Agalsidase alfa (Replagal™) in the treatment of Anderson-Fabry disease

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Abstract: Anderson-Fabry disease (AFD) is an X-linked storage disorder caused by a deficiency of the lysosomal hydrolase a-galactosidase A (AGAL) and the resultant accumulation of its glycosphingolipid substrate (Gb3) in several tissue types. Major morbidity and reduced life expectancy among affected individuals are a consequence of renal, cardiac and cerebrovascular involvement. Symptomatic males and females with AFD have been described, although the onset of clinical manifestations may be delayed and more variable among the latter patient group, partly attributed to lyonization. Agalsidase alfa (Replagal™) is a recombinant formulation of human AGAL which has been demonstrated to modify the course of AFD in treated patients. Factors that may influence clinical outcomes include disease stage at the point of treatment initiation and antibody formation. There is incomplete understanding of AFD pathophysiology. Early diagnosis and timely intervention may be essential. The use of adjunctive therapies, directed at risk reduction (eg, aspirin for stroke prophylaxis), require careful scrutiny, but such agents are likely to be vital components of a comprehensive approach to patient care. Long-term studies may clarify the optimal dose and frequency of enzyme administration. Meanwhile, budding strategies such as chaperone-mediated enzyme enhancement may offer the potential for an alternative or multimodality approach to the management of AFD.

Keywords: adjunctive therapy, agalsidase alfa, agalsidase beta, enzyme replacement therapy

Background

Anderson-Fabry disease (AFD) is a multi-systemic disorder, associated with major renal, cardiac and cerebrovascular complications; consequent to the progressive deposition of an incompletely metabolized substrate (Gb3) in multiple cell types, and attendant mechanisms of tissue injury that remain to be more fully defined (Clarke 2007). Alterations in vascular reactivity and a propensity for thrombo-embolic disease are believed to play a role in the increased risk for particular problems, such as stroke (Moore et al 2007). Renal and cardiac failure represent major sources of morbidity, and likely account for the reduced survival among affected males and females (wherein median age of death is 50–57 and 70–72 years, respectively) (MacDermot et al 2001a, 2001b). In conjunction, pain crises, acroparesthesia, hearing loss and gastrointestinal problems lead to significant reduction in patients' health-related quality of life (Gold et al 2002).

The series of events that have led to development of treatment directed at the underlying biochemical basis of AFD include: (1) elucidation of the causal enzyme deficiency (a-galactosidase A (AGAL)); (2) characterization of the relevant receptor-mediated endocytic pathway (mannose-6-phosphate [M6P] receptor) and thus, enabling intracellular uptake of the intravenously administered therapeutic protein; and (3) over-expression of the recombinant form of AGAL in a mammalian cell line with the requisite glycosylation pattern for delivery to the appropriate cell types (see Pastores 2003).

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The incidence of AFD is about 0.85 per 100,000, although recent studies suggest that the condition may be missed, particularly among patient populations at high risk (eg, patients with ESRD on dialysis, and young individuals with cyrptogeneic stroke) (Ichinose et al 2005; Rolfs et al 2005; Tanaka et al 2005).

As an "orphan disorder" the development of treatment for AFD qualified for fast-track approval, with several incentives provided to the manufacturers to stimulate industrial drug development (Pastores 2003). The current review examines the clinical experience with agalsidase alfa which supports the safety and efficacy of enzyme therapy for AFD.

Agalsidase alfa and special considerations

The recombinant enzyme agalsidase alfa (Replagal™, Shire Human Genetic Therapies (HGT), Inc., Cambridge, MA, USA) is produced in a cultured human cell line following the activation of the relevant encoding gene. It is currently approved for the treatment of AFD in 38 countries, but not in the United States.

Attributes

Shire HGT's manufacturing process ensures an appropriate and species-specific post-translational modification of the primary amino-acid sequence, a factor that is critical in the targeted delivery of the therapeutic enzyme to the cellular sites of substrate storage (van de Weert et al 2005). Furthermore, intrinsic glycosylation and other differences between the recombinant formulation and the native (endogenous, non-mutant) enzyme may be a factor in the development of antibodies against the intravenously administered protein (Baker and Jones 2007).

