Primary hyperoxaluria: from gene defects to designer drugs?

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Enzyme deficiencies and metabolic abnormalities

Primary hyperoxaluria is a name given to a group of hereditary disorders characterized by increased synthesis and excretion of the metabolic end-product oxalate, and deposition of insoluble calcium oxalate (CaOx) in the kidney and urinary tract [1]. Only two of the primary hyperoxalurias have been well characterized—type 1 (PH1, MIM 259900) and type 2 (PH2, MIM260000). PH1 is caused by a deficiency of the liver-specific peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT, EC 2.6.1.44), and PH2 is caused by a deficiency of the cytosolic/mitochondrial enzyme glyoxylate/hydroxy-pyruvate reductase (GR/HPR, EC 1.1.1.26/79) [2]. Although the mutant genes and the dysfunctional enzymes are different in PH1 and PH2, the clinical outcome is very similar (i.e. hyperoxaluria, urolithiasis and/or nephrocalcinosis) (Figure 1). Especially in the case of PH1, renal CaOx deposition is likely to lead to kidney failure, after which the combined effects of increased oxalate synthesis and failure to remove it from the body allow widespread deposition of CaOx throughout the body. In addition to hyperoxaluria, PH1 patients often have hyperglycolic aciduria and PH2 patients hyper-l-glyceric aciduria. However, the calcium salts of glycolate and l-glycerate are soluble and, therefore, do not result in any pathology.

Genotype–phenotype correlations

AGT is encoded by the AGXT gene located on chromosome 2q37.3 [3,4], and GR/HPR is encoded by the GRHPR gene located in the pericentromeric region of chromosome 9 [5,6]. Over 50 mutations have been identified in the AGXT gene and >12 in the GRHPR gene. The enzyme phenotype of PH2 appears to be fairly uniform, with most patients having a complete, or nearly complete, absence of both GR/HPR catalytic activity and immunoreactivity. In PH1, however, the enzyme phenotype is much more varied, some patients having no AGT catalytic activity or immunoreactivity, some having no AGT catalytic activity but normal, or only slightly reduced, levels of AGT immunoreactivity, and yet others having significant levels of both AGT catalytic activity and immunoreactivity [7]. In these latter patients, PH1 is caused by an unparalleled intracellular protein trafficking defect in which AGT is mistargeted from its normal location in the peroxisomes to the mitochondria [8].

Most of the mutations identified in the GRHPR gene can be categorized as insertions, deletions or splice site mutations. Loss of meaningful coding sequence means that, in most cases, GR/HPR is not synthesized or, if it is, it is rapidly degraded. Although many mutations in the AGXT gene also fit into this category, about half are point missense or nonsense mutations. Many of the missense mutations can be related to specific enzyme phenotypes. For example, Ile244Thr is associated with aggregation and accelerated degradation [9,10]; Gly41Arg is also associated with accelerated degradation as well as intra-peroxisomal aggregation [11]. Gly82Glu leads to loss of AGT catalytic activity due to blockage of cofactor (pyridoxal phosphate) binding [9]. Gly170Arg, which is the most common mutation in PH1 accounting for 30–40% of mutant alleles, is found in patients with the peroxisome-to-mitochondrion AGT trafficking defect [12].

In PH1, the relationship between genotype and enzyme phenotype is often complicated by the presence or absence of a common Pro11Leu polymorphism [12]. A number of mutations, including...
Gly170Arg and Ile244Thr, not only segregate with this polymorphism, but also actually require its presence on the same allele in order to achieve their untoward effects [9].

The determination of the crystal structure of AGT has enabled the effects of many of the missense mutations and polymorphisms found in PH1 to be rationalized in terms of their likely effects on AGT conformation and stability [13] (Figure 2). For example, it explains how the combined presence of Pro11Leu and Gly170Arg inhibits AGT dimerization and redirects it away from the peroxisomes towards the mitochondria. It explains how Gly82Glu prevents the cofactor from binding to the active site of AGT, and it suggests a mechanism by which Gly41Arg might block AGT dimerization, leading to its aggregation into peroxisomal cores.

Treating the symptoms

The classic treatments for PH1 and PH2 target the more downstream components of the disease process, in other words the symptoms [1]. Among other things, these treatments are aimed at decreasing the solubility product of CaOx in the urine (by increasing fluid and decreasing oxalate intake), decreasing the chances of CaOx crystallization, crystal growth and crystal adherence to the tubule or collecting duct wall (by the use of crystallization inhibitors), removal of the CaOx accretions once formed (by lithotripsy or surgery), and dealing with the effects of kidney failure (by dialysis and kidney transplantation). Although potentially life saving, at least in the short term, these treatments at best slow down the rate of disease progression, rather than cure it. This is because they do not tackle the basic defects (i.e. AGT deficiency in the case of PH1 and GR/HPR deficiency in PH2). Kidney transplantation, especially, is problematic because even though the new kidney may very well provide an immediate solution to the problem of kidney failure, it is highly likely to succumb to the ravages of CaOx deposition, as did the original organ.

Treating the causes

Although treatments aimed at the symptoms of PH1 and PH2 have the advantage that they are ‘catch-all’ (i.e. they address the problem of increased urinary oxalate excretion and its consequences, irrespective of their causes), they can only be temporary holding operations, as the basic causes of the diseases still continue to operate. On the other hand, whereas treatments targeted at the causes of the diseases are
more likely to provide long-term benefits, they are more likely to require specific tailoring to the disease in question (Figure 1).

