The Primary Hyperoxalurias

Amy E. Bobrowski, MD, MSCI, and Craig B. Langman, MD

Summary: The primary hyperoxalurias (PHs) are rare autosomal-recessive inborn errors of metabolism. In the most severe form (type 1), recurrent kidney stones and progressive nephrocalcinosis lead to the loss of kidney function, accompanied by systemic oxalosis, and often requires dialysis and/or transplantation. The variety of genetic mutations leading to PH increasingly are being defined, resulting in the ability to diagnose most patients accurately via minimally invasive means. During and after definitive diagnosis, supportive therapies with pyridoxine supplementation, urinary crystallization inhibitors, and hydration should be used, but have varying success. Emerging information about the renal tubular and intestinal transport of oxalate is leading to increasing evidence to support the use of oxalate-degrading bacteria (probiotics) and enzymes in the treatment of PH. Organ transplantation historically has offered the only potential cure for PH, and may include kidney-alone, combined liver-kidney, or pre-emptive liver-alone transplantation. Exciting new approaches in the treatment of type 1 PH, however, are under investigation. These include the restoration of defective enzymatic activity through the use of chemical chaperones, hepatocyte cell transplantation, or enzyme replacement by recombinant gene therapy. These novel approaches illustrate the goal for the ideal treatment of PH: correcting the genetic defect without exposing patients to the life-long risks associated with organ transplantation.

Semin Nephrol 28:152-162

Keywords: *Alanine:glyoxylate aminotransferase, glyoxylate reductase/hydroxypyruvate reductase, hyperoxaluria, kidney stones, peroxisomal disorders, probiotics, pyridoxine, systemic oxalosis*

The diseases termed *primary hyperoxalurias* (PHs) represent rare, autosomal-recessive, inborn errors of metabolism in which one of several specific enzyme defects result in the endogenous overproduction of oxalate. The subsequent increase in blood oxalate is counterbalanced by increased kidney excretion, with perhaps the gastrointestinal tract, based on recent evidence, playing a role as well to offset the potential increase in blood oxalate levels. Untreated, these diseases lead to recurrent calcium oxalate nephrolithiasis and progressive nephrocalcinosis, most often with a progressive decline in glomerular filtration rate. As kidney function declines, this in turn leads to an increase in plasma oxalate concentration above its threshold for calcium-oxalate supersaturation (>30 μmol/L), and subsequent deposition of calcium oxalate crystals in solid organs, termed *systemic oxalosis*. Oxalosis commonly involves the bones, bone marrow, myocardium, retina, and joints, but no tissue may be spared.

There are 2 known types of PH. Type 1 (PH1) is caused by a deficiency in the function of the liver-specific peroxisomal enzyme, alanine:glyoxylate aminotransferase-1 (AGT1), and thereby results in excessive generation of oxalate from disordered glyoxylate metabolism (Fig. 1). Disease severity in PH1 varies widely. The course may be mild and fully responsive to medical therapy such as vitamin B_6, or may present aggressively in infancy with rapid kidney failure and severe systemic manifestations. Type 2 (PH2) results from a deficiency of activity in the enzyme glyoxylate reductase/hydroxypyruvate reductase (GRHPR) (Fig. 1), which is distributed more widely in human beings than

Feinberg School of Medicine, Northwestern University, Chicago, IL, and the Division of Kidney Diseases, Department of Pediatrics, Children's Memorial Hospital, Chicago, IL.

Address reprint requests to Amy E. Bobrowski, MD, MSCI, Feinberg School of Medicine, Northwestern University, Division of Kidney Diseases, Department of Pediatrics, Children's Memorial Hospital, 2300 Children's Plaza #37, Chicago, IL 60614.