Pharmacokinetics and pharmacodynamics

There is limited information on tissue distribution and enzyme half-life following the administration of agalsidase alfa to humans. Less than 1% of the maximal plasma concentration of agalsidase alfa is detectable 8 hours after infusion (data on file, Shire HGT), and the intracellular half-life of the enzyme is estimated to be 24–48 hours (Schiffmann et al 2000).

Recently, detailed information was published on the relative tissue and cellular distribution of agalsidase alfa in a mouse knockout model of AFD (Murray et al 2007). Specific immunostaining for the recombinant enzyme was found in the liver, kidney, heart, testes, adrenal gland, spleen and bone marrow. The patterns of cellular uptake were varied; from enzyme presence in parenchymal as well as endothelial cells in the liver and the adrenal glands, to selective uptake into certain cell types such as in the kidney and spleen, to the heart and bone marrow where immunostaining was observed only in the vascular endothelial cells. AGAL-specific activity in tissue homogenates matched the relative extent of tissue distribution.

The mechanism of the differential uptake of agalsidase alfa in the different organs of the mouse model, as in humans, is likely complex, and only partly explained by the distribution and density of M6P receptors. Furthermore, tissue distribution in these experiments did not did not correlate directly with the relative organ blood flows or the presence or absence of fenestrated capillaries in various organs (Murray et al 2007). However, it should be noted that these findings resulted from a single intravenous injection of the enzyme and that repeated administration, as occurs in the clinical setting, may lead to a different profile. Moreover, the mouse model (while exhibiting the biochemical features of AFD) does not follow the characteristic course of disease seen in human patients. This has limited its utility in establishing therapeutic guidelines, with studies in the mouse having found their greatest utility in establishing pre-clinical "proof of concept" and the effects of enzyme therapy on substrate deposits.

The issue of targeted enzyme delivery to cellular sites of pathology is an important one. Currently, there are two enzyme formulations given to patients with AFD (Pastores and Thadhani 2002). As experiments in the mouse model for the two recombinant enzymes were conducted separately, it is not certain whether the use of an identical treatment protocol would reveal any differences in tissue uptake between the two drugs that may be relevant clinically. Anyhow, these investigations appear to have established a dose-response relationship; ie, tissue clearance is greater when using a higher dose of enzyme. However, as the correlation between tissue substrate accumulation and organ dysfunction is uncertain, the advantages of using a higher treatment dose remains to be determined. Whatever the case may be enzyme therapy regimens must be chosen judiciously and ultimately decisions regarding treatment should based on clinical outcome.

Should delivery to certain compartments be a limiting factor, one approach that has been suggested as a means to enhance tissue distribution of AGAL might be to add a protein transduction domain such as the 11-amino acid TAT peptide, as has been done with ß-galactosidase (a lysosomal enzyme deficient in GM1-gangliosidosis) (Cai et al 2006).

The pharmacokinetics of agalsidase alfa in humans has been examined in a study involving 18 adult male AFD patients

naïve to enzyme replacement therapy; randomized to one of five regimens: 0.1, 0.2, or 0.4 mg/kg weekly, and 0.2 or 0.4 mg/kg every other week (Clarke et al 2007). The observed mean half-life was 56–76 minutes, and the mean volume of distribution at steady state was 17%–18% of body weight. No significant association was noted between dose and half-life, clearance, or volume of distribution at steady state. The AUC was linearly proportional to dose from 0.1–0.4 mg/kg. Plasma Gb3 (average at baseline 9.12 ± 2.61 nmol/mL) declined by 50% after 10 weeks of treatment, with no statistically significant differences between enzyme therapy regimens. It is interesting that the circulating half-life of the recombinant enzyme is significantly longer than that for the therapeutic protein (imiglucerase) administered to patients with Gaucher disease. Whether the presence of a neutral pH in plasma and the potential for processing of the exogeneous enzyme during its transit to target organs may adversely affect the concentration of functional enzyme in the various tissues is not known.

As nearly all classically affected males with AFD experience kidney dysfunction, the pharmacokinetics (PK) of agalsidase alfa was also examined in patient with progression to end-stage renal disease (ESRD). Twenty-two AFD patients (20 men and 2 women) receiving dialysis or who had a history of kidney transplantation were treated with agalsidase alfa in an open label setting using the same dosing regimen given to patients without ESRD (0.2 mg/kg every other week) (Pastores et al 2007). A typical biphasic plasma elimination profile was seen in both dialysis and transplant patients, similar to that observed in 18 non-ESRD patients with AFD. Calculated PK parameters were similar among these three patient groups. In the male patients, plasma Gb3 level declined by 43% after 6 months ($p < 0.001$).

