Towards a molecular therapy for glycogen storage disease type II (Pompe disease)

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Glycogen storage disease type II (GSD-II), also known as Pompe disease, is a fatal genetic muscle disorder caused by a deficiency of acid α-glucosidase, a glycogen-degrading lysosomal enzyme. Currently, there is no treatment for this fatal disorder. However, several lines of research suggest the possibility of future treatment. Enzyme replacement strategies hold the greatest hope for patients currently affected by GSD-II, but future strategies could include *in vivo* or *ex vivo* gene therapy approaches and/or mesenchymal stem cell or bone-marrow transplantation approaches. Each of the approaches might eventually be combined to further improve the overall clinical efficacy of any one treatment regimen. The lessons learned from GSD-II research will also benefit a great number of individuals affected by other genetic disorders.
unsuccessful, owing to an inadequate understanding of the route that interventions have not been successful. However, to date, these interventions have not been successful in GSD-II patients in an attempt to provide GAA via secretion from donor cells. However, to date, these interventions have not been successful because glycogen accumulation in the GSD-II patient is primarily located within lysosomes, whereas these drugs work in the cytosol. A high-protein diet has also been tested, based on previous evidence that increased muscle catabolism occurs in GSD-II (Ref. 7).

Recent therapeutic approaches
Two approaches to the treatment of GSD-II are currently being pursued: enzyme replacement and gene therapy. These are described below.

Enzyme replacement therapy for GSD-II
General considerations
The discovery of cell-surface receptors that can mediate the delivery of lysosomal enzymes to target tissues renews the possibility of enzyme replacement for the treatment of lysosomal storage diseases. We will start by reviewing the current understanding of lysosomal enzyme trafficking and receptor-mediated endocytosis.

Lysosomal enzymes are synthesized in the rough endoplasmic reticulum, where a preformed oligosaccharide is transferred from the endoplasmic reticulum to the Golgi network where it acquires the mannose 6-phosphate (M-6-P) marker by the sequential action of N-acetylglucosaminylphosphotransferase (GnPT) and N-acetylgalactosamine-1-phosphotransferase. N-acetylgalactosamine-1-phosphotransferase, an uncovering enzyme (UCE) (pathway 2). The enzyme containing M-6-P residues then binds to M-6-P receptors and is transported to the lysosome. Alternatively, it undergoes trimming of mannose, addition of sugars and formation of a complex oligosaccharide and is secreted (pathway 1).

![Figure 1. Trafficking of lysosomal enzymes. The newly synthesized α-glucosidase contains a high-mannose oligosaccharide, which is transferred to the Golgi network where it acquires the mannose 6-phosphate (M-6-P) marker by the sequential action of N-acetylglucosaminylphosphotransferase (GnPT) and N-acetylgalactosamine-1-phosphotransferase, an uncovering enzyme (UCE) (pathway 2). The enzyme containing M-6-P residues then binds to M-6-P receptors.](image-url)
condition resulting from a lack of effective phosphorylation of mannose residues. Furthermore, the plasma membrane is known to contain carbohydrate receptors other than the M-6-P receptor such as the asialoglycoprotein receptor (which can internalize glycoproteins containing galactose or N-acetylgalactosamine) and mannose receptors (which bind mannose-containing glycoproteins). The mannose receptor (present in macrophages) is believed to be significantly involved in the uptake of mannose-terminated β-glucocerebrosidase by the macrophages of Gaucher disease patients. These observations suggest that, in lysosomal storage diseases, the compatibility between the carbohydrate chains of the administered enzyme and the type of receptors expressed on the plasma membrane of the target cells is critical for therapeutic success. Along these lines of reasoning, an effective therapy is now available for Gaucher disease and Niemann–Pick disease.

