CASE PRESENTATIONS

Patient 1. A 50-year-old man with end-stage renal disease (ESRD) possibly secondary to Fabry disease commenced chronic ambulatory peritoneal dialysis (CAPD) 19 months ago. In childhood he presented with acroparesthesias and pain crises with fever, which were considered to be of psychosomatic origin. Remission of the symptoms occurred when he was 15 years old, but he continued to have atypical abdominal pain and a low threshold for pain. In childhood he also developed angiokeratomas.

From the age of 29, he suffered from asthma and since then has been treated with anti-asthmatic inhalers. Fifteen years ago he underwent a slit-lamp examination, in which the typical ophthalmic findings of Fabry disease (cornea verticillata, lenticular opacities) were observed and the diagnosis of the disease was established. Eleven years ago, hypertrophic cardiomyopathy with arrhythmias was treated with anti-arrhythmic agents, including amiodarone. Renal insufficiency was detected when he was 38 years old. At the time of onset of renal insufficiency, proteinuria was approximately 2500 mg/24 hours and continued at this level during all the years of follow-up until the patient reached ESRD, when a reduction to 300 mg/24 hours was noted. The patient’s mild to moderate hypertension was well controlled at a level of 130/84 mm Hg with an angiotensin-converting enzyme (ACE) inhibitor until 1 year before the onset of ESRD, when a calcium channel blocker was added. During the 10-year follow-up, the patient’s creatinine clearance (C_{cr}) declined at a rate of 7.9 mL/min/year (Fig. 1). The CAPD program is 4 × 1 L dialysis fluid with 1.5% glucose concentration. The small size of the patient (54 kg body weight and 156 cm height) led to an intolerance of larger intraperitoneal volumes. Before he commenced CAPD, echocardiography revealed a left ventricular mass (LVM) of 430 g and an ejection fraction (EF) of 47%. In view of the renal findings and cardiac dysfunction, enzyme replacement treatment (ERT) with intravenous agalsidase beta (Fabrazyme®), 1 mg/kg/body weight initially every other week, was initiated 1 month after he began CAPD.

Before the commencement of treatment and after 1.5, 3, 6, 12, and 15 months, weekly residual renal creatinine clearance (wC_{rCr}) and renal Kt/V urea (wK_{t/V}urea) were determined according to standard equations. Peritoneal dialysis adequacy was also estimated by weekly peritoneal Kt/V urea (wK_{p/V}urea) and creatinine clearance (wC_{pCr}). The latter was adjusted for the patient’s size using a nomogram for body surface area. Table 1 displays routine laboratory investigation before and after ERT.

The wC_{rCr} rose after 1.5 months of therapy from 29 to 82 L/week/1.73 m². It remained at approximately 80 L/week/1.73 m² after 3 and 6 months of therapy; a decrease to 60 L/week/1.73 m² was noticed at month 12 and continued until month 15 of therapy [these values are represented in the figure as C_{cr} (mL/min)]. Also, the wK_{t/V}urea rose strikingly, from 0.8 to 2.3 after 1.5 months, remained around 2.0 after 3 and 6 months, dropped to 1.5 after 12 months, and remained stable after 15 months of treatment. The wC_{pCr}, on the other hand, did not change significantly during the same period. Similarly,
wKp/Vurea decreased from 1.6 to 1.1 after 15 months of enzyme replacement treatment (ERT). Fig. 1. Creatinine clearance values of Patients 1 and 2 before and after enzyme replacement treatment (ERT).

Patient 1. A 38-year-old man, diagnosed with Fabry disease during evaluation for chronic renal insufficiency (CRI), presented with angiokeratomas on his legs. He was 180 cm tall and weighed 70 kg. He had not received an ACE inhibitor. His blood pressure was 120/80 mm Hg, and his heart rate was 74 bpm. Physical examination was unremarkable except for the angiokeratomas on his legs. He had severe pain crises and hypohidrosis. Significant laboratory findings included anemia, proteinuria, and microalbuminuria. The diagnosis of Fabry disease was confirmed by skin biopsy and slit-lamp examination. The patient's renal function remained stable during the years of follow-up.

Physical examination was unremarkable apart from the angiokeratomas on his legs. He was 160 cm tall and weighed 71 kg. His blood pressure was well controlled (134/84 mm Hg) with one to two antihypertensive agents. He had never received an ACE inhibitor. He was about 39 years old when mild CRI was diagnosed (serum creatinine, 1.3 mg/dL). At the same time, he presented with microalbuminuria (~150 mg/24 hours), which remained unchanged during the years of follow-up. During a follow-up of about 10 years, his Ccr decreased at a rate of 2.4 mL/min/year (Fig. 1). Although his Ccr was approximately 45 mL/min, and ultrasound showed that the right and left kidneys were 10.4 cm and 9.8 cm with cortices of 15.5 mm and 14.3 mm, respectively, he declined renal biopsy. The likely diagnosis was Fabry nephropathy, and he started treatment with IV agalsidase beta, 1 mg/kg/body weight every other week. The Ccr rose gradually during the 15 months of treatment and reached 65 mL/min; microalbuminuria remained stable at 150 mg/24 hours, as it was before the start of therapy. This patient also gained about 4 kg. Echocardiography revealed parameters similar to his brother's. The LVM showed small alterations: from 503 g at the beginning to 490 g after 18 months. In contrast, the EF increased even after 12 months from 46% to 56% and moreover to 82% after 18 months. Table 1 illustrates his laboratory data before the initiation of the ERT and after 15 months.