The proportion of infused enzyme delivered to the various organs, the fraction that remains active within these different compartments, and the length of activity are unknown. It is possible that there are factors in plasma that may adversely affect the circulating enzyme whose optimal activity is within the acidified environment of the lysosome. The latter may include antibodies which may facilitate the rapid clearance of the exogeneous enzyme and\or neutralize its function. Additionally, the formation of antibody-antigen complexes may result in altered targeting to other cells such as phagocytes. Non-specific enzyme uptake by cells without evident Gb3 storage is unlikely to reduce systemic substrate burden.

Disease-modifying effects

Several studies, including a placebo-controlled clinical trial and the analyses of data derived from an observational survey (FOS), have demonstrated the beneficial effects of agalsidase alfa. (FOS stands for Fabry Outcome Survey, a collaborative effort established to pool data (initially from European clinics) on the natural history of AFD and the long-term efficacy and safety of treatment (Mehta et al 2004).)

Various reports have noted small reductions in the incidence and severity of neuropathic pain and gastrointestinal symptoms, and improvements in the perception threshold for warm and cold sensation (Altarescu et al 2001; Schiffmann et al 2001). Additionally, increased sweating and slowing of the progression of renal disease in adult male AFD patients have also been documented (Schiffmann et al 2003). Furthermore, treatment has also been shown to lead to a reduction in left ventricular mass; although it is not certain whether this finding translates to a reduction in the incidence of cardiac-related events (eg, arrhythmias, myocardial infarction) (Hughes et al 2007). There is uncertainty about whether enzyme therapy results in a reduction in the incidence of stroke. During a 4- to 4.5-year period of follow-up for the original NIH cohort which enrolled 26 hemizygous males, four patients suffered a cerebrovascular accident or a transient ischemic attack, one patient suffered a myocardial infarction and required a coronary bypass, and another patient underwent a coronary angioplasty (Schiffmann et al 2006). The latter observations underscore the need to incorporate adjunctive measures for secondary and probably primary prevention (Close and Elliott 2007).

All told, these observations have led to the current clinical impression that enzyme therapy for AFD appears to positively influence the patients' health-related quality of life (QoL) and ameliorates the natural course of disease. However, its influence on survival and on the incidence and severity of disabling disease complications (and the associated utilization of resources, such as dialysis, the use of an implantable pacemaker or defibrillator) remains to be established.

It should also be pointed out that the quality of most of the publications has been viewed as less than optimal, primarily because most focused on surrogate endpoints and not on the assessment of clinical endpoints (Lidove et al 2007).

It is likely that enzyme therapy for AFD is most efficacious when administered prior to established disease changes (eg, glomerulosclerosis, myocardial fibrosis). As progressive disease expression is partly influenced by age, there has been interest in examining the effects of treatment in children. Although it is too soon to ascertain whether the use of agalsidase alfa in children eliminates the risk of major organ failure, or delays the inception of renal insufficiency and

other problems, treatment is well tolerated and, in the short term, appears to improve autonomic function, decrease pain and improve pain-related QoL (Ries et al 2006; Ramaswami et al 2007).

Enzyme therapy regimens

Symptomatic patients with AFD are conventionally treated with 0.2 mg/kg of agalsidase alfa q 2 weeks. The current regimen that is used for most AFD patients may have been chosen, as the bi-monthly infusion schedule had been shown to be adequate for another glycosphingolipid (GSL) disorder (Gaucher disease) and is deemed to be a convenient schedule by most. Almost all of the current outcome data on agalsidase alfa is from patients treated at 0.2 mg/kg every other week (EOW). On this regimen, patients with stage I or II chronic kidney disease (CKD) appear to show stabilization of their glomerular filtration rate (GFR); whereas patients with stage III CKD (30–59 mL/min/1.73 m^2) demonstrate a slower rate of decline in renal function (about 5.2 mL/min/1.73 m^2/year), when compared with a historical control of untreated AFD male patients (12 mL/min/1.73 m^2) (Schiffmann et al 2006). Thus, treatment benefit appeared to occur mostly in patients with a relatively preserved baseline glomerular filtration rate (GFR) (>60 mL/min/1.73 m^2).