0270-9295/08/$ - see front matter

 doi:10.1016/j.semnephrol.2008.01.008

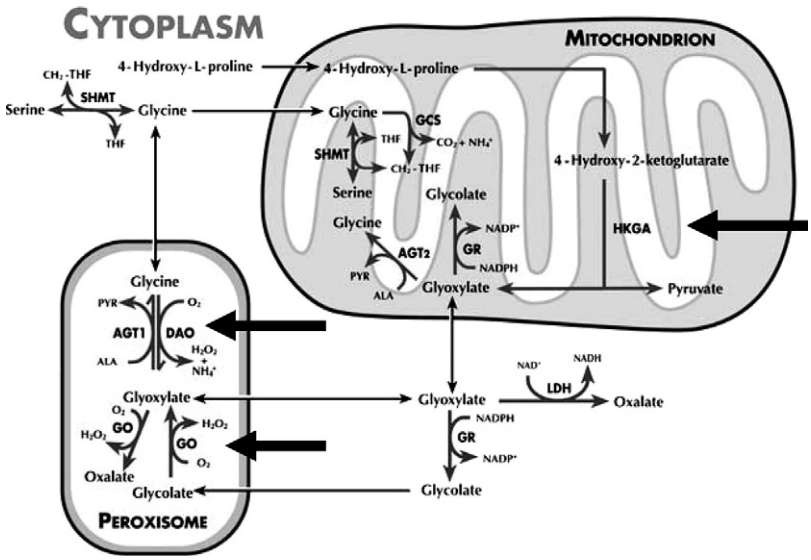

Figure 1. Oxalate synthesis in hepatocytes, highlighting 3 potential sites/steps where the production of the major oxalate precursor, glyoxylate, could be decreased (thick arrows). DAO, D-amino acid oxidase; GCS, glycine cleavage system; GO, glycolate oxidase; GR, glyoxylate reductase; HKGA, 4-hydroxy-2-ketoglutarate lyase; LDH, lactate dehydrogenase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate. Reprinted with permission from Baker et al.[33]

AGT1, with a predominance in muscle, liver, and kidney. As a group, patients with PH2 seem to have less morbidity and mortality than those with PH1, with a lower incidence of end-stage kidney disease (ESKD) and an older age at onset of symptoms.[1,2] In addition, there appear to be a small number of patients who fit the diagnostic criteria for having primary hyperoxaluria, but in whom the 2 enzymes described earlier have been found to be fully functional when studied in vitro. This group constitutes about 7% of the 95 PH patients reported in the Mayo Clinic's International Registry for Primary Hyperoxaluria, and such patients are termed as either *unclassified PH*, a term we prefer, or *type III PH*.[3,4]

THE GENETICS OF PH

The gene that encodes AGT (*AGXT*) is located on chromosome 2q37.3. To date, at least 83 mutations have been described in this gene that either eliminate, or decrease substantially, enzyme activity. The crystal structure of AGT has been deduced to within several angstrom, and we have learned the mechanism whereby the more common mutations in *AGXT* lead to reduced or absent enzyme activity. Some of these mutations act by mistargeting the gene product from the peroxisome to the mitochondria, where it is inactive because glyoxylate cannot enter this organelle, whereas other mutations lead to protein misfolding, or enhanced degradation, and lack of function.[5]

The most common *AGXT* mutation found in patients with PH1 is G170R, which results in mitochondrial location of AGT. This mutation was found in 37% of 64 PH1 patients (55 probands) from the Mayo Clinic that recently were analyzed by Monico et al,[6] using comprehensive sequencing of the gene. This same study found the second-most common mutation to be c.33_34insC, with a frequency of 11%. F152I and G156R had frequencies of 6.3% and 4.5%, respectively. In this population, only 2.7% of the mutations represented the I244T mutation, which previously has been reported as the third most common molecular change in PH1. However, it seems that affected patients in the Canary Islands have a predominance of the I224T mutation, suggesting a founder effect. Overall, this 2007 publication reports 28 new variants in *AGXT*, with the highest frequencies of mutations being located on exons 1, 4, and 7.[6]

P11L is the most prevalent of 3 known minor allele variants, which alone result in reduced AGT activity, but without apparent clinical con-

sequence. The minor allele frequency ranges from 2% to 28% in general populations,[7] but is as high as 50% in the PH1 population.[8] Protein aggregation occurs[9] and clinical disease ensues when this minor allele is co-expressed (cosegregates) with 1 of at least 4 common mutations (G41R, G170R, F152I, or I244T).