The feasibility of using receptor-mediated endocytosis of GAA for the replacement therapy of GSD-II was first demonstrated in pre-clinical studies. When normal mice were treated with GAA containing M-6-P (purified from bovine testis), the GAA was taken up by the heart and skeletal muscles. Uptake of GAA was less evident with placenta-derived GAA, a form of the enzyme that lacks the M-6-P residues. Although these studies demonstrated the feasibility of using appropriately glycosylated GAA to exploit receptor-mediated uptake into target tissues, it is clear that interspecies antigenicity of bovine GAA precludes the use of this source of enzyme in human therapy. A nonhuman, human form of enzyme is required.

Recombinant enzyme therapy

Recombinant human α-glucosidase (rhGAA) has recently been produced in its precursor form in Chinese hamster ovary (CHO) cell lines and in transgenic mouse and rabbit milk. Incubation of the precursor form of rhGAA with primary fibroblasts derived from patients with infantile-onset GSD-II resulted in the uptake of the enzyme and normalization of GAA activity and glycogen levels in the fibroblasts. This uptake is inhibited by M-6-P, suggesting mediation of uptake by M-6-P receptors. Injection of purified rhGAA into guinea pigs resulted in increased GAA activity levels in liver, heart, and skeletal muscle. To study in vivo efficacy, however, an appropriate animal model of GSD-II was required. Naturally occurring GAA deficiency has been reported in Shorthorn cattle, Brahman cattle and a strain of Japanese quail. Recently, mouse models generated by targeted gene disruption have also become available. The Japanese quail with acid-maltase deficiency (referred to as the AMD quail) has been shown to correct GAA deficiency and reduce glycogen storage in heart and skeletal muscle of GSD-II knockout mice. The observation that rhGAA improves muscle strength (in the quail model) and histopathology and biochemical parameters (in both quail and mouse models) suggests that rhGAA is a promising enzyme replacement therapy for human GSD-II. Based on these results, two clinical trials using different enzyme sources have recently been initiated to investigate the potential of rhGAA to safely treat GSD-II patients. A Phase II study is being conducted in the Netherlands (Sophia’s Children Hospital, Rotterdam) using GAA purified from the milk of transgenic rabbits, and a Phase III study using rhGAA purified from CHO cells is being conducted in the USA (Duke University Medical Center, Durham, NC).

Although rhGAA could offer a promising therapy for GSD-II, two potential problems can be foreseen. First, will the correction of the...
The specific cause of GSD-II is mutations within the GAA gene that result in either lack of adequate transcription of the gene, or the expression and translation of a significantly altered GAA protein. Lack of adequate GAA activity results in the abnormal accumulation of glycogen, and its detrimental effects in muscle cells. The ultimate cure for GSD-II would be to correct the responsible mutations in all of the cells of an affected individual. However, this form of genetic therapy is currently unachievable, and alternative modes of gene correction are being investigated not only for GSD-II specifically, but also for genetic diseases in general. Recently, it has been demonstrated that utilization of RNA–DNA chimeric oligonucleotides can specifically target and repair (via DNA mismatch repair mechanisms) specific one or two base pair alterations in the genomes of mammalian cells. The most dramatic results have been achieved in hepatocytes (both in vitro and in vivo), although confirmation of the results by other laboratories at this time is still lacking. Direct genetic correction of DNA mutations causing GSD-II has not been investigated. It is also not clear whether the primary target cells for such an intervention in GSD-II patients (skeletal and cardiac muscle cells) are amenable to the mismatch repair mechanisms noted in hepatocytes.

**Gene therapy for GSD-II**

**General considerations**

The immune response is more likely to develop in the infantile patients as many of them carry a null mutation. In contrast, missense mutations resulting in residual enzyme activity in juvenile and adult patients might render these late-onset patients more immunologically tolerant.

**Gene therapy and ‘augmenting’ GAA activity**

As the direct genetic correction of mutant DNA is still in its infancy, the next level of genetic therapy would be the addition of normal GAA encoding sequences into cells that are deficient for GAA activity. This ‘augmentative’ form of gene therapy holds the most pragmatic view at present for potential genetic therapies of GSD-II. In order to ‘add back’ the genetic information encoding GAA to cells that lack GAA activity, a vector is required to deliver the necessary information.