In a study of adult male AFD patients (N = 11) on 2–4 years of this regimen and who demonstrated a continuing decline in renal function (specifically, of eGFR of >5 mL/min per 1.73 m^2/year), an improved slope of decline was noted with weekly administration on the same dosage per infusion (Schiffmann et al 2007). Before switching to weekly dosing, eGFR was 53.7 ± 6.3 mL/min per 1.73 m^2 (mean ± SEM), and mean rate of change in eGFR was −8.0 ± 0.8 mL/min per 1.73 m^2/year. During the 24-month follow-up period on the weekly dosing, the mean rate of change in eGFR was observed to slow to −3.3 ± 1.4 mL/min/1.73 m^2/year ($p = 0.01$ vs EOW). Essentially only two patients failed to improve their eGFR slope. A multiple regression model confirmed that the weekly infusion regimen was the strongest explanatory variable for the change in eGFR ($p = 0.0008$), with a weaker contribution from the concomitant use of angiotensin converting enzyme inhibitors/angiotensin receptor blockers ($p = 0.02$). No information on kidney histology was available to ascertain the extent of renal structural abnormalities (eg, focal and global glomerular sclerosis, tubular atrophy, interstitial fibrosis) and its "chronicity". Proteinuria is an important risk factor for progression of kidney involvement in a number of disorders, and may be a useful factor to consider in AFD patients. Unfortunately, at the currently approved dose/frequency of administration enzyme therapy does not have a significant impact on urinary protein excretion (Warnock 2007).

To some extent it appears logical to tailor the enzyme therapy regimen to substrate flux (and ensure clearance of pre-existing substrate and the prevention of its re-accumulation), assuming that the Gb3 is the major pathogenic factor. On the other hand, neither the amount nor the type of glomerular Gb3 deposition has been correlated with degree of renal dysfunction, or with functional improvement among treated patients (Warnock 2007). Earlier studies, which were undertaken prior to the introduction of more sophisticated quantitative assessments (as by tandem MS), indicated turnover times for plasma Gb3 of about 4–8 days, with a turnover rate of 1–6 μmol/day (Vance et al 1975). Given the relatively short intracellular half-life of the enzyme, it is possible that prior to the next infusion (when given at 2 week intervals), 'susceptible cells' may be left "unprotected". This hypothesis is in agreement with clinical report given by some patients who note a return of some of their AFD symptoms such as neuropathic pain and the lack of sweating as well as loss of energy or vitality after the first post-infusion week. However, these observations require further study, and the issue of optimal dose\frequency of administration remains to be resolved.

Until lately, there has been no direct assessment (ie, a head-to-head clinical trial) of the two available enzyme formulations. Recently, the results of a comparative trial involving 34 AFD patients treated with either agalsidase alfa or agalsidase beta at an equal dose of 0.2 mg/kg every 2 weeks were published (Vedder et al 2007). No significant difference was found in reduction of left ventricular mass (the primary endpoint) or changes in other disease parameters (ie, glomerular filtration rate, pain, anti-agalsidase antibodies, and plasma and urine Gb3 levels), after 12 and 24 months of treatment with either enzyme formulation. Treatment failure, which appeared to be related to age and severe pre-treatment disease, occurred frequently in both treatment groups. Moreover, further progression of disease could not be prevented after a switch to 1.0 mg/kg of agalsidase beta.

Safety and tolerability concerns

Intravenous agalsidase alfa is reasonably well tolerated, with reported infusion reaction of about 10% and mostly consisting of fever and rigors, of mild to moderate intensity and transient. IgG antibodies have been noted in about 55% of treated patients, but none have developed IgE antibodies and there are no reports of an anaphylactic reaction to the infused enzyme. Reactions were mitigated by reducing the

rate of infusion and/or with the administration of appropriate premedications (eg, antihistamines, corticosteroids). The short infusion time for agalsidase alfa and the limited number and transient nature of adverse events that have been observed in treated patients have prompted consideration of home-based infusions.

Sero-conversion: determinants and clinical implications

The use of recombinant proteins as therapeutics is frequently associated with the generation of antibodies, which may impact clinical efficacy. Many factors influence the immunogenicity of a protein, including structural properties (eg, sequence variation and glycosylation), and dose and length of treatment (Schellekens 2005).