The patient discontinued taking his antihypertensive medication after 6 months of ERT, and his blood pressure remains normal at 130/80 mm Hg without therapy. Plasma GL-3 levels decreased from 9.1 μmol/L to 3.7 μmol/dL. The patient reported sweating for the first time in his life, especially of the hands, and he enjoyed his first summer without suffering from the heat. Moreover, the vertigo has improved significantly so that he has discontinued the three medications he was taking for dizziness and vertigo.

DNA analysis, which was performed for both patients at the Genetic Testing Laboratory of the Mount Sinai School of Medicine, revealed that the brothers are heterozygotes for Fabry disease with the same missense mutation T385P in exon 7. Detection of plasma α-galactosidase A (α-Gal A) activity performed immediately before the start of ERT (in the Department of Biochemistry of the Academic Medical Center, Amsterdam, The Netherlands) revealed similar activities for both patients (0.8 and 0.7 nmol/hour/mL, respectively). Treatment with agalsidase beta was well tolerated by both patients.

DISCUSSION

Dr. Kostas C. Siamopoulos (Professor of Medicine/Nephrology, Chief, Division of Nephrology; Department of Medicine, University of Ioannina, Ioannina, Greece): Fabry disease is an X-linked metabolic disorder caused by the deficient activity of the lysosomal enzyme α-Gal A,
Table 1. Laboratory investigation in Patients 1 and 2 before and after enzyme replacement treatment (ERT)

<table>
<thead>
<tr>
<th></th>
<th>Before ERT</th>
<th>After 15 months</th>
<th>Before ERT</th>
<th>After 15 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit/hemoglobin % g/dL</td>
<td>34.1/10.3</td>
<td>37.3/11.8</td>
<td>36.1/11.6</td>
<td>37.1/12.1</td>
</tr>
<tr>
<td>White blood cells µL</td>
<td>5070</td>
<td>6570</td>
<td>8570</td>
<td>6740</td>
</tr>
<tr>
<td>Platelets µL</td>
<td>297,000</td>
<td>300,000</td>
<td>202,000</td>
<td>191,000</td>
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<tr>
<td>Urea mg/dL</td>
<td>142</td>
<td>121</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>Creatinine mg/dL</td>
<td>5.7</td>
<td>6.7</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Glucose mg/dL</td>
<td>87</td>
<td>95</td>
<td>96</td>
<td>81</td>
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<tr>
<td>Uric acid mg/dL</td>
<td>7.4</td>
<td>6.1</td>
<td>9.7</td>
<td>6.8</td>
</tr>
<tr>
<td>Calcium/phosphate mg/dL</td>
<td>143/4.7</td>
<td>144/4.3</td>
<td>142/4.3</td>
<td>140/4.8</td>
</tr>
<tr>
<td>Transaminase (GOT/GPT) IU/L</td>
<td>11/16</td>
<td>16/11</td>
<td>21/14</td>
<td>26/14</td>
</tr>
<tr>
<td>Total bilirubin/direct bilirubin mg/dL</td>
<td>0.4/0.07</td>
<td>0.4</td>
<td>0.9/0.16</td>
<td>0.8</td>
</tr>
<tr>
<td>Creatinine kinase/lactic dehydrogenase IU/L</td>
<td>73/498</td>
<td>214/612</td>
<td>111/404</td>
<td>218/477</td>
</tr>
<tr>
<td>Proteins/albumin g/dL</td>
<td>6.1/4.0</td>
<td>6.4/3.4</td>
<td>8.3/5.0</td>
<td>7.1/3.6</td>
</tr>
<tr>
<td>Total cholesterol/triglycerides/high-density lipoprotein cholesterol mg/dL</td>
<td>204/112/45</td>
<td>207/151/51</td>
<td>176/136/38</td>
<td>167/79/41</td>
</tr>
</tbody>
</table>

Abbreviations are: ALP/-GT, alkaline phosphatase/gamma glutamyl transferase; GOT/GPT, glutamic oxalacetic transaminase/glutamic pyruvic transaminase.

a glycoprotein of approximately 101 kD with a homodimeric structure. The deficiency of α-Gal A results in the progressive lysosomal accumulation of neutral glycosphingolipids with terminal α-galactosidase residues, predominantly GL-3. The accumulation of GL-3 occurs primarily in body fluids and in the lysosomes of many cells, particularly in renal epithelial cells, endothelial cells, pericytes, vascular smooth muscle cells, cardiomyocytes, and neurons of the autonomic nervous system [1]. The disease is rare: the prevalence in a predominantly white population is one in 117,000 males [2]; according to another estimate, the disease affects approximately one in 40,000 males [1]. Renal insufficiency is the most frequent and serious late complication, leading to ESRD and renal replacement therapy. In the past, treatment of Fabry disease has been symptomatic and directed to palliative management of disease complications without correcting the underlying disorder. However, the availability of ERT remarkably improved the outcome for patients with this disease. My aim in this Forum is to persuade the reader that some of the serious complications of Fabry disease, a previously devastating disease, are curable today, particularly when the disease is diagnosed early. In this presentation, I will review the clinical diversity of renal involvement in Fabry disease and will discuss the effects of ERT on renal function and pathology.

