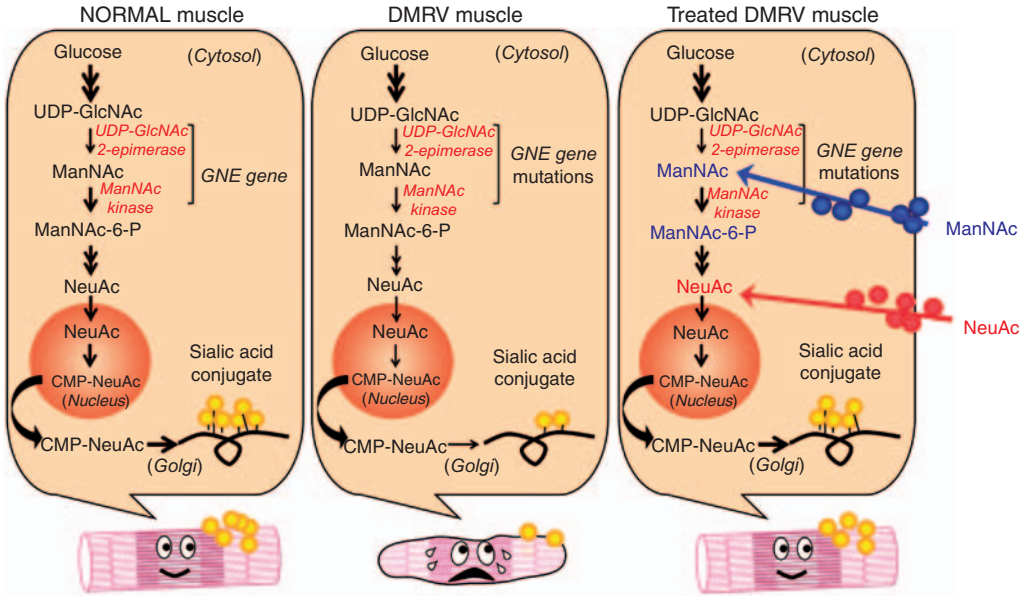

Figure 2. Incorporation of exogenous metabolites into sialic acid biosynthesis. **Left panel** shows the sialic acid biosynthetic pathway initiated within the cytosol, which starts with a series of steps whereby glucose is sequentially converted to UDP-GlcNAc. The succeeding essential steps involve the bifunctional enzymes encoded by *GNE: UDP-GlcNAc 2-epimerase* which epimerizes UDP-GlcNac into ManNAc, and *ManNAc kinase* which catalyzes the phosphorylation of ManNAc into ManNAc-6-P. The next two steps in the pathway eventually convert ManNAc-6-P into NeuAc. Activation of free NeuAc into CMP-NeuAc occurs in the nucleus. CMP-NeuAc is later transported to the Golgi apparatus. Sialic acids are ultimately transferred to oligosaccharide chains of gangliosides or sialoglycoproteins become sialylated (sialic acid conjugates). **Right panel** depicts a situation whereby the activity of the bifunctional enzyme is reduced because of mutations in *GNE,* resulting into decreased sialic acid production (and sialylation) of gangliosides or sialoglycoproteins, and reduced functional status of skeletal muscles in DMRV/hIBM. **Middle panel** shows the steps whereby exogenous sialic acids are incorporated into the pathway, improving sialylation status of sialic acid conjugates, and contributing to the improvement of skeletal muscle function in DMRV/hIBM.

end of the treatment, the phenotypes of DMRV/hIBM mouse were evaluated and compared with control-treated DMRV/hIBM mice and non-affected littermates. At all doses, serum creatine kinase activity, motor performance of mice, physiological contractile properties of isolated skeletal muscles as well as muscle pathology were notably improved to a level almost similar to that of the non-affected littermates. Intracellular protein deposits and rimmed vacuoles were rarely seen in the skeletal muscles of mice treated with ManNAc. Sialic acid levels in the blood and tissues were elevated, and more importantly, the levels of sialic acid in the muscle were recovered to an almost normal level after treatment [Malicdan *et al.* 2009], providing evidence that prophylactic oral administration of ManNAc to DMRV/hIBM mice was remarkably effective. Figure 2 (middle panel) is a simplified schema depicting the purported decrease of sialic acid levels on the glycoproteins and glycolipids in the muscle, which may contribute to the generation of symptoms in DMRV/hIBM by an unidentified mechanism. Figure 2 (right panel) also shows the incorporation of exogenously administered sialic acid metabolites into the sialic acid biosynthetic pathway. Once incorporated, sialic acids are supposedly utilized by glycoproteins and glycolipids in the muscle, preventing muscle weakness, or at least enhancing its physiologic function. We could not see, however, any correlation of the improvement of phenotype to the doses of administered ManNAc, except in the survival rate of the mice and sialic acid levels in the blood. This absence of dose response could potentially be explained by the following concepts: (a) administered ManNAc is effective in improving phenotypes completely only with the lowest dose used in the study, that is, 20 mg/kg bodyweight/day; (b) when large amounts of ManNAc are given, excess amounts of administered ManNAc will be excreted rapidly in the urine and only small aliquots of ManNAc will be retained in the blood for a certain time; and