**Ex vivo approaches**

A number of intriguing studies have been performed to investigate several potential modalities for the gene therapy of GSD-II. In one example, a viral vector might be used to transfer the GAA gene into fibroblasts and myoblasts isolated directly from individuals affected by GSD-II. It is hoped that, in this form of gene therapy, myoblasts from GSD-II patients could be infected with the vector (transduced) ex vivo, then implanted back into the muscles of the affected individuals. The corrected myoblasts would then either directly fuse with neighboring cells (supplying them with GAA enzyme) and/or secrete the enzyme for uptake by other cells not in direct contact with the genetically corrected cells.

Zaretsky and colleagues demonstrated that myoblasts transduced with a GAA-encoding retrovirus were capable of expressing large amounts of GAA, 30-fold higher than the levels found in myoblasts from unaffected individuals. Furthermore, the retrovirally encoded GAA protein was capable of targeting lysosomes and reducing the amount of accumulated glycogen in treated cells. The retrovirally transduced cells were also capable of secreting the GAA enzyme, allowing cross-correction of non-transduced GSD-II myoblasts in tissue culture systems. Nicolino and colleagues further demonstrated that a first-generation adenovirus (Ad) vector was also capable of transducing the GAA gene into fibroblasts and muscle cells isolated from affected patients. The adenovirus-transduced cells were phenotypically corrected, and were also capable of secreting the recombinant GAA enzyme. However, further studies addressing the efficacy of ex vivo myoblast therapy in animal models of GSD-II have yet to be demonstrated.

**Muscle symptoms of GSD-II (over time) unmask neurologic symptoms in treated patients**

This possibility exists because intravenously administered rhGAA is unlikely to cross the blood–brain barrier, and excessive glycogen is known to be stored in the nervous tissues, particularly in infantile patients. Second, will antibodies develop against the rhGAA and interfere with the effectiveness of the therapy? In muscular patients, rhGAA administered was capable of decreasing the glycogen deposits, and improving muscle symptoms. The immune response is more likely to develop in the infantile patients as many of them carry a null mutation. In contrast, missense mutations resulting in residual enzyme activity in juvenile and adult patients might render these late-onset patients more immunologically tolerant.

**Glossary**

**Dyspnea** – Shortness of breath.

**Gaucher disease** – A lysosomal storage disease resulting from a deficiency of glucocerebrosidase, an enzyme that breakdowns cellular glycolipids.

**Hepatomegaly** – Enlargement of liver.

**Hypertrophic cardiomyopathy** – Enlargement of heart due to increased thickness of the heart wall.

**Macroglossia** – Enlargement of tongue.

**Mannose 6-phosphate receptor** – A cell receptor that recognizes molecules containing mannose 6-phosphate.

**Orthopnea** – Discomfort of breathing that is relieved by reverting to a sitting or standing position.

**Reviews**

50 (b) and (e) sections stained with periodic acid–Schiff (PAS) from normal (a, d and g), AMD control treated (h) and (i) and AMD high-dose rhGAA treated (c, f and i) quad. Scale bar = 50 μm. Reproduced with permission from Ref. 25.
Both studies highlight the potential of \textit{ex vivo} gene therapy for GSD-II, but other studies have also demonstrated the known limitations of myoblast transfer in patients affected by Duchenne muscular dystrophy. These limitations include: (1) the inherent difficulties of growing large quantities of cultured myoblasts; (2) the decreased ability of cultured human myoblasts to fuse after implantation in vivo; (3) the uncertainty over enzyme production from successfully implanted myoblasts would be capable of cross-correcting all of the muscles in an affected individual. Clinical studies of myoblast transfer in patients affected by Duchenne muscular dystrophy demonstrated that \textit{in vivo} fusion of previously cultured human myoblasts can occur, but at a very low efficiency\cite{16}. \textit{Ex vivo} genetic correction of affected myoblasts is a promising approach, but further studies will be required before myoblast transfer achieves clinical reality.