GRHPR, the defective enzyme in PH2, is encoded by a gene (*GRHPR*) located on chromosome 9p11. Thirteen mutations have been identified, all leading to a loss of enzyme expression or function. The most common mutation in PH2 is 103delG, which is a frameshift mutation that results in a premature stop.[10] It is present in about 40% of patients with PH2.[11]

A SUGGESTED DIAGNOSTIC APPROACH TO PATIENTS WITH SUSPECTED PH

Patients with PH often present with urolithiasis, nephrocalcinosis, or recurrent urinary tract infections. Unfortunately, up to a third of patients in some case series present only at ESKD, when uremia develops.[4,12,13] For this reason, we recommend that PH be considered in patients with recurrent calcium oxalate nephrolithiasis, unexplained nephrocalcinosis, or unexplained ESKD in which the kidneys are echodense with calcium. Table 1 describes the frequency of various clinical presentations of PH.

Once the diagnosis of PH is suspected, the analysis of urine glycolate and L-glyceric acid levels can be helpful at the initiation of the evaluation because they can distinguish PH1 (increased urinary glycolate) from PH2 (increased L-glyceric acid). However, these diagnostic findings are not always present, with up to one third of PH1 patients having normal urinary glycolate levels, and, of course, are not available in patients with ESKD. For this reason, the definitive diagnosis of PH rested, until recently, with the use of a liver biopsy for in vitro measurement of AGT and GRHPR immunopresence and enzymatic activity. This now may be supplanted with the use of molecular diagnostic techniques.

Table 1. Clinical Presentations of PH1

	Frequency
Urolithiasis	71%[13]–90%[4]
Nephrocalcinosis	48%[4]–60%[13]
Recurrent urinary tract infection	40%[13]
Uremia/ESKD	11%,[50] 30%,[12] 34%[13]

The relative ease in modern laboratory medicine with performing sequence analysis, and the delineation of the molecular basis of many of the mutations behind PH1 and PH2, as described earlier, has led to the proposal of molecular diagnostic algorithms that may obviate the need for invasive biopsy procedures. In 2004, Rumsby et al[14] described a method of restriction enzyme-based screening for the 3 most common *AGXT* mutations thought to be relevant at the time (G170R, c.33_34insC, I244T) and the one most common *GRHPR* mutation noted earlier. This method was able to identify the approximate one third of the liver-biopsy–proven PH patients in the reported population who were homozygous or compound heterozygous for one of these mutations. At least one mutant allele was found in 62% of the PH1 patients in that study.[14] The same investigators have reported more recently on the utility of selected exon sequencing (exons 1, 4, and 7) for 300 PH1 patients. Therein, the investigators were able to find 2 mutations in 50% of the subjects, and at least 1 mutation in 75%, thereby increasing the diagnostic sensitivity for PH. Twenty-nine sequence changes were found, of which 15 were novel.[15]

Interestingly, Monico et al[6] recently reported comprehensive mutation screening across the entire *AGXT* coding region in 55 probands with PH1. In this population, 2 disease alleles were detected in 96%, and at least 1 allele was detected in 98%. When limited to sequencing of exons 1, 4, and 7, the sensitivity was 77%. Given the relatively small size of the gene, the investigators advise that complete molecular analysis does not involve prohibitive expense. An algorithm beginning with limited sequencing of exons 1, 4, and 7, followed by direct sequencing of the entire gene if inconclusive, would make intuitive sense, and would eliminate the need for liver biopsy in most patients.[8] As a rule, pyridoxine (vitamin B_6) therapy

should be initiated (see later) while this diagnostic process is initiated, to ascertain early in the clinical course whether a patient will be pyridoxine-responsive.

A THERAPEUTIC APPROACH TO PATIENTS WITH PH1

Unfortunately, no randomized controlled trials exist to support any particular treatment protocol in the management of PH1. Although great strides have been made in the molecular biology underlying PH1 and its utility in diagnosis, treatment options have remained largely unchanged over several decades. Available therapies have focused on supportive treatments to decrease oxalate production, to increase urine calcium oxalate solubility, and to decrease crystal deposition within the kidney. Ideally, the urinary concentration of oxalate should be lowered to less than 0.45 mmol/d, and urine calcium should be maintained at less than 4 mg/kg/d. Unfortunately, despite optimal medical management, ESKD often follows.