(c) skeletal muscle has a maximum limit for actually incorporating ManNAc, or there might be other systems that can regulate the amounts of cellular ManNAc (e.g. by GlcNAc 2-epimerase, which is the catabolic enzyme). Nonetheless, these results suggest the need for a detailed analysis on the incorporation and metabolism of chronically administered ManNAc in living animals.

We also examined the effect of oral NeuAc and sialyllactose together with ManNAc using the minimum dose used in ManNAc treatment (20 mg/kg bodyweight/day) on the phenotypes of DMRV/hIBM mice starting at the preclinical age of 10–20 weeks. Treatment was also continued up to 54–57 weeks of age. Similar beneficial effects on motor performance, force generation of skeletal muscles, and muscle pathology were obtained after the treatment, as compared with the ManNAc trial. When the different compounds were compared as regards efficacy, we did not find any conspicuous differences, suggesting that these are equally good options for treatment.

One of the characteristic features in DMRV/hIBM is the accumulation of numerous autophagic vacuoles in the myofibers in which the transport and function of lysosomes may also be affected. As NeuAc is known to be incorporated into cells via the macropinocytotic/lysosomal pathway, one might question how NeuAc could actually be incorporated; however, from our data, treatment with NeuAc and sialyllactose improved sialic acid levels in various tissues with almost the same efficacy as with the ManNAc treatment. As the compounds were given at a preclinical stage, the accumulation of autophagic vacuoles, which is a phenomenon at a later stage, was prevented by the prophylactic treatment of sialic acid compounds. These issues on the preference and efficacy of the compounds at different stages of disease can only be answered by further studies that will focus on the systematic treatment in various and later ages of DMRV/hIBM mice.

Toxicology of the compounds

Long-time administration of low dose ManNAc and sialic acid metabolites was tolerated well in DMRV/hIBM mice [Malicdan *et al.* 2009]. In a previous paper where *N*-acyl-mannosamines and their O-acetylated derivatives were administered for 2 weeks to normal mice, no significant difference was seen in mice given the drug or phosphate-buffered saline alone, at least in terms of morphology of the investigated organs, histochemical data on various kinds of metabolic enzymes, and the expression of several markers of blood cells [Gagiannis *et al.* 2007]. Interestingly, however, O-acetylated ManNAc showed cytotoxicity of cultured cells in higher concentrations [Schwartz *et al.* 1983]. This could imply that the rapid excretion rate of ManNAc in rodents may actually prevent the toxic effects of the compounds.

Can reduction in sialic acid cause myopathy?

Whether decreased sialic acid production causes myopathy has been controversial. Although we and others demonstrated that hyposialylation does exist in DMRV/hIBM cells, others reported that there is no overall hyposialylation in the myoblasts and lymphoblastoid cell line from DMRV/hIBM patients [Salama *et al.* 2005; Hinderlich *et al.* 2004]. In addition, *GNE*-gene product has been proposed to have functions and roles outside sialic acid biosynthesis [Amsili *et al.* 2007; Wang *et al.* 2006]. We also do not completely discount that there might be other factors that could contribute to the pathogenesis of DMRV/hIBM. Nevertheless, the fact that increasing sialylation status, at least in skeletal muscles, can prevent the development of the myopathic phenotype in the majority of sialic acid metabolite-treated DMRV/hIBM mice clearly suggests that hyposialylation is one of the key factors in the pathomechanism of DMRV/hIBM (Figure 2, right panel). Addressing this metabolic impairment is a logical option for therapy, before a definitive cure is developed. Several issues still need to be clarified, however, including the role of sialic acid in muscle biology and the mechanism by which perturbation of sialic acid levels could lead to muscle atrophy, weakness, and degeneration.

Based on the concept of reduced sialylation in DMRV/hIBM, Sparks *et al.* tried *in vivo* sialic acid administration in human patients [Sparks *et al.* 2007]. This was an open-label study on the efficacy of intravenous immunoglobulin on four patients with DMRV/hIBM. The basis for this study was that 1 g immunoglobulin contains 8 μmol sialic acid and thus could be used to deliver sialic acid into patients' cells. After a few doses of intravenous immunoglobulin, patients subjectively experienced a mild improvement in muscle strength. However, for a robust effect to be seen, a placebo-controlled study may be needed.