Alongside the factors noted above that may determine a protein's antigenicity, the patient's CRIM (cross-reacting immunologic material)-status is also an important consideration. This issue is particularly relevant among individuals with the "classic" phenotype in whom a deleterious mutation results in the generation of a severely truncated or defective protein that is rapidly cleared. In these cases, particularly among hemizygote males, the recombinant enzyme formulation may be seen a "foreign" protein leading to the induction of antibody formation. As the enzyme is encoded by a gene on the X chromosome (at Xq22), affected males only produce a mutant gene product, whereas carrier (heterozygous) females may produce a normal gene product in various cell types; the extent of which is dependent on the pattern of lyonization. (The latter partly explains the difficulty of identifying female carriers, based on measuring residual enzyme activity in plasma and leukocytes; as the value obtained (in up to 20% of cases) may overlap with that encountered in the general healthy population. The presence of residual enzyme activity among symptomatic females may be the basis for their attenuated phenotype and the greater variability in clinical expression when compared to that seen among affected males. A later disease onset and relatively milder clinical disease course are also found among affected male patients with missense mutations associated with residual enzyme activity.)

In vitro studies have revealed that antibodies that develop in patients on one of the two enzyme formulations cross-react with the other and result in the neutralization of AGAL activity. In a study involving a subgroup of patients (n = 8) with a persistent IgG antibody response, mean urine Gb3 levels were observed to rise, reaching mean baseline (Linthorst et al 2004). Further studies are required to ascertain the long-term implications of antibody formation, although it is reassuring to note that titers have been noted to decline in some patients receiving on-going treatment.

Home infusion

The protracted requirement for regular intravenous infusion of the recombinant enzyme, the costs related to a clinic- or hospital-based administration, and the potential interference with lifestyle and other encumbrance on the patient have prompted consideration of home-based therapy. Fortunately, the safety profile of agalsidase alfa and its ease of administration over a minimum period of 40 minutes have enabled this approach.

In a single center study involving twenty-two patients whose infusions had been transitioned to the home setting, a total of 1528 treatments were administered (range of 42–73 infusions per patient) (Schiffmann et al 2006). No additional safety concerns were raised by administration of agalsidase alfa in the home setting by a visiting nurse. Specifically, no patient experienced the new onset of infusion reactions after switching to home therapy.

Palliative measures and adjunctive therapies

As a large proportion of AFD patients currently on enzyme therapy are adults, with a lifetime of tissue Gb3 storage and varying degrees of renal, cardiac, and cerebrovascular dysfunction, there is a continuing need for palliative measures and adjunctive therapies to optimize clinical outcome.

The rationale for the use of adjunctive therapies is based on their conventional or demonstrated value in modulating risks associated with other chronic conditions, such as hypertension and stroke (Berthiaume et al 2007; Kikano and Brown 2007). These measures may include anti-platelet agents (eg, aspirin or clopidogrel) to reduce the incidence of transient ischemic attacks and stroke. Other therapies that have been suggested include the use of angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (sartans) for patients with proteinuria (>30 mg albumin/g creatinine), the regulation of blood pressure and statins (Ferrari 2007). Statins may improve renal and cardiac outcomes in AFD by decreasing circulating levels of low-density lipoproteins (LDL), improving endothelial function, or decreasing patterns of LDL oxidation (Campese and Park 2007). Investigations of disease mechanisms in AFD are essential, and may clarify the role of adjunctive therapies in the overall management of affected individuals.

Among palliative approaches, the introduction of renal replacement therapy (ie, dialysis and renal transplant) for

ESRD during the period preceding enzyme therapy resulted in an increased survival by a decade or more (Sessa et al 2004). A few patients have had implantable pacemakers and defibrillators for bradycardia and arrhythmias, respectively; but the number of lives saved through these measures has not been as closely examined (Shah et al 2005). It is hoped the availability of enzyme therapy and greater use of adjunctive therapies will at least delay, if not prevent the onset of major organ failure. These developments would offset the acquisition costs of agalsidase alfa, while significantly improving patient quality of life.