The gene of α-Gal A, comprised of 7 exons, has been isolated, sequenced, and localized to the chromosomal region Xq22.1 [3–6]. To date, a total of 271 mutations of the gene have been identified in all 7 exons (Human Gene Mutation Database, http://uwcmmls.uwcm.ac.uk/ uwcm/mg/search/119272.html), including 198 nucleotide substitutions (missense/nonsense, splicing), 60 microdeletions (deletions, insertions, indels), and 13 gross lesions (deletions, insertions and duplications, and complex rearrangements). Nevertheless, the crystallographic structure of the human α-Gal A protein is not yet available, and only recently was the construction of a model of the human α-Gal A enzyme achieved [7]. According to this knowledgeable review, the locations of the human α-Gal A point mutations reveal two major classes of Fabry disease protein effects on enzymatic activity: site mutations and folding mutations. Site mutations reduce enzymatic activity by perturbing the active site without necessarily affecting the overall structure of the enzyme; folding mutations destabilize the α-Gal A protein by disrupting its hydrophobic core. This instability possibly leads to the mild variant of Fabry disease.

Genetically the disease is described as an X-linked recessive disorder in which affected males have absent or very low levels of the lysosomal enzyme α-Gal A. These patients usually have the so-called classic phenotype with onset of the disease during childhood and manifestations that include angiokeratomas, hypohidrosis/anhidrosis, and acroparesthesias. Later in life, corneal/lenticular opacities develop as well as major clinical manifestations, such as cardiac involvement (congestive heart failure, cardiac conduction abnormalities, myocardial infarction), and cerebrovascular disease, which includes dizziness, transient ischemic attacks, and stroke [1]. In the surviving patients, progressive renal disease leads to ESRD, which limited the patient’s life span to the early
forties and was the rule in the absence of dialysis or renal transplantation [8]. It is promising that ERT will not only substantially increase the life span of these patients but in time also will reduce their need for dialysis and transplantation.

Two variants of the classic phenotype are the renal and cardiac variants. Patients with the renal variant usually have low plasma α-Gal A activity, a milder phenotype, or the absence of early presenting symptoms of classic Fabry disease, but they can reach ESRD and receive dialysis. According to a recent study, 1% to 2% of ESRD patients in Japanese, Italian, and American dialysis centers might have an undiagnosed renal variant of Fabry disease [abstract; Desnick RJ et al, Nephol Dial Transplant 17 (Suppl 1):4A, 2002]. Patients with the cardiac variant can develop late onset cardiac or cardiopulmonary disease with some degree of proteinuria and renal insufficiency [9, 10]. It is of note that in male patients with hypertrophic (mainly concentric) cardiomyopathy in whom the disease was diagnosed at ≥40 years of age, retrospective clinical examination revealed the prevalence of Fabry disease to be 6.3% [11]. Thus, early identification of patients with both renal and cardiac variants could prevent end-stage organ damage by early intervention and at the same time help detect affected relatives.

The third variant of the classic phenotype comprises heterozygous women from families with the classic phenotype. Although most of these women remain asymptomatic and have a normal life span, some experience a few minor manifestations. Approximately 70% have whorl-like corneal dystrophy, 30% exhibit a few angrokeratomas, and less than 10% suffer from acroparesthesias [1]. Cardiac and renal manifestations in heterozygous women with Fabry disease are usually lacking. However, these patients are potential victims for severe organ damage; severe manifestations and death due to heart and kidney involvement have been reported. The 1995 ERFEDTA registry included 10 females among the 83 patients with Fabry disease who started renal replacement therapy [12]. Similarly, among 95 patients with Fabry disease who began dialysis between 1985 and 1993 in the United States, 12% were females [13]. Given these considerations, perhaps Fabry disease should be included in the differential diagnosis of females with evidence of renal and/or cardiac involvement, especially those with a positive family history. The increasing recognition of clinical manifestations of the disease in female heterozygotes supports the hypothesis that Fabry disease is an X-linked dominant, and not recessive, disorder [14, 15]. However, such variable phenotypic expression is expected in females heterozygous for most X-linked diseases because of the random inactivation of one X chromosome.