Pyridoxine (vitamin B_6) is a necessary cofactor for optimal AGT1 activity. A review of the available PH1 literature to date, albeit with protocols for determining B_6 responsiveness not coordinated across different investigators, reveals that about 20% of PH1 patients have a normalization of urine oxalate concentration with the use of some dose of pyridoxine, and an additional 30% of patients have a partial response (defined conservatively as a >30% reduction in urine oxalate). Fully 50% of patients have no response at all.[16] In one study, the use of AGT1 mutation analysis resulted in the ability to predict, retrospectively, pyridoxine responsiveness, with patients homozygous for either G170R and F152I genotypes (which have residual AGT activity) being associated with responsiveness to pyridoxine therapy and relative preservation of residual kidney function,[17] when compared with nonresponders. Although patients homozygous for the G170R mutation may have normalization of oxalate excretion with treatment, another study found that heterozygous patients had only a partial reduction. A dose of up to 5 mg/kg/d of pyridoxine was sufficient to show these responses in this population.[18] We suggest that pyridoxine dosing should be started at 1 to 2 mg/kg/d, with increases in steps every 2 months if needed. This caution is the result of a severe sensory neuropathy that is associated with excessive doses.[19]

Crystallization inhibition is another mainstay in the therapy of PH1. Doses of citrate of 100 to 150 mg/kg/d, in the form of potassium or sodium citrate, have been shown to decrease urinary calcium oxalate supersaturation and stabilize kidney function in small numbers of PH1 patients.[20] Alkalinization with sodium bicarbonate, using a target urine pH of 6.2 to 6.8, also will increase urine citrate. *Orthophosphate* also has been used for this purpose in doses of 30 to 40 mg/kg. In combination with pyridoxine therapy, one study found a reduction in urinary supersaturation for calcium-oxalate, and longer ESKD-free patient survival rates (74% at 15-20 y), when compared with historical controls.[21]

Dietary restriction of foods high in oxalate (eg, rhubarb, coffee, tea, nuts, spinach, and strawberries) often is advised in patients with PH. The efficacy of this advice is limited, however, because urinary oxalate levels reflect excessive endogenous production much more than dietary oxalate consumption and subsequent absorption. However, calcium-rich foods should be encouraged because this mineral complexes with oxalate in the intestine to form insoluble, nonabsorbable complexes. Conversely, the use of supplemental vitamins containing vitamin C should be cautioned against because ascorbic acid is metabolized to oxalate, and can lead to a substantial increase in oxalate burden in PH patients. Most importantly, high fluid intake should be encouraged, with a goal of greater than 2 L/m^2 body surface area/d. This may require nasogastric or gastrostomy tube–based hydration strategies in infants and young children.

FUTURE DIRECTIONS IN THE TREATMENT OF PH1

Several major developments in the biology of oxalate have led to promising new directions in therapy: the demonstration of specific transport-mediated and bidirectional pathways for oxalate in discrete segments of the intestine, an understanding of the role of probiotics in the intestinal handling of oxalate, an enhanced un-

derstanding of the tubular toxicity of oxalate, and the solving of the crystal structure of AGT1.

Oxalate Transport

New information on the role of renal tubular and intestinal transport mechanisms in oxalate handling has been obtained from studies in knockout (KO) mice that lack a specific anion transporter, Slc26a6. Among other anions, this transporter acts to mediate chloride-oxalate exchange in kidney proximal tubular and intestinal epithelial cells. New information from this model illustrates how the intestine serves an excretory function for oxalate, and in whom the absence of the Slc26a6 transporter leads to hyperoxaluria. Slc26a6 KO animals have a high incidence of calcium oxalate stones, marked hyperoxaluria, and their proximal tubule brush-border membranes are deficient in the transport of oxalate seen in preparations from wild-type mice.[22] Freel et al[23] were able to show a net oxalate absorption across isolated ileum from Slc26a6 KO mice, as compared with a net secretion of oxalate across the wild-type ileum. Urinary oxalate excretion was also 4 times greater in the null mice as compared with their wild-type littermates.[23]