Final remarks

Our recent preclinical trial on the effect of sialic acid metabolites has shown promising results for preventing the onset of myopathy in the DMRV/hIBM mouse model. We have shown that these sialic acid metabolites were equally effective and well tolerated, thus may be considered for therapeutic trial in DMRV/hIBM patients. It is important to point out, however, that the study design involved the treatment of mice before they developed obvious myopathic symptoms. This prophylactic treatment and its study design may, therefore, need careful interpretation, as we usually do not encounter pre-symptomatic patients. Notwithstanding the complex issues surrounding therapeutic trials in humans, we hope that the next step should include a careful evaluation of these metabolites in preparation for a formal clinical trial. Even though our results on sialic acid administration to DMRV mice are encouraging, further steps are needed to define precisely the metabolism and incorporation of ManNAc and sialic acid. In addition, the application of the present strategy to DMRV mice at different stages of the disease may benefit translation into clinical trial in the future. Another important issue is the fact that DMRV/hIBM is a rare disease. For a clinical trial to be set in motion there is clearly a need for international collaboration among clinicians and scientists working on this disabling myopathy.

Acknowledgments

This study is supported partly by: the Research on Psychiatric and Neurological Diseases and Mental Health from the Japanese Health Sciences Foundation; the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO); the Research Grant (19A–7) for Nervous and Mental Disorders from the Ministry of Health Labor and Welfare; the Kato Memorial Trust for Nambyo Research; and the Neuromuscular Disease Foundation.

Conflicts of interest statement

The authors have declared that there is no conflict of interest.

References

Amsili, S., Shlomai, Z., Levitzki, R., Krause, S., Lochmuller, H., Ben-Bassat, H. *et al.* (2007) Characterization of hereditary inclusion body myopathy myoblasts: possible primary impairment of apoptotic events. *Cell Death Differ* 14: 1916–1924.

Argov, Z. and Yarom, R. (1984) 'Rimmed vacuole myopathy' sparing the quadriceps. A unique disorder in Iranian Jews. *J Neurol Sci* 64: 33–43.

Bardor, M., Nguyen, D.H., Diaz, S. and Varki, A. (2005) Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells. *J Biol Chem* 280: 4228–4237.

Carlson, S.E. and House, S.G. (1986) Oral and intraperitoneal administration of *N*-acetylneuraminic acid: effect in rat cerebral and cerebellar *N*-acetylneuraminic acid. *J Nutr* 116: 881–886.

Eisenberg, I., Avidan, N., Potikha, T., Hochner, H., Chen, M., Olender, T. *et al.* (2001) The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy. *Nat Genet* 29: 83–87.

Gagiannis, D., Gossrau, R., Reutter, W., Zimmermann-Kordmann, M. and Horstkorte, R. (2007) Engineering the sialic acid in organs of mice using N-propanoylmannosamine. *Biochim Biophys Acta* 1770: 297–306.

Galeano, B., Klootwijk, R., Manoli, I., Sun, M., Ciccone, C., Darvish, D. *et al.* (2007) Mutation in the key enzyme of sialic acid biosynthesis causes severe glomerular proteinuria and is rescued by *N*-acetylmannosamine. *J Clin Invest* 117: 1585–1594.

Hinderlich, S., Berger, M., Keppler, O.T., Pawlita, M. and Reutter, W. (2001) Biosynthesis of N-acetylneuraminic acid in cells lacking UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase. *Biol Chem* 382: 291–297.

Hinderlich, S., Salama, I., Eisenberg, I., Potikha, T., Mantey, L.R., Yarema, K.J. *et al.* (2004) The homozygous M712T mutation of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase results in reduced enzyme activities but not in altered overall cellular sialylation in hereditary inclusion body myopathy. *FEBS Lett* 566: 105–109.

Hirschberg, C.B., Goodman, S.R. and Green, C. (1976) Sialic acid uptake by fibroblasts. *Biochemistry* 15: 3591–3599.

Hong, Y. and Stanley, P. (2003) Lec3 Chinese hamster ovary mutants lack UDP-N-acetylglucosamine 2-epimerase activity because of mutations in the epimerase domain of the Gne gene. *J Biol Chem* 278: 53045–53054.

Keppler, O.T., Hinderlich, S., Langner, J., Schwartz-Albiez, R., Reutter, W. and Pawlita, M. (1999) UDP-GlcNAc 2-epimerase: a regulator of cell surface sialylation. *Science* 284: 1372–1376.