Since most mutations are private, occurring in a single Fabry disease family, attempts at recognizing genotype/phenotype correlations have been limited. Moreover, a variety of phenotypes can occur in identical hemizygous patients, and the same mutations have been identified in mild hemizygous patients and severely affected heterozygous patients. Such observations suggest that other factors are also involved in disease expression [16]. It is also well known that apart from the primary disease, genetic or nongenetic factors can influence the severity and progression of cardiovascular and renal disease. Whether such factors were involved in the completely different progression of the renal dysfunction of the two patients we are considering today is a matter of discussion. Although the two brothers differ in age by only 2 years, with the CAPD patient being the younger, their age at onset of renal failure was similar, they have the same mutation (T385P on the same exon 7), and they had comparable, very low plasma α-Gal A activities. Nevertheless, they exhibited a different progression of renal dysfunction. This profile contrasts with data from Branton et al [17], which show that patients with conservative substitution mutations in exons 1, 2, and 5 have a higher residual α-Gal A level and a later onset of CRI in comparison to patients with nonconservative mutations in exons 3, 6, and 7, who have earlier progression of renal insufficiency and absent or low levels of α-Gal A. However, as the authors stated, their paper has several limitations: the data were obtained by retrospective chart review, and the study was not designed to examine renal manifestations. Different blood groups also might be related to the severity of disease progression, with blood groups AB and B associated with a more aggressive disease course [18]. The blood group of the two brothers we are discussing is blood group O.

Renal involvement, which starts in early adulthood, is expressed by an inability to concentrate the urine, proteinuria, and progressive renal failure, which can lead to ESRD. According to data from the NIH studies [17], 78 of 105 patients with Fabry disease had proteinuria, CRI, or both. Proteinuria was present at some time in the clinical course of 66 of those 78 patients. The age at onset of nonnephrotic proteinuria was 34 years (range, 14–55 years). Fifty percent of all Fabry disease patients developed proteinuria by age 35, and 100% of surviving patients developed proteinuria by age 52. Nephrotic-range proteinuria developed in 19 of 78 patients (18%), and the age at onset was 40 years (range, 26–55 years). The onset of nephrotic-range proteinuria did not correlate with the development of CRI; nephrotic-range proteinuria appeared (with similar percentages) either before or after CRI, or it did not appear at all. Of 105 patients, 39 developed CRI. The median age at CRI onset was 42 years (range, 19–54 years). Twenty-four of 105 patients (23%) developed ESRD at a median age of 47 years (range, 21–56 years). The time of progression from the onset of CRI to ESRD was 4 years (range, 1–13 years) in 14 patients for whom ages at onset for both CRI and ESRD were available. The same data report a mean decline of glomerular filtration rate (GFR) of about 12.2 mL/min/year, a value
comparable to the decline observed in patients with diabetic nephropathy who do not receive therapy to block the renin-angiotensin system [19–21].

A close correlation between blood pressure and the rate of decline of GFR exists in patients with diabetic nephropathy [22–24] and in those with a variety of non-diabetic nephropathies [25]. In one Fabry disease population [17], the prevalence of hypertension was 30%. According to Branton and colleagues, hypertension was more likely to be essential or secondary to established renal disease. However, the possibility that increased blood pressure is a consequence of Fabry disease itself cannot be disregarded. The two patients we are considering were hypertensive and had been receiving antihypertensive agents that were discontinued after some months of ERT even though the patients had gained weight and their ejection fractions had increased. Although the improvement of renal function in the second patient and CAPD replacement therapy in the first might be responsible for the lowering of blood pressure, an improvement of the vascular endothelial dysfunction also might be a possible antihypertensive mechanism.

In support of this speculative mechanism are the findings from studies that confirmed an elevation of markers of endothelial cell injury and activation. These abnormalities suggest that the vascular endothelial cells of Fabry disease patients are in a chronic proinflammatory and prothrombotic state [26, 27]. Also, proteinuria plays a major role in the progressive loss of GFR in patients with diabetic and nondiabetic nephropathies [22, 25, 28]. On the other hand, the role of antihypertensive treatment in slowing the decline of GFR is documented by long-term clinical studies both in patients with diabetic nephropathy [19, 29] and nondiabetic nephropathies [25]. The beneficial effect of ACE inhibitors on proteinuria and the rate of decline in GFR, mainly in type 1 diabetic nephropathy [30, 31] and nondiabetic nephropathies [26], is well recognized, and as Björck et al [32] suggested, this beneficial effect on renal function and structure is above and beyond that expected from the blood pressure reduction alone. It is of interest that Patient 1 had received an ACE inhibitor since the onset of his CRI. Although a delay in the decline of renal function was observed, renoprotection was not achieved, possibly because of his long-lasting, permanent, severe proteinuria. Compatible with this patient’s case are the results of a recent study showing that 42% of patients with nondiabetic renal disease escape the antiproteinuric effect of ACE inhibitors and subsequently develop an exacerbation of renal dysfunction [33].