Role of Probiotics

An exciting discovery about the potential role of *Oxalobacter formigenes* in the treatment of PH also has shed some light on the role of the intestine in maintaining oxalate balance. This area of investigation, the use of the intestine to alter oxalate balance when kidney excretion alone is inadequate, is now among the therapeutic options on the horizon for the treatment of PH that is perhaps the most exciting and immediately applicable. Studies in rats have shown that intestinal colonization with *O formigenes* can induce colonic secretion of oxalate, in part by producing a favorable concentration gradient through oxalate degradation.[24] This has led to the investigation of the use of this anaerobic bacterium as a probiotic, or, in another approach, to the use of specific oxalate-degrading enzymes taken in a stable oral form, to degrade endogenously produced oxalate that has been secreted into the colon. Hoppe et al[25] reported on the efficacy of oral *O formigenes* administration to human beings with PH1, with the majority of subjects with normal kidney function showing a 22% to 92% reduction in urinary oxalate. Two of 3 dialysis patients had a significant reduction in plasma oxalate levels as well, and with improvement in clinical symptoms. These data suggest that this therapy could be useful at any stage of PH disease. By using the same idea for the intestine to be responsible for altering oxalate balance in hyperoxaluric conditions, a highly specific oxalate-degrading enzyme, formulated as cross-linked crystals, is being studied in mice with diet-induced enteric hyperoxaluria, and in ethylene glycol–challenged AGT1 KO mice (a mouse model of genetic PH1). Four weeks of oral treatment led to a 25% to 50% reduction in urinary oxalate excretion in both models, as compared with controls.[26] Long-term studies are in progress, but it is hoped that this formulation may provide stable activity in the gut environment, lending to sustained clinical efficacy. Interestingly, it is possible that such enzymatic treatments promote gut secretion of oxalate by encouraging oxalate transport down a concentration gradient from blood to intestinal lumen, as intraluminal oxalate is degraded. Therefore, one might hypothesize that the Slc26a6 transporter discussed earlier may play a role in mediating this gradient-induced transport.

Oxalate Toxicity in the Renal Tubules

Another possible adjunctive target for therapy of PH would be to limit the renal tubular epithelial toxicity induced by calcium oxalate crystals in the hyperoxaluric state. Antioxidant therapy with vitamin E, given to rabbits with ethylene glycol–induced hyperoxaluria, showed a decrease in epithelial cell apoptosis.[27] Unfortunately, no human clinical studies have yet shown any benefit in preventing renal damage or progression of kidney failure in hyperoxaluria. Another potential agent, pyridoxamine, is an inhibitor of advanced glycation reactions. It acts by scavenging reactive carbonyl products that result from glucose and lipid degradation, and thus far has been investigated as a potential therapy for diabetic nephropathy.[28] It is hypothesized that pyridoxamine also may have

the ability to scavenge carbonyl intermediates produced in the glyoxylate metabolic pathway, resulting in a reduction of oxalate production by limiting its precursor. In fact, pyridoxamine treatment in a rat model of ethylene glycol–induced hyperoxaluria did result in a reduction of calcium oxalate crystallization in the kidneys of treated rats, as well as lower levels of urinary oxalate, when compared with untreated animals.[29] Human trials of pyridoxamine treatment in hyperoxaluria are ongoing, and we await proof-of-principle results eagerly.

Crystal Structure of AGT1

Crystallization studies of the proteins encoded by known *AGXT* mutations have revealed potential approaches that may result in the ability to convert some of these proteins into functional conformations. Certain mutation combinations that result in peroxisomal-to-mitochondrial mistargeting or cytosolic aggregation and degradation have been shown to be correctable in vitro with the addition of nonspecific, chemical chaperones such as glycerol,[30] betaine,[31] or with decreased temperature. The utility of chaperones in vivo await design and testing, but based on other diseases may be available for PH as well.[32]

Precursor Depletion

Other potential interventions in the therapeutic future for oxalosis could include a more targeted interruption of oxalate synthesis. Currently, no clearly defined functional roles have been established for either oxalate or the metabolic pathways leading to its formation. Because glyoxylate is the major precursor of oxalate, inhibiting its production should lead to a decrease in endogenous oxalate production that potentially could be clinically relevant. Glyoxylate production could be inhibited at 3 sites: (1) the oxidation of glycolate by peroxisomal glycolate oxidase; (2) the oxidative deamination of glycine by peroxisomal D-amino acid oxidase; and (3) the metabolism of hydroxyproline by a mitochondrial pathway[33] (Fig. 1). These approaches await proof-of-principle testing as well.