Malicdan, M.C., Noguchi, S., Hayashi, Y.K., Nonaka, I. and Nishino, I. (2009) Prophylactic treatment with sialic acid metabolites preclude

the development of a myopathic phenotype in the DMRV-hIBM mouse model. *Nat Med* 15: 690–695.

Malicdan, M.C., Noguchi, S., Hayashi, Y.K. and Nishino, I. (2008) Muscle weakness correlates with muscle atrophy and precedes the development of inclusion body or rimmed vacuoles in the mouse model of DMRV/hIBM. *Physiol Genomics* 17: 106–115.

Malicdan, M.C., Noguchi, S. and Nishino, I. (2007a) Autophagy in a mouse model of distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy. *Autophagy* 3: 396–398.

Malicdan, M.C., Noguchi, S., Nonaka, I., Hayashi, Y.K. and Nishino, I. (2007b) A Gne knockout mouse expressing human GNE D176V mutation develops features similar to distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy. *Hum Mol Genet* 16: 2669–2682.

Nishino, I., Malicdan, M.C., Murayama, K., Nonaka, I., Hayashi, Y.K. and Noguchi, S. (2005) Molecular pathomechanism of distal myopathy with rimmed vacuoles. *Acta Myol* 24: 80–83.

Nishino, I., Noguchi, S., Murayama, K., Driss, A., Sugie, K., Oya, Y. *et al.* (2002) Distal myopathy with rimmed vacuoles is allelic to hereditary inclusion body myopathy. *Neurology* 59: 1689–1693.

Noguchi, S., Keira, Y., Murayama, K., Ogawa, M., Fujita, M., Kawahara, G. *et al.* (2004) Reduction of UDP-N-acetylglucosamine 2-epimerase/ N-acetylmannosamine kinase activity and sialylation in distal myopathy with rimmed vacuoles. *J Biol Chem* 279: 11402–11407.

Nonaka, I., Noguchi, S. and Nishino, I. (2005) Distal myopathy with rimmed vacuoles and hereditary inclusion body myopathy. *Curr Neurol Neurosci Rep* 5: 61–65.

Nöule, U. and Schauer, R. (1981) Uptake, metabolism and excretion of orally and intravenously administered, 14C- and 3H-labeled N-acetylneuraminic acid mixture in the mouse and rat. *Hoppe Seylers Z Physiol Chem* 362: 1495–1506.

Oetke, C., Hinderlich, S., Brossmer, R., Reutter, W., Pawlita, M. and Keppler, O.T. (2001) Evidence for efficient uptake and incorporation of sialic acid by eukaryotic cells. *Eur J Biochem* 268: 4553–4561.

Ritskes-Hoitinga, M. (2004) Nutrition of laboratory mice. In: Hedrich, H. and Bullock, G. (eds). *The laboratory mouse*. Elsevier Academic Press: London.

Salama, I., Hinderlich, S., Shlomai, Z., Eisenberg, I., Krause, S., Yarema, K. *et al.* (2005) No overall hypo-sialylation in hereditary inclusion body myopathy myoblasts carrying the homozygous M712T GNE mutation. *Biochem Biophys Res Commun* 328: 221–226.

Schwartz, E.L., Hadfield, A.F., Brown, A.E. and Sartorelli, A.C. (1983) Modification of sialic acid metabolism of murine erythroleukemia cells by analogs of N-acetylmannosamine. *Biochem Biophys Acta* 762: 489–497.

Schwarzkopf, M., Knobeloch, K.P., Rohde, E., Hinderlich, S., Wiechens, N., Lucka, L. *et al.* (2002) Sialylation is essential for early development in mice. *Proc Natl Acad Sci U S A* 99: 5267–5270.

Sparks, S., Rakocevic, G., Joe, G., Manoli, I., Shrader, J., Harris-Love, M. *et al.* (2007) Intravenous immune globulin in hereditary inclusion body myopathy: a pilot study. *BMC Neurol* 7: 3.

Yamaguchi, S., Ohnishi, J., Maru, I. and Ohta, Y. (2006) Simple and large-scale production of N-acetylneuraminic acid and N-acetyl-D-mannosamine. *Trends Glycosci Glycotechnol* 18: 245–252.

Wang, Z., Sun, Z., Li, A.V. and Yarema, K.J. (2006) Roles for GNE outside of sialic acid biosynthesis: modulation of sialyltransferase and BiP expression, GM3 and GD3 biosynthesis, proliferation and apoptosis, and ERK1/2 phosphorylation. *J Biol Chem* 281: 27016–27028.