Surrogate markers and measuring therapeutic response

In the phase III pivotal study, agalsidase alfa treatment resulted in a 54% reduction in plasma Gb3 levels from baseline, as measured by HPLC. In urine sediment, the magnitude of reduction in Gb3 was about 50% (Schiffmann et al 2001). A clinical endpoint (ie improvement in neuropathic pain, as measured with the Brief Pain Inventory) was chosen as the primary outcome variable. Although agalsidase alfa was shown to significantly reduce "pain at its worst", "overall pain severity", and "pain-related quality of life" when compared to placebo, several concerns (eg, inconsistencies in reporting of pain medication usage, disparity in baseline pain scores between treatment groups) were raised during the process of regulatory review by the US Food and Drug Administration regarding the interpretation of study findings (see Pastores 2004).

A decline in plasma levels and endothelial cell storage of Gb3, the primary substrate of AGAL, supported the conditional approval of agalsidase beta (Fabrazyme, Genzyme Corp, Boston, MA, USA) (Eng et al 2001). This situation for an 'orphan disorder' was justified based on the outcome of preclinical studies in the AFD mouse model, which established delivery of the recombinant enzyme to and the clearance of stored substrate in several target organs, such as the liver, heart and kidneys. Unfortunately, the validity of Gb3 as a surrogate marker of disease burden and its clinical utility had not been established beforehand, and currently there is debate about its usefulness in the serial evaluation of treated and untreated AFD patients.

A recent study involving 96 patients with AFD revealed a limited relationship between plasma Gb3 and various disease manifestations, and that individual symptoms did not correlate with either elevated urinary or plasma Gb3 levels (Vedder et al 2007). Additionally, a separate report noted that despite the virtual absence of storage material in plasma and skin vascular endothelial cells, a wide spectrum of clinical abnormalities was seen in a group of carrier women (N = 61) (Gupta et al 2005). In the latter study, all patients had normal plasma Gb3 levels and only a single patient had visible storage material in the superficial dermal vascular endothelial cells. Yet, cardiac, renal, or cerebrovascular abnormalities were documented in 52/57 patients (91%) (Gupta et al 2005). Moreover, no studies have reported on the correlation between degree of reduction in plasma or cellular Gb3 levels and clinical benefit.

The source of Gb3 found in plasma is not known, and may reflect a surplus from the cellular turnover of membranes from a variety of organs and newly synthesized substrate in transit. It is possible that the detrimental health effects of Gb3 may be a consequence primarily of its intracellular storage; resulting in a disruption of GSL metabolism and the activation of additional complex cellular abnormalities. Indeed, a recent study suggested the presence of a potentially toxic factor (a growth-promoting substance) present in the plasma of AFD patients may stimulate the proliferation of vascular smooth muscle cells and cardiomyocytes (based on in vitro studies in the rat and mouse) (Barbey et al 2006). The significance of parallel or downstream pathologic events within AGAL deficient cells is underscored by the lack of perfect concordance between organ pathology and the amount of tissue Gb3 storage. This is evident when considering that in a severely hypertrophic heart of an AFD patient, Gb3 accounted for only about 0.3% of the total weight (Elleder et al 1990). In addition, systolic dysfunction or a severe restrictive hemodynamic filling pattern is not usually encountered in these cases until later in the course of disease. Interestingly, an increase in common carotid artery intima-media thickness has been noted among males and females with AFD, suggesting a congruence of disease mechanisms that is independent of the level of residual plasma AGAL activity (characteristic of most female carriers and some male patients with the "variant" phenotype). Additionally, in male patients with AFD receiving enzyme therapy there was no significant improvement of coronary microvascular function, in spite of Gb3 clearance from endothelial cells (Barbey et al 2006). Failure to fully correct the microvascular dysfunction in AFD despite enzyme therapy may partly explain the persistent risk for stroke, even with reversal of the exaggerated cerebrovascular dynamics.

Thus, besides cardiac Gb3 storage there must be other mechanisms that play a contributory role and account for the observed myocardial remodeling. A recent study involving 29 adult AFD patients, revealed the presence of

abnormal extracellular matrix turnover (based on elevated serum MMP-9 levels), which was negatively correlated with endocardial and mid-wall fractional shortening (Shah et al 2007). The distribution of fibrosis in the mid-myocardial and left ventricular free wall puts it beyond the reach of conventional biopsy. On the other hand, cardiac magnetic resonance imaging may be useful for the characterization of myocardial tissue in AFD, and it may be important to establish the presence and extent of myocardial fibrosis and corresponding functional changes prior to therapy (Imbriaco et al 2007).