THE ROLE OF DIALYSIS IN PH1

In PH1 patients with progressive loss of glomerular filtration rate (progressive chronic kidney disease), dialysis might be initiated before the development of ESKD. The risk for systemic oxalosis increases significantly once plasma oxalate concentrations are greater than 30 μmol/L, and such levels may occur at clearances as high as 25 to 30 mL/min/1.73 m^2.[34] Systemic oxalosis should be avoided if at all possible because the accumulated body oxalate burden determines the long-term outcomes of patients with PH1.[35]

Conventional maintenance hemodialysis (HD) schedules of 3 times a week procedures are not sufficient to compensate for continued increases of endogenous oxalate production. Dialyzer membranes with large surface areas and composed of high-flux polysulfone or cellulose triacetate have been found to have the highest in vitro clearances of oxalate.[36] Unfortunately, no in vivo comparisons of dialyzer oxalate clearances exist. Daily HD sessions of 6 to 8 hours' duration would be ideal, although not practical. One case report does describe the use of daily high-flux HD sessions of 6 to 7 hours for 67 consecutive days in an 18-year-old girl with PH1 and severe systemic oxalosis. Her plasma oxalate concentrations and systemic complications were normalized in this way before liver-kidney transplantation (LKT).[37] This case, however, is clearly exceptional.

Peritoneal dialysis (PD) also does not appear to provide an optimal method of oxalate clearance. One small comparison of PH1 patients on HD (5 hours 3 times/wk) and PD (4-5 daily exchanges) showed similar mean weekly oxalate removal. However, plasma oxalate levels remained increased in all of the PH1 patients (70-148 μmol/L), regardless of dialysis modality.[38] Combinations of intermittent HD and nightly PD also have been attempted, with varying success reported anecdotally.

SOLID ORGAN TRANSPLANTATION IN PH1

Thus far, only successful liver transplantation, with concomitant removal of the oxalate-overproducing native liver, has offered a potential

Table 2. Patient and Graft 5-Year Survival Rates by Organ Transplanted for PH

	USRDS 1988–1998[40]	International Registry–Mayo Clinic[53]	European Registry[13,35]
Patient survival			
Kidney alone	80%	89% (4.5 y)	10%–50% (5–10 y)
Combined	80%	78% (3.5 y)	80%
Graft survival			
Kidney alone	47.9% (8 y)	36%	17%–23% (1990 data)
Combined	76% (8 y)	52%	72%

As compared with 5-year patient survival rates of 76% to 80% for kidney transplants, 72% 5-year patient survival rate for liver transplants, 52% to 59% 5-year kidney graft survival rate, and 64% 5-year liver graft survival rates in patients with oxalosis, as per a query of the United Network for Organ Sharing database. These are inferior to the 80% to 90% patient and 65% to 79% graft survival rates at 5 years reported for those organs transplanted for other diagnoses.[33]

cure for the enzyme defect causing PH1 in those patients unresponsive to pyridoxine therapy. If performed before kidney failure, oxaluria will continue until body tissue–accumulated stores are excreted fully, and this occasionally may last up to more than 1 year after liver transplantation. Therefore, supportive treatment for hyperoxaluria must continue during this time to prevent further kidney damage. Kidney transplantation by itself, on the other hand, will increase kidney clearance oxalate, but the graft remains at high risk for disease recurrence by virtue of the excess urinary concentration of oxalate.

Kidney-alone transplantation in PH1 has an overall long-term kidney graft survival of only 20% to 50%, and ranks much lower than for either living-related or deceased-donor kidney transplantation survival rates in non–PH-affected recipients. In fact, our query of the United Network for Organ Sharing database on 228 kidney transplants between 1992 and 2005 revealed a 52.1% 5-year living-donor graft survival in oxalosis patients, as compared with a 78.7% 5-year living donor graft survival in all patients receiving a kidney transplant during that time.[39] Similarly, a study looking at the death-censored renal graft survivals in 190 hyperoxaluric patients in the US Renal Data System (USRDS) transplant registry, transplanted between 1988 and 1998, showed only a 47.9% death-censored graft survival.[40] This is consistent with the European experience, in which patient survival rates for isolated kidney transplantation ranged from 10% to 50%.[35,41] This appears to be a fairly consistent experience despite the use of crystal inhibition therapy, high fluid protocols, and pyridoxine therapy. Intuitively, kidney-alone transplantation still may be a feasible option for patients with confirmed mutations denoting pyridoxine responsiveness, but such a protocol has not been studied prospectively.