Kidney biopsies in patients with Fabry disease have shown extensive accumulation of GL-3 in nearly all renal cell types, but it varies considerably in quantity and morphology among the different cells. In a well-conducted and detailed histologic study, Thurberg et al analyzed pre- and post-ERT renal biopsy data from 58 FD patients (56 males, 2 females) [34]. Of these patients, one-half received treatment for 6 months, the other half 11 months. At baseline, podocytes and epithelial cells of distal convoluted tubules and collecting ducts contained the highest concentrations of GL-3 deposits; proximal tubular epithelial cells were relatively unaffected. Vascular endothelial and smooth muscle cells as well as mesangial and interstitial cells also accumulated moderate amounts of GL-3. Lipid-laden distal tubular epithelial cells desquamate and can be detected in the urine [35]. In later stages of the disease, the histologic findings correspond to those of ESRD, with severe arteriolar and glomerular sclerosis, tubular atrophy, and diffuse interstitial fibrosis [1]. According to Gubler et al [36], these lesions might be related to ischemic changes due to necrosis of smooth muscle cells fatally overloaded with GL-3 deposits. However, podocyte injury due to toxic accumulation of GL-3 and tubulointerstitial injury could constitute two other important mechanisms that lead to ESRD [37]. Lipid material in epithelial cells of the glomeruli and within tubular epithelial cells and the interstitium also was detected in a renal biopsy from a patient with Fabry disease who did not have the typical skin lesions [38].

Enzyme replacement therapy with recombinant α-Gal A became available recently and two placebo-controlled clinical trials were published in 2001. In the first trial [39], agalsidase alfa (Replagal), a human cell line product, was administered intravenously every 14 days at a dose of 0.2 mg/kg/body weight for 24 weeks (12 infusions) to 26 hemizygous male patients. Although the evaluation of renal function was not the main outcome measure, the investigators found an improvement in glomerular histology in patients treated with agalsidase alfa. There was a 21% increase in the fraction of normal glomeruli (glomeruli without mesangial widening or sclerosis) in treated patients, whereas a 27% decrease in the fraction of normal glomeruli was observed in patients randomized to placebo (P = 0.01). The authors also noted a decrease in glycolipid inclusions within the vascular endothelium in the treated group compared to an increase in the placebo group. Renal function was assessed by Ccr and inulin clearance in 15 patients undergoing active treatment and in 11 of the placebo group. Both tests showed a trend in favor of enzyme treatment. There was no consistent change in urinary protein excretion in 5 of the patients from the active treatment group and in 3 from the placebo group who had proteinuria > 1 g/24 hours.

The second study included 58 patients (56 males and 2 females) who received agalsidase beta (Fabrazyme), which is produced in a hamster cell line [40]. Twenty-nine patients received the drug intravenously in a dose of 1 mg/kg/body weight; the other 29 received placebo every 14 days. The patients had normal or slightly impaired renal function. The primary end point was the
disappearance ("0" score) of microvascular endothelial GL-3 deposits in renal biopsy specimens after 20 weeks of treatment (11 infusions). Of 29 patients in the active treatment group, 20 (69%) reached this end point compared to none in the placebo group. Thereafter, all patients were enrolled in an open label extension study. After 6 months of treatment with recombinant agalsidase beta, 98% of patients in whom a biopsy was performed (42 of 43) had a score of 0 on histologic analysis of microvascular endothelial deposits of GL-3 in kidney specimens. Glycolipid completely disappeared from the endothelium of all vasculature as well as from the mesangial cells of the glomerulus and interstitial cells of the cortex. Moderate reduction was noted from the smooth muscle cells of arterioles and small arteries. Podocytes and distal tubular epithelium also demonstrated decreased GL-3, although this reduction was more limited than that observed in other cell types. No evidence of immune complex disease was found by immunofluorescence despite circulating anti-r-hu-Gal A IgG antibodies [34]. Baseline serum creatinine and GFR were normal (treatment group, 0.8 ± 0.2 mg/dL and 83 ± 22.0 mL/min; placebo group, 0.8 ± 0.2 mg/dL and 96.6 ± 35.3 mL/min, respectively) and did not change substantially in either group after week 20 of the double-blind study (P = 0.19) or after 6 months of the open-label treatment (P = 0.81). The safety and efficacy of ERT have been demonstrated with the two enzyme formulations, and both the ERT and placebo groups experienced significant improvement in the severity of pain and quality of life. Plasma and urine concentration of GL-3 decreased significantly in the treated groups of both studies in contrast to patients who received placebo.

In Patient 2 under discussion today, a gradual increase in Ccr was noticed after 6 months of ERT. This important finding suggests that in a Fabry disease patient with CRI and without overt proteinuria, ERT can reverse the decline of renal function. Moreover, Patient 1 also had a slight increase of residual renal function. Proteinuria, on the other hand, is a marker of podocyte injury and, as I already mentioned, the removal of GL-3 from the podocytes was limited compared to that in other cells. This is possibly the reason why proteinuria did not change in the 5 patients who received ERT with agalsidase beta [39].