It should be noted that the state of affairs may be entirely different with respect to the brain and kidneys; wherein intracellular Gb3 may exert a more direct injurious effect. Clearly, these are issues that require further investigation. Recently, a systems biology approach has been undertaken to demonstrate multiple and complex parallel cellular abnormalities in AFD. The observed abnormalities may be used to identify potential surrogate markers that can be used as a measure of total body or organ-specific burden of disease, which may also be useful in monitoring response to enzyme therapy (Moore et al 2007).

Albuminuria is a cardiovascular and renal risk factor in patients with comorbid conditions (eg, diabetes and hypertension); with relative risk shown to increase continuously with increasing urinary albumin levels, even after adjusting for numerous other factors (Basi and Lewis 2006). The pathophysiological process of albuminuria is not definitively known, but may be related to endothelial dysfunction, inflammation, and/or abnormalities in the renin-angiotensin-aldosterone system; mechanisms that may be relevant in AFD but require further exploration. Interestingly, microalbuminuria (ie, 30–300 mg of albumin in a 24-hour urine collection) has been associated with other markers of vascular dysfunction, such as increased intima-media thickness; the latter a finding described in AFD patients, but whose predictive value remains to be established (Barbey et al 2006; Bigazzi 1995).

In the recently reported phase IIIB\IV placebo-controlled study involving agalsidase beta, a statistically significant difference in composite primary endpoint (ie, worsening of kidney function, myocardial infarction, stroke or death) in favor of enzyme therapy was seen, only when the patients were stratified by baseline proteinuria (in a per-protocol analysis) (Banikazemi et al 2007). On the other hand, those with more severe disease and proteinuria did not exhibit the same benefits. These observations were similar to that seen for a subset of patients (N = 6) in the original agalsidase-beta phase III who initially had well-preserved GFR values and overt proteinuria and went on to show a continuing decline of their kidney function at a rate that approached –10 ml/min per 1.73 m^2/year (Germain et al 2007). The predictive value of baseline proteinuria in AFD requires further investigation. In IgA nephropathy, baseline proteinuria was not significantly related to renal progression; nor was hypertension or impaired renal function at onset (Coppo and D'Amico 2005). On the other hand, a multivariate Cox analysis revealed age and mean proteinuria during follow-up were powerful independent prognostic predictors. Interestingly, IgA nephropathy in patients with AFD has been described (Whybra et al 2006). A comparative analysis of these patients may provide some insight into relevant prognostic factors.

Expert opinion

On final analysis, the value of enzyme therapy for AFD will be judged based on its effectiveness in leading to a normal (event-free) survival and sustained improvement of patient health-related quality of life. Such an outcome would translate into healthcare cost-savings from reduction in resource utilization (eg, dialysis, cardiac or renal transplant, stroke rehabilitation), along with contributions made during a productive life by affected individuals (with deferment of disability or death). These represent our therapeutic goals; however, at the moment we should focus on examining the factors that influence treatment response, and the identification of clinical markers that may be useful in projecting disease trajectory and individualizing the therapeutic regimen. We need to devise a staging system to stratify patients' by disease state. This may entail a combination of clinical evaluations (eg, of endurance and cardiopulmonary function with the 6-minute walk test), imaging (eg, cardiac MRI to ascertain areas of fibrosis) and the histological characterization of major target organs (such as the heart and kidney); during this initial period post introduction of enzyme therapy. Of particular importance is the need, on first principle, to demonstrate that significant Gb3 deposits are cleared from other cells types and not just endothelium. This concern is highlighted by two recent reports that examined cardiac myocyte in two patients treated with agalsidase beta. In one patient, a 47-year-old man treated for about 2.5 years, numerous lamellar inclusions were found in cardiac myocytes (Schiffmann et al 2006). In the other patient, a 51-year-old male who received agalsidase beta for over 18 months, light microscopy revealed glycolipid storage typical for untreated AFD. Although the cardiac myocytes did not show the typical lamellar inclusions there were extensive

aggregates and single tubular crystalline structures, giant secondary lysosomes (5–6 μm) as well as abnormal branched chain glycogen (Owens et al 2006). These observations are particularly fascinating as there are patients with AFD who exhibit residual enzyme activity with a restricted phenotype, in which cardiac involvement represents the predominant feature. Thus, cardiac muscle may not be sufficiently targeted by exogenous enzyme, or heart involvement may be a feature of AFD requiring prolonged treatment to show a response. There are no comparable studies in agalsidase alfa-treated patients; thus, it is not certain whether therapeutic outcome may be different in such treated individuals.