\textbf{In vivo approaches} An optimum gene-transfer vector would be capable of transducing the GAA gene into all of the affected cells of a patient after a simple direct administration, as well as allowing for long-term expression of the GAA protein either to prevent worsening of disease, or to effect a cure. At present, no one vector can achieve all of these lofty goals; however, several classes of vector have shown promise. DNA transfer mediated by non-viral vectors \textit{in vivo} is generally an inefficient process, although skeletal muscles appear to be capable of uptake and expression of DNA after a simple intramuscular injection\cite{17}. Unfortunately, gene expression is limited only to those muscle fibers close to the injection site. It has yet to be demonstrated whether direct DNA injection of the GAA gene would result in adequate expression (and secretion) of the GAA protein to allow for cross-correction of non-injected muscle groups.

Direct intramuscular injection of viral vectors encoding GAA has also been investigated. Pauli and colleagues demonstrated that direct intramuscular injection of a first-generation adenoviral vector (encoding human GAA) into the cardiac or hind-limb muscles of normal neonatal rats resulted in over-expression of GAA activity in each of the injected tissues\cite{18}. However, systemic secretion of GAA was not demonstrated. Tsujino et al. confirmed that adenoviral vectors were also capable of transducing the human GAA gene after direct injection into the pectoralis muscles of the AMD qual model of GSD-II (Ref. \cite{19}). Importantly, the latter studies confirmed that the \textit{in vivo} delivery of the GAA gene could result in adequate enzyme expression, as well as reduce glycogen accumulation in the treated muscle groups\cite{18}. However, cross-correction of other muscle groups was not achieved in either study. These important investigations demonstrated the potential of \textit{in vivo} approaches, but also highlighted their limitations, especially the inability to correct a significant number of muscle cells beyond the vicinity of the local injection site. Surgically invasive techniques such as the vascular isolation of a limb, followed by the re-circulation of a vector-containing solution, allow for more dispersed transduction of muscle cells by viral vectors\cite{19}. The latter techniques are highly experimental, but with further investigation might allow an increased number of muscle cells to be transduced with GAA-encoding vectors. Furthermore, the targeting of specific muscle groups (for example the intra-cranial delivery of a vector for cardiac transduction) might improve the function of the muscles primarily causing morbidity in some forms of GSD-II (Ref. \cite{19}).

\textbf{Hepatic gene therapy for a systemic muscle disorder} In light of the previously discussed limitations, our group is developing alternative strategies for the genetic therapy of a systemic muscle disorder such as GSD-II. The conceptualization of one such approach is outlined in Fig. 4. This approach capitalizes on the known propensity of adenoviral vectors to efficiently transduce hepatocytes after a simple intravenous administration. The limitations include: (1) the need to neutralize vectors in vivo to prevent infection of nontarget tissues; (2) the decreased ability of cultured human hepatocytes to fuse after implantation in vivo; (3) the uncertainty over enzyme production from successfully implanted hepatocytes would be capable of cross-correcting all of the muscles in an affected individual. Once secreted, the enzyme could be systemically distributed, resulting in the simultaneous cross-correction of major muscle groups, without having to directly inject the gene transfer vector into each of the respective muscles. We recently confirmed that intravenous administration of a new class of modified adenoviral vector allowed for extremely efficient transduction of the hepatocytes of mice, including GAA-knockout (GAA-KO) mice\cite{20}. Specifically, the livers of adenovirally treated GAA-KO mice expressed nearly 100-fold more GAA enzyme than is normally detected in the tissues of wild-type mice. Furthermore, the overexpression resulted in secretion of the precursor form of the enzyme into the bloodstream of these mice, which was then subsequently taken up and targeted to the lysosomes of multiple muscle groups. Within 12 days of a single injection, the heart, diaphragm, quadriceps and gastrocnemius (calf) muscles were all essentially devoid of their pathologic glycogen accumulations, as compared with untreated, age-matched control animals\cite{20}. Recently, other studies in animal models of Fabry disease have also confirmed that lysosomal storage disorders (other than GSD-II) can also benefit from adenovirus-mediated transduction of the liver, with subsequent secretion and targeting of lysosomal enzymes to other tissues\cite{21}.