Despite some differences in comparisons of patient survival rates, LKT for PH1 does appear to provide a definitive graft-survival advantage over kidney-alone transplantation, despite the added intraoperative and postoperative morbidity associated with liver transplantation compared with kidney transplantation (Table 2). The European experience found that overall LKT patient survival rates ranged from 69% to 86%.[35,41] In addition, the USRDS registry data report a death-censored graft survival rate of 76% in those receiving LKT between 1988 and 1998, which was significantly better than the 47.9% reported in the kidney-only recipients ($P < .001$).[40] Overall health condition and degree of systemic oxalosis at the time of transplantation seems to affect these outcomes, with superior 5-year patient survival rates (100%) in patients described in one study to be in good condition by their physicians at the time of transplant. Those with a history of prolonged dialysis or complications related to systemic oxalosis had inferior survival rates.[35]

In addition, a French series detailing 8 children with PH1, and receiving LKT between 1990 and 2000, reported 2 patients who died

intraoperatively, whereas the other 6 had normal urine oxalate excretion and were deemed healthy 5 to 11 years later. Notably, 3 patients with oxalosis involving the bones had a complete healing of their bony lesions on follow-up evaluation.[42] An Israeli series of 9 LKTs reported 2 deaths during the immediate postoperative period. The remaining 7 patients had normal urinary oxalate excretion and were deemed healthy at 6 to 54 months of follow-up evaluation.[43] Overall, based on a limited worldwide experience, we suggest that if perioperative mortality can be avoided, LKT is a successful and definitive treatment for PH1.

LKT can be either sequential or simultaneous, and either living or deceased donor sources may be used. Simultaneous combined transplantation may confer some immunologic benefit, with lower rates of acute rejection seen in simultaneous versus sequential recipients in one small series.[44] Simultaneous living donor LKT would seem ideal to maximize the potential benefit to the recipient, although the possibility of surgical morbidity to the single, living donor cannot be ignored. A case report of at least one such successful LKT from a living donor has been published.[45]

Pre-emptive liver transplantation is practically difficult to achieve in the United States because the scarcity of donor livers often delays transplantation until significant kidney damage has taken place, and kidney transplantation often is needed to provide optimal systemic oxalate clearance. However, if liver transplantation is performed before the occurrence of significant renal dysfunction, the complications of systemic oxalosis may be prevented. This approach is of course controversial because liver transplantation and its associated immunosuppressive therapy are risky, and the time course of PH disease progression is impossible to predict, even when disease genotype is known. Small case series from Germany and Israel have reported on a total of 7 children with PH1 receiving pre-emptive liver transplantation. In the German cohort, plasma and urinary oxalate levels normalized quickly, and kidney function remained stable over 3 to 6 years of follow-up evaluation.[46] Over 3 years of follow-up evaluation in the Israeli group, 1 of the 3 patients had a slight decrease in kidney function, but the remainder had improved or stable function.[43]

FUTURE DIRECTIONS OF GENE REPLACEMENT THERAPY FOR PH1

To date, whole liver transplantation has been the only feasible form of gene or enzyme replacement therapy available to potentially cure PH1, although with the drawback of converting the patient's condition to that of a chronic organ transplant patient, with all of the concomitant possible complications. The development of an AGT1 knockout mouse to serve as an animal model of PH1, however, has led to novel approaches to other methods of functional enzyme replacement.

Attempts at hepatocyte cell transplantation have been challenging because native liver cells, if remaining functional, continue to overproduce oxalate. The number of cells that can be transplanted feasibly in a single procedure is too small to compensate for this, and multiple procedures with multiple donors would increase rejection risks. Methods are therefore being studied to encourage the preferential proliferation and survival of transplanted hepatocytes, while inducing cell death in native hepatocytes. AGT1 KO mice given transplanted wild-type hepatocytes, in conjunction with hepatocyte growth factor and pretransplant preparative native liver irradiation, have been described to have a 90% to 95% wild-type hepatic cell repopulation rate over the liver cells containing the mutant AGT. These animals also had a significant decrease in urinary oxalate:creatinine ratios 8 weeks after the procedure. Partial native hepatectomy and apoptosis-inducing monoclonal antibodies were not as effective.[47]

Provision of recombinant AGT by gene replacement therapy also has had some recent success in vitro. Amplified *AGXT* complementary DNA has been cloned and fused with green fluorescent protein to trace its distribution when transferred via liposomes into hepatocytes. Such transfection was shown to result in localization of the recombinant gene product to peroxisomes, with an efficiency of 60% to 90% in one study.[48] Such novel approaches to correcting the underlying defect in PH1 by less

invasive means than whole liver transplantation hopefully will lead to human-based trials in the near future.