The majority of reported studies have described the treatment outcome in the adult male with AFD. As a subset of these treated patients remain symptomatic and show disease progression, several investigators have argued in favor of starting treatment earlier (ie, in childhood or juvenile period). This contention appears to be supported by the recent head to head trial, in which treatment failure was not observed in the AFD patients younger than 40 years and with a disease severity score <17 (Vedder et al 2007). Similarly, the treatment of symptomatic females has also gained favor as the burden of disease among heterozygotes has clearly been underestimated until recently (Eng et al 2007). The major issues in this regard, are at what age and which women; as the cumulative direct and indirect cost of treatment would be high and could involve risks related to infusion reactions.

The systemic nature of AFD necessitates a multimodality approach that integrates adjunctive therapies. A chief predicament in interpreting outcome data and determining the scope of treatment effect(s) from enzyme therapy lies in the fact that therapeutic responses are presented for each of the major organs (kidney, heart and brain) for cohorts of patients in relative isolation; that is, with a paucity of information on co-existing problems. This issue may be partly addressed by utilizing a staging system that allows for the stratification of patients by disease severity at baseline. For instance, stratifying patients according to baseline by age and renal function may provide a better insight regarding overall therapeutic response profile. In a prospective, open-label study involving 26 patients (with one early death), impaired renal function (ie, GFR <60 mL/min) was associated with a less favorable outcome and greater risk for developing cardiovascular and renal end points (Breunig et al 2006). Furthermore, most of the current reports do not provide data on the types (and doses) of adjunctive agents that are used. Thus, a complete analysis of the effectiveness of enzyme therapy will have to take into account the use of these other medications that may influence outcome. At the moment, based on currently available clinical outcomes data and taking into account the outlay in healthcare dollars for enzyme therapy, to declare that this option represents an unqualified treatment advance for symptomatic AFD requires a leap of faith.

Summary

Regulatory approval for the use of agalsidase alfa in patients with AFD has been granted within the member states of the European Union. There is increasing evidence to support claims for its safety and disease modifying effects; leading to its greater use, which includes administration within the home setting. Although the bulk of treatment experience is with the use of an enzyme dose of 0.2 mg/kg every other week, further studies are required to define the optimal treatment regimen (ie, enzyme dose and frequency of administration) for the individual patient.

Concerns have been raised regarding the cost of ERT, as estimations indicate a perpetual net deficit to society under current costing and ERT efficacy as determined by the QALY metric (Moore et al 2007). Thus, it has been suggested that rules of fair cooperation should govern decision making both for ERT in AFD and for funding therapeutic advances in other rare diseases belonging to the orphan and ultra-orphan categories.

Management issues which remain outstanding include the following: (1) the inability to assign disease stage for the purposes of prognostication and determination of the appropriate time to intervene, (2) uncertainty regarding the repertoire of clinical investigations and diagnostic modalities to establish pretreatment disease severity, (3) the optimal regimen individualized to the patient's current disease status, and (4) the combination of adjunctive therapies that may be useful in the primary and secondary prevention of adverse outcomes. There is an active search for surrogate markers, which may lead to greater understanding of pathogenesis and the identification of rational therapeutic targets. There is a pressing need to focus on the assessment of the true benefit from enzyme therapy, in terms of event-free survival and patient health-related quality of life. Medical practice in the specialized field of AFD demands an expanded model of partnerships, involving healthcare providers, biotechnology companies, third party payers and healthcare policy makers; to ensure delivery of the most advantageous and high quality patient care. The current organizational culture drives collaborative relationships, with therapeutic guidelines from key opinion-leaders, based primarily on data derived from observational databases or open-label studies, mostly conducted

under the auspices of industry; clearly transparency is crucial. After almost a decade of clinical investigation, one would hope to have achieved several major therapeutic milestones, or at least a significant inroad in the care of AFD patients. Verily, we still are at the beginning of our journey towards better health for patients.

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