Pyridoxine therapy

A minority (10–30%) of PH1 patients are responsive to pharmacological doses of pyridoxine. Its efficacy at reducing oxalate excretion seems to be restricted to those patients who have disease due to peroxisome-to-mitochondrion AGT mistargeting [14,15]. In the body, pyridoxine is metabolized to pyridoxal phosphate, the essential cofactor of AGT. Although its therapeutic mechanism of action is almost certainly related to its role as a cofactor, how it works at the molecular and cellular levels is unclear. This form of treatment would be completely inappropriate for PH2 patients for the simple reason that GR/HPR does not require pyridoxal phosphate as a cofactor.

Enzyme replacement therapy

Both PH1 and PH2 are caused by single (but different) enzyme deficiencies and, as such, are potentially treatable by enzyme replacement therapy (ERT). The discovery that almost all the body’s AGT is concentrated in the liver opened up the possibility that liver transplantation could be used as a form of ERT. Such a procedure has a number of benefits over more conventional forms of ERT. In particular, liver transplantation is expected to reintroduce most of the body’s requirements for AGT, already in the correct cell and intracellular compartment [16]. Over the past 20 years, several hundred liver transplantations have been carried out in PH1 patients throughout the world. Because most patients were in renal failure at the time, most procedures have been combined liver–kidney transplantations. Liver transplantation is able to correct the metabolic defects in PH1 (i.e. elevated oxalate and glycolate synthesis and excretion) [17–20] and reverses at least some of the
pathological consequences of chronic CaOx deposition [1,21]. Rapid metabolic normalization is demonstrated by the immediate return to normal of urinary glycolate levels. However, oxalate excretion usually takes much longer to normalize due to the resolubilization of CaOx deposited throughout the body during periods of poor renal function or dialysis. Although liver transplantation would probably also work for PH2, it has yet to be carried out. One slight drawback is that, although GR/HPR is present in the liver at higher levels than found in other tissues, its distribution in the body is more widespread than that of AGT.

Gene therapy

Liver transplantation is really as much a form of gene therapy as it is a form of ERT [22]. However, from a philosophical point of view, it would appear to be rather wasteful, as tens of thousands of perfectly normal genes have to be replaced simply to replace one defective gene. One of the potential problems highlighted very early on in discussions about appropriate strategies for ‘more conventional’ gene therapy was the particular need to be able to transduce a large percentage of the patient’s hepatocytes with the normal AGT gene, rather than maximizing the total amount of AGT expressed in the liver as a whole [22]. Although no PH1 patients have received gene therapy to date, it remains a viable proposition for the future once better (i.e. more efficient) vectors have been designed. Gene therapy would also be expected to work for PH2. However, the more widespread distribution of GR/HPR, compared with that of AGT, would suggest that the gene would have to target more than just hepatocytes.

Chemical chaperones

It is now recognized that many, possibly most, missense mutations in human genetic diseases cause their effects by decreasing protein stability, leading to a multitude of downstream effects, such as aggregation, accelerated degradation and mistargeting. Several studies have shown that if the destabilizing effects of mutations can be overcome, for example by the use of chemical chaperones, then the newly stabilized, but still mutant, protein re-acquires its functional activity. In this respect, PH1 is no different. Structural analyses, as well as a number of biochemical studies, have suggested that many of the missense mutations in AGT, including the two most common mutation–polymorphism combinations (i.e. Gly170Arg + Pro11Leu and Ile244Thr + Pro11Leu), exert their effects, at least partly, by reducing AGT stability. Non-specific protein-stabilizing agents are able to normalize the mistargeting of AGT caused by Gly170Arg + Pro11Leu, as well as prevent the aggregation of AGT caused by Ile244Thr + Pro11Leu, at least in vitro [10,23]. Some missense mutations which produce their effects for reasons unrelated to protein stability, as well as nonsense mutations, insertions, deletions and splice site mutations would not be open to this approach. Unfortunately, this rules out about half the mutations in AGT in PH1, as well as most of the mutations in GR/HPR in PH2.

At the moment, the only ‘curative’ option for PH1 patients who are unresponsive to pyridoxine is liver transplantation. In the future, at least for those patients with the ‘right’ kind of mutations, pharmacological approaches to treatment might offer a real alternative. However, for this to be realized, much more research will need to be done to identify more specific chemical chaperones that bind to and stabilize AGT with very much greater affinity than the nonspecific agents used so far. Knowledge of the structure of normal AGT, and how it is perturbed by mutations and polymorphisms, should allow the design of high affinity small molecule drugs that will counteract these perturbations. Such designer drugs may remove the requirement for liver transplantation and diminish the need to develop high-efficiency gene therapy strategies.

Conflict of interest statement. None declared.

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Toll-like receptors recognize uropathogenic *Escherichia coli* and trigger inflammation in the urinary tract

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**Introduction**

Urinary tract infection (UTI) is one of the most common types of infection. In the USA, >US$1.6 billion per year are spent in the management of UTI [1]. Amongst the pathogens that can cause UTI, uropathogenic *Escherichia coli* (UPEC) has been found to be the causative organism in ~80% of cases. A number of pathogen-related virulence factors have been identified that relate mostly to pathogen adhesion to the epithelial lining of the urinary tract [2–4]. In addition, patient-related factors determine the prevalence and severity of UTI. Diabetes, immunosuppression, reflux and obstruction due to structural abnormalities of the urinary tract or pregnancy are known to predispose to UTI. These conditions affect either physiological urine flow or cellular immunity against uropathogenic agents. However, the patient-related risk for UTI may also be determined by pathogen recognition and pathogen-induced signalling for host defence. Host defence in UTI is based on innate immunity. Thus, which receptors recognize uropathogenic pathogens, and what are the subsequent signalling cascades that concert antibacterial immunity? The recent discovery of the Toll-like receptor (TLR) family has identified a group of such receptors that appear to have important functions not only for pathogen recognition but also for innate immune activation [5]. In this article, we present the current view on TLRs in UTI. Based on these data, we propose a working hypothesis...