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INVITED REVIEW

Molecular basis of primary hyperoxaluria: clues to innovative treatments

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Abstract

Primary hyperoxalurias (PHs) are rare inherited disorders of liver glyoxylate metabolism, characterized by the abnormal production of endogenous oxalate, a metabolic end-product that is eliminated by urine. The main symptoms are related to the precipitation of calcium oxalate crystals in the urinary tract with progressive renal damage and, in the most severe form named Primary Hyperoxaluria Type I (PH1), to systemic oxalosis. The therapies currently available for PH are either poorly effective, because they address the symptoms and not the causes of the disease, or highly invasive. In the last years, advances in our understanding of the molecular bases of PH have paved the way for the development of new therapeutic strategies. They include (i) substrate-reduction therapies based on small-molecule inhibitors or the RNA interference technology, (ii) gene therapy, (iii) enzyme administration approaches, (iv) colonization with oxalate-degrading intestinal microorganisms, and, in PH1, (v) design of pharmacological chaperones. This paper reviews the basic principles of these new therapeutic strategies and what is currently known about their application to PH.

Keywords Rare disorder · Primary hyperoxaluria · Drug discovery · Molecular basis of disease · Substrate-reduction therapies · Pharmacological chaperones

Introduction

In humans, oxalate is a metabolic end product that has to be excreted by urine [97]. The main risk associated with oxalate accumulation is the formation of the poorly soluble calcium oxalate (CaOx) salt, which is prone to crystallize and deposit as stones in the kidneys and urinary tract [6]. The excretion of more than 40–45 mg /24 h of urinary CaOx is considered pathological and gives rise to a clinical condition named hyperoxaluria [97]. Hyperoxaluria can be generally divided into two categories: (i) primary hyperoxalurias (PHs) are rare inborn errors of glyoxylate metabolism that result in a high endogenous oxalate production, mainly by the liver [27, 48, 55]; (ii) secondary hyperoxalurias (SHs) generate from an increased exogenous oxalate absorption, as a consequence of various alterations including intestinal inflammation, bariatric surgery or excessive intake of oxalate precursors [60, 85, 97]. Despite the fact that the clinical manifestations of PH and SH are qualitatively similar, and comprise recurrent urolithiasis and nephrocalcinosis, PHs are usually associated with a more severe phenotype and serious consequences for human health. In fact, the continuous CaOx deposition that occurs in the kidneys of PH patients in most cases leads to a remarkable kidney damage and to the consequent End Stage Renal Disease (ESRD). Under ESRD conditions, huge oxalate production is compounded by impaired oxalate elimination, thus causing a step-by-step increase of plasmatic oxalate, which in turn results in the accumulation of CaOx crystals in extra-renal tissues, such as in particular skin, retina, bones and heart. The latter state, named systemic oxalosis, is often fatal [9, 55].

In this review, we briefly describe the molecular bases of PHs and the new treatment strategies under investigation for the cure of this life-threatening disease.

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

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PHs are inherited disorders of glyoxylate metabolism

In mammals oxalate is mainly synthesized in the liver, and glyoxylate is thought to be its main precursor [53]. In human hepatocytes, glyoxylate is produced by two main pathways that take place in different cellular compartments, mitochondria and peroxisomes (Fig. 1). Mitochondrial glyoxylate arises from the catabolism of hydroxyproline, derived by either collagen turnover and the metabolism of proteins from animal food [63, 66]. 4-hydroxy-2-oxoglutarate aldolase (HOGA1), a liver-specific enzyme, is involved in the last step of hydroxyproline metabolism, and catalyses the conversion of 4-hydroxy-2-oxoglutarate (HOG) into glyoxylate and pyruvate [70, 80]. In mitochondria, glyoxylate can be further metabolized by the NADPH/NADH dependent glyoxylate reductase/hydroxypyruvate reductase (GRHPR), which reduces glyoxylate and hydroxypyruvate to glycolate and D-glycerate, respectively [12, 44, 73]. GRHPR displays a double mitochondrial/cytosolic localization, and thus it is also involved in the metabolism of cytosolic glyoxylate. In peroxisomes, glyoxylate derives either from the intake of vegetable and fruits containing glycolate, which is oxidised by glycolate oxidase (GO), or from the oxidation of glycine by D-amino acid oxidase (DAO) [102]. Peroxisomal glyoxylate is metabolized by alanine:glyoxylate aminotransferase (AGT), a pyridoxal 5′-phosphate (PLP)-dependent enzyme that catalyses the transamination of L-alanine and glyoxylate to pyruvate and glycine, respectively. Since the equilibrium constant of the reaction catalysed by AGT is about 4700, it can be considered irreversible in the cellular milieu, in line with the supposed role of the enzyme in glyoxylate detoxification [17].

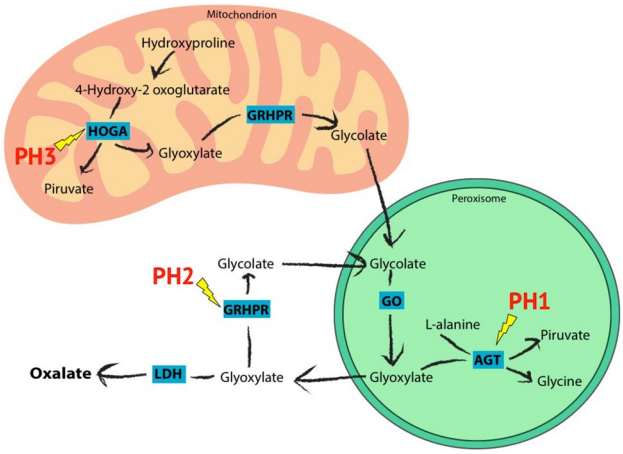

Fig. 1 Molecular mechanisms leading to PH1, PH2 and PH3. *HOGA* 4-hydroxy-2-oxoglutarate aldolase; *GRHPR* glyoxylate reductase/hydroxypyruvate reductase; *AGT* alanine:glyoxylate aminotransferase; *GO* glycolate oxidase and *LDH* lactate dehydrogenase

In the last years, many efforts have been directed to a better understanding of glyoxylate metabolism in human liver. It has been reported that the intermediates of different pathways are exchanged between mitochondria, peroxisomes and the cytosol [73]. In particular, mitochondrial glyoxylate can be delivered to the peroxisome through its conversion to glycolate by mitochondrial GRHPR followed by its oxidation to glyoxylate by peroxisomal GO [10]. In this regard, the finding that AGT is not inhibited by high glyoxylate concentration and that the peroxisomal membrane is permeable to both glycolate and glyoxylate, prompted to identify the peroxisome as the main site of glyoxylate degradation in humans [98].

The deficit of liver enzymes involved in glyoxylate metabolism, due to inherited mutations associated with PHs, leads to the formation of huge amounts of glyoxylate. Three forms of PH have been identified until now and classified as Type I (PH1), Type II (PH2) or Type III (PH3) depending on the mutated gene (Fig. 1) [25–27, 34, 48, 55]. Mutations in the *AGXT* gene, encoding human AGT, give rise to PH1 (OMIM 259900) [35]. Among the three forms, PH1 is the most severe and most frequent one, with an estimated prevalence of 1–3 per million population and an incidence of approximately 1:120,000 live births [114]. Mutations in the *GRHPR* gene, encoding human GRHPR, give rise to PH2 (OMIM, 260000). Although disease prevalence is unknown, PH2 is less frequent than PH1 and it is usually characterized by a milder clinical phenotype and by the presence of glycolic aciduria [31, 