THERAPIES FOR PH2

Overall, PH2 is a less severe disease than PH1, based on our own anecdotal experience and the few reports about it. The treatment options for PH2 include many of the supportive measures used in PH1 to prevent calcium oxalate crystallization. These may include potassium or sodium citrate supplementation, magnesium administration, dietary oxalate restriction, and maintenance of a high fluid intake. Theoretically, oral probiotic or degradative enzyme therapies also could prove to be beneficial in promoting transport and excretion of excess oxalate in the gut in these patients, although this hypothesis has not yet been tested experimentally or in human beings.

Pyridoxine (vitamin B_6) is not a cofactor for the defective enzyme in PH2 (GRHPR), so it probably is not useful in the treatment of this disease. It has been hypothesized that pyridoxine may be able to reduce liver oxalate production by increasing AGT activity in those with normal AGT function, but this has not been tested rigorously in human studies.

In addition, because GRHPR is present in tissues outside of the liver, liver transplantation would have no specific curative role. At present, it is not clear how much of the GRHPR activity resides outside the liver in PH2 patients. Kidney transplantation, on the other hand, is the treatment of choice for the small minority of PH2 patients in whom ESKD does occur. With good supportive care posttransplant, this disease can have a far more favorable prognosis when compared with kidney-alone transplantation for PH1. One PH2 patient has been reported to have excellent long-term graft survival after kidney transplantation, with normal kidney graft function at 20 years of follow-up evaluation.[49]

CURRENT OUTCOMES AND PROGNOSIS FOR PH

As noted earlier, PH2 has less morbidity and mortality than PH1. PH2 patients form fewer kidney stones. Also, the incidence of ESKD is estimated at 12% in PH2, as compared with 54% in PH1. The mean age at symptom onset is also greater in PH2 patients (15.7 y) than in PH1 patients (9 y).[1,2] It is unclear what these numbers would be if these diseases were detected, diagnosed, and appropriately treated earlier because these data come from populations in which 11% to 30% of patients were not diagnosed before reaching ESKD.[50,51]

Unfortunately, despite marked advances in characterizing various mutations leading to PH1, attempts to correlate genotype to exact phenotype and prognosis have been unsuccessful to date. This difficulty is thought to be caused by the large number of mutations that have been found throughout the entire 11 exons of the *AGXT* gene, and the fact that most patients from nonconsanguineous families are compound heterozygotes.[51] Even in a very homogenous group of PH1 patients in the Canary Islands, where almost all patients are homozygous for the I244T missense mutation, phenotype was found to be variable. Onset of symptoms was as early as infancy in some, and as late as the fourth decade of life in others.[52] For this reason, the possibility of modifier genes and environmental factors (such as endogenous gut flora populations) affecting disease expression and progression are being investigated.[51]

LKT appears to afford the best long-term outcomes for PH1 patients who reach ESKD, provided that the systemic complications of oxalosis can be minimized before transplantation. The optimal timing of various dialytic and transplant options remains uncertain, and the effect of these choices on prognosis remains to be evaluated.

CONCLUSIONS

Primary hyperoxaluria, and PH1 in particular, often can be a very grave systemic disease. Its management can be challenging, and the outcomes often are poor. Currently, outcomes may be optimized with early and accurate diagnosis, including assignment of genotype where possible. Early initiation of supportive therapies, including adequate pyridoxine trials for proven or suspected PH1 and for certain genotypes, may help delay disease progression. Once significant organ dysfunction is present, however,

the timing of transplant procedures and the difficulties associated with dialysis remain a major challenge. Even when a PH1 cure is achieved through successful liver transplantation or LKT, the condition merely is traded for that of a transplant patient, with the morbidity and mortality associated with the life-long use of potent immunosuppressive medications. It is our hope that potential new therapies, already being tested in human trials, will prove helpful in the management of the primary hyperoxalurias by using the newly discovered transport function of the intestine to reduce body oxalate burden. Even more exciting, however, is the prospect of human therapies evolving from advances in the use of chemical chaperones to modulate the function of certain mutant forms of AGT, and from the possibility of using an adenoviral vector system for human AGT gene therapy. Ideally, the goal is to correct the underlying genetic defect causing PH, without subjecting patients to the life-long risk of organ transplantation.

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