\textbf{Future directions} At present, enzyme replacement strategies hold the greatest hope for patients currently affected by GSD-II, but future strategies might include \textit{ex vivo} or \textit{in vivo} gene therapy, or mesenchymal-stem-cell bone-marrow transplantation approaches. Currently, there are two ongoing clinical trials of rhGAA replacement therapy in GSD-II (see above). No efficacy data are yet available, but the pre-clinical data suggest that enzyme replacement therapy will be successful. However, there remain challenges, particularly for the infantile patients. Extensive muscle damage at the time of diagnosis might be beyond repair, and rapid progression of the disease (patients die, on average, three months after diagnosis) might not allow sufficient time for muscle regeneration. A successful treatment might unmask the underlying neurologic problem. Furthermore, immune responses could limit the efficacy of the treatments. These potential problems might also be encountered with gene therapy approaches.

For gene therapy, the liver secretion of lysosomal enzymes such as the GAA protein for GSD-II offers much potential for the therapy of GSD-II disease specifically, and a number of other lysosomal disorders in general. Critical questions that have yet to be addressed include whether or not removal of glycogen actually results in improved muscle function in the treated animals, and how long the therapeutic effects can persist. The latter point is a complex matter that depends not only on duration of expression and secretion of the GAA gene from the liver, but also on the lysosomal half-life of GAA in muscle cells, and the kinetics of re-accumulation of glycogen in muscle cells. In addition, immune responses (specifically, neutralizing antibody to the secreted GAA, and/or cytotoxic T-Cell mediated elimination of adenovirus-infected hepatocytes) might limit the overall efficacy of this approach. The use of modified vectors and/or other gene-transfer vectors (adenovirus-based vectors) might avoid these problems.
The usefulness of alternative vectors will, however, be dictated by whether or not the vector can transduce enough cells, or secrete enough GAA protein, to allow for the systemic cross-correction of GSD-II.

Bone-marrow transplantation has not been successful. However, recent data suggested that bone-marrow cells can participate in the repair process of muscle39,40, and donor cells have been found to be incorporated into multi-(muscular dystrophy, X-linked) mouse muscles following enzymatically active GAA. (f) Secretion of precursor hGAA from hepatocyte. (g) Systemic distribution of precursor hGAA via the bloodstream.

The outstanding questions

● What mechanisms are involved in muscle targeting of hGAA enzyme by either enzyme replacement therapy or hepatic gene therapy? Is the mannose 6-phosphate receptor primarily involved, or do other carbohydrate receptors and/or other mechanisms play a significant role?

● Can existing muscular damage be cured, or will current approaches only prevent further damage? How much muscle regeneration can be expected in the various forms of GSD-II?

● Will the correction of the muscular symptoms of GSD-II reveal underlying neurological involvement in treated patients?

● Will immune responses interfere with the effectiveness of the therapies?

● Is the human liver as amenable to viral-mediated gene transfer as some animal species’ livers appear to be?

References


The diagram illustrates the important steps required to achieve systemic distribution and uptake of hepatically produced GAA enzyme in hepatocytes or muscle cells. (a) Adenovirus entry into the hepatocyte after intravenous administration. (b) Human GAA mRNA transcription/translation. (c) Binding of precursor GAA by mannose-6-phosphate receptor. (d) Receptor mediated targeting of GAA to lysosomal compartment. (e) Cleavage of precursor form of GAA upon entry into lysosomes, generating enzymatically active GAA. (f) Secretion of precursor hGAA from hepatocyte. (g) Systemic distribution of precursor hGAA via the bloodstream.
26 Blattner, R.G. et al. (1999) Restoration of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. Am. J. Physiol. 279, 251-259