In conclusion, I would argue that early therapeutic intervention with ERT for renoprotection in patients with Fabry disease is effective and safe. Although renal transplantation successfully corrects renal failure and the graft is protected by itself because its cells have the ability to produce the enzyme, ERT also might confer benefit to transplant and dialysis patients in protecting other vital organs. However, no available studies support this argument at the moment; future experience will show whether this treatment is efficient and cost-effective.

QUESTIONS AND ANSWERS

DR. NICOLAOS E. MADIAS (Dean ad interim, Tufts University School of Medicine, Boston, Massachusetts, USA): Considering the different clearance of GL-3 deposits from various cells, what do we know about the mechanisms of recombinant α-Gal A entry into various cells? What information do we have about tissue kinetics of the enzyme?

DR. SIAMOPOULOS: Studies on the synthesis of α-Gal A in cultured human cells have shown that the enzyme is synthesized as a precursor peptide that is processed in the mature lysosomal subunit [41, 42]. After intravenous administration, the enzyme is rapidly removed from the circulation and taken up by vascular endothelial and parenchymal cells into the lysosomes. The transportation to the lysosomes is dependent on the mannose-6-phosphate receptors located at the plasma membrane of the cells. Initial studies with different recombinant α-Gal A glycoforms, as regards their glycosylation and/or phosphorylation, showed that at doses of 1 to 10 mg/kg body weight, more than 90% of recovered enzyme activity of all four glycoforms was found in the liver and spleen, with 1% to 3% recovered in the heart and kidney, and none detectable in the brain. The half-life in plasma was less than 5 minutes for each glycoform [1]. Single doses of administered α-Gal A (0.3 to 10.0 mg enzyme/kg body weight) demonstrated that the reduction of GL-3 in liver, spleen, heart, and kidney is dose-dependent [43]. In an open-label, dose-escalation study of agalsidase beta treatment in 15 patients, each of whom received 5 infusions at one of the 5 dose regimens, clearance of the enzyme from the circulation appeared to be biphasic for all biweekly dose groups, with the more rapid elimination phase lasting 1 to 2 hours after the infusion [44]. In patients with pre- and post-treatment biopsies, mean GL-3 content was decreased by 84% in the liver, markedly reduced in the kidney in 4 of the 5 patients and, after 5 doses, was modestly lowered in the endomyocardium of 4 of the 7 patients.

DR. AIKATERINI PAPAGIANNI (Lecturer in Nephrology, Medical School, Aristotelion University of Thessaloniki, Thessaloniki, Greece): The persistence of GL-3 accumulation in podocytes in the study you mentioned is at least partly due to the extremely low turnover of these cells and underlies the need for longer treatment (more than 11 months) to achieve significant clearance of the deposits. However, does the extent of GL-3 accumulation in podocytes, and therefore the degree of proteinuria, play an important role in the development of renal function impairment? We know that there are heterozygous asymptomatic females with extensive deposits in podocytes but well-preserved renal function for a long period. Could you comment on this?
DR. SIAMOPOULOS: Podocytes are highly differentiated cells and, along with parietal epithelial cells, have the slowest turnover rate among the renal cell populations [45–47]. On the other hand, podocytes are among the cells with the greatest glycolipid inclusions. The combination of slow turnover with the high rate of endogenous substrate generation and the relatively short duration of treatment, along with an anticipated difficulty of the enzyme to pass through various cellular and acellular renal tissue barriers to reach the podocytes, most likely explains the low clearance of GL-3. Apart from the very well known role of severe proteinuria in the development and progression of renal failure, on which I have already commented, it seems that the accumulation of GL-3 in podocytes alone is not enough to cause severe renal dysfunction. As you mentioned, heterozygous females with significant accumulations of GL-3 in the podocytes have little alteration in renal function [48–51].

DR. JOHN PAPADAKIS (Director, Renal Unit, General Hospital “Thriasion” of Elefsina, Athens, Greece): Could you please tell us what criteria we should use in deciding whether to order a renal biopsy?

DR. SIAMOPOULOS: Patients with normal renal function and mild proteinuria have a substantial accumulation of GL-3 in renal biopsies. Therefore, renal biopsy is recommended in patients with significant proteinuria (300 mg/24 hours). Besides, most patients in two placebo-controlled trials [39, 40] had normal renal function or some degree of renal insufficiency with significant GL-3 deposits in the biopsied renal tissue. In the absence of significant proteinuria or renal insufficiency, and if the diagnosis of Fabry disease is difficult to establish, a renal biopsy should be considered, either to make the diagnosis or to exclude co-existing renal disease. In 19% of patients without a family history of Fabry disease, the diagnosis was made by nephrologists [17].

DR. AIMILIOS ANDRIKOS (Registrar in Nephrology, Department of Nephrology, General Hospital “G. Hatzikosta” of Ioannina, Ioannina, Greece): Are there any guidelines regarding the initiation of enzyme replacement therapy?

DR. SIAMOPOULOS: According to one recent review [52], ERT should be initiated in all patients with Fabry disease. Treatment ideally should begin as soon as clinical signs and symptoms, such as pain or isostenuria, are observed. Affected children and carriers with substantial disease manifestations also should be treated. Patients undergoing dialysis and those who have received a kidney transplant are at high risk for cardiac and cerebrovascular complications; this is why ERT is also recommended in these patients.

DR. CHRISTOS IATROU (Director, Center for Nephrology “G. Papadakis,” General Hospital ofNikea, Piraeus, Greece): In the case of a patient with Fabry disease and significant proteinuria who doesn’t respond to the suggested doses of ERT, would you add an ACE inhibitor or increase the dose of the enzyme?

DR. SIAMOPOULOS: As I mentioned earlier, the beneficial effect of an ACE inhibitor on proteinuria and the rate of decline in GFR in diabetic [30, 31] and non-diabetic nephropathies [26] is well recognized, and this beneficial effect on renal function and structure is above and beyond that expected from the blood pressure reduction alone [32]. It is of interest that the CAPD patient I presented was receiving an ACE inhibitor since the onset of CRI. Although he reached ESRD, a delay in the decline of renal function was observed (rate of decline of renal function of approximately 7.9 mL/min/year). No data are available on the efficacy of ACE inhibitors or angiotensin receptor blockers in delaying the progression of Fabry renal disease. Extrapolating the results from the use of such drugs in other nephropathies, I could suggest that their administration in combination with ERT is a reasonable medical practice. The increase of the dose of the enzyme is in fact a matter of discussion, because the optimal doses for the reversal, maintenance, and prevention of GL-3 accumulation in the kidney, heart, and vasculature are unknown. Perhaps higher doses or longer treatment are required to clear the massive accumulation of GL-3 in podocytes.

DR. IATROU: If the patient’s body weight is low, as in the case of the first patient (50 kg), and since each vial of Fabrazyme contains 30 mg of the enzyme, an expensive drug, how can we use the remaining amount of the drug from the second vial? Can we administer it to the patient or should we discard it?

DR. SIAMOPOULOS: The results of the phase I-II dose-escalation clinical trial [44] indicated that the clearance of GL-3 from tissue lysosomes was dose-dependent. Therefore, I see no reason in discarding the rest of the enzyme, especially in a patient with severe cardiomyopathy. Like podocytes, cardiomyocytes also are resistant to GL-3 clearance by ERT.

DR. IATROU: What are the guidelines for the dosage? Are the levels of GL-3 in the blood an appropriate and sensitive index?

DR. SIAMOPOULOS: According to a recent review of clinical guidelines [52], doses of 0.2 mg/kg for agalsidase alpha (Replagal) and 1 mg/kg for agalsidase beta (Fabrazyme) every 2 weeks are recommended. Patients receiving ERT should be followed at least once annually. If plasma GL-3 levels prove to be a useful marker of the disease burden and treatment efficacy, a goal of therapy should be to normalize plasma levels. For children, monitoring the frequency and severity of peripheral pain and gastrointestinal symptoms also could indicate clinical effectiveness.
DR. MADIAS: Are patients with no detectable endogenous protein at a higher risk of developing an immune response to exogenous enzyme? Do anti-α-Gal A antibodies have neutralizing activity? What is the significance of these antibodies regarding the effectiveness and safety of ERT?

DR. SIAMOPOULOS: Seroconversion rates, with largely IgG antibodies, in the two placebo-controlled trials were 64% for agalsidase alpha [39] and 88% for agalsidase beta [40]. Patients with the classic phenotype have low or undetectable levels of enzyme, so the antibody response is expected to be high. However, no evidence exists that antibody formation alters the efficacy of enzyme treatment; this hypothesis is based on the continued reduction in histologic scores for cellular clearance of GL-3 even in patients who developed transiently higher titers [40]. Patients with IgG antibodies might have a higher risk of hypersensitivity reactions such as fever/chills, headache, chest pain, and dyspnea. The available evidence suggests, however, that over time, the number of patients experiencing infusion reactions to agalsidase beta is reduced to 10% to 20% [53].

DR. MADIAS: What are the prospects of alternative therapies in Fabry disease, namely gene therapy, as well as inhibition of synthesis or accumulation of GL-3?

DR. SIAMOPOULOS: A number of other therapeutic endeavors have been attempted [1]. A variety of inhibitors to deplete the accumulated GL-3 have been used experimentally [54]. Although a reduction of GL-3 in lymphoblasts was found in vitro, the safety and efficiency of these compounds have not been clinically evaluated. Depletion of GL-3 from the circulation also was achieved by plasmapheresis, with the plasma substrate levels returning to pre-plasmapheresis levels in 5 days [55]. Chronic plasmapheresis, however, is not yet acknowledged as an important intervention for the therapy of Fabry disease. Finally, for cardiac variant patients with residual α-Gal A activity, the use of competitive inhibitors such as “chemical chaperons” at subinhibitory intracellular concentrations might prove efficacious. The prospect of gene therapy in Fabry disease is promising. After the early experimental studies that focused on the construction of retroviral and other vectors that expressed the α-Gal A cDNA in vitro, treatment with the recombinant adenoviral vector of Fabry knockout mice increased the α-Gal A activity in all tissues analyzed, including the liver, lung, spleen, muscle, heart, and kidney [56]. However, expression of α-Gal A did not persist, although a decrease of the accumulated GL-3 to nearly normal levels for as long as 6 months after treatment was found in the tissues studied. With time, overcoming some of the initial limitations will give the green light for gene therapy in Fabry disease patients.

DR. ANDREAS ZOURIDAKIS (Director, Renal Unit, General Hospital of Filiates, Thesprotia, Greece): Could liver transplantation in early Fabry disease provide a sufficient amount of α-Gal A to the patient?

DR. SIAMOPOULOS: Following transplantation of fetal liver in 3 hemizygotes with Fabry disease, the α-Gal A levels in serum and leukocytes remained unchanged, and the GL-3 levels in urine and serum slightly decreased [57]. As far as bone marrow transplantation is concerned, no data are available in humans. Normal murine bone marrow transplantation into α-Gal A-deficient mice lowered the concentration of GL-3 in the liver, spleen, and heart, but not in the kidney [58] [abstract; Simonara CM et al, Am J Hum Genet 65: A503, 1999].

DR. VASSILIOS VARGEMEZIS (Professor of Nephrology, Medical School, Democritus University of Thrace, Thrace, Greece): What are the kinetics of the enzyme after its administration in patients on hemodialysis or peritoneal dialysis? Is there any loss from the dialyser or peritoneal membrane?

DR. SIAMOPOULOS: Administration of the enzyme during hemodialysis does not change the kinetics or efficacy of agalsidase beta in patients with Fabry disease. According to a recent study [abstract; Kosch M, et al, Nephrol Dial Transplant 18:632, 2003], replacement therapy with recombinant human agalsidase beta performed in the intervals between dialysis or during hemodialysis resulted in no loss of enzyme activity into the dialysate using either low- or high-flux dialyzers. Similarly, the amount of enzyme lost in the peritoneal fluid is minimal (about 3.0 μg) and is mainly detected in the first 2 dialysate draining bags (personal data).

DR. DIMITRIOS TSAKIRIS (Consultant Nephrologist, Director, Department of Nephrology, General Hospital of Veria, Veria, Greece): Experience from the ERA-EDTA Registry showed that transplantation is not contraindicated in patients with Fabry disease, as both graft and patient survival were comparable to graft and patient survival in patients with other primary renal diseases [12]. That study, however, did not show meaningful results with regard to two points: (1) improvement of α-Gal A activity after transplantation, and (2) possible recurrence of the disease in the graft. Could you comment on these two points? Also, do Fabry disease patients with a renal transplant benefit from ERT?

DR. SIAMOPOULOS: The paper you mentioned [12] along with another more recent report from the United States Renal Data System registry [60] indicate a clear benefit of transplantation in Fabry disease with excellent outcomes. Successful renal transplantation corrects renal function, and the engrafted kidneys function normally for several years in the absence of clinical signs or loss due to recurrence [60, 61], although isolated GL-3 deposits have been detected histologically [62]. However, although the normal α-Gal A activity of the graft catabolizes endogenous renal glycosphingolipid substrates, the enzyme is ineffective in correcting the systemic metabolic
abnormality [6]. Transplantation of kidneys from Fabry carriers should be avoided, as these grafts can contain GL-3 deposits.

Regarding your question about ERT in these patients, no studies have been reported of ERT in patients with Fabry disease who have received a kidney transplant or who are undergoing dialysis. According to a recent small study [abstract; Mignani R, et al, Nephrol Dial Transplant 18:633, 2003] in 6 patients with Fabry disease and cadaveric kidney transplantation who received agalsidase beta mainly for severe cardiomyopathy, ERT was found to be safe and effective and yielded improvement of symptoms, such as pain, and echocardiographic findings such as LVH.

DR. TSAKIRIS: Is there an international registry for Fabry disease patients that coordinates the answers to the many questions raised in this new era of ERT? The disease is very rare, the therapy is very costly, and it is applied by more than one speciality. Only an international registry will provide meaningful answers to novel questions and assist in the planning of prospective studies.

DR. SIAMOPOULOS: Thank you for this interesting comment. Recently a web-based, central data collection system was developed and a registry program established (www.fabryregistry.com). The objective of the Fabry registry is to (1) enhance the understanding of the variability, progression, and natural history in males and females, including the development of recommendations for monitoring patients; (2) optimize patient care; and (3) evaluate long-term effectiveness of ERT [abstract; Wanner C, et al, Nephrol Dial Transplant 18:634, 2003].

DR. ALKIS PIERIDES (Consultant Nephrologist; Director, Department of Nephrology, General Hospital of Nicosia, Cyprus): What is the optimal diagnostic approach for women from families with classic Fabry disease?

DR. SIAMOPOULOS: The detection of a female carrier by the α-Gal A assay is not reliable because some obligate heterozygotes have normal α-Gal A activity. Therefore, all females at risk for carrying the gene of Fabry disease should have molecular studies to detect the family’s private mutation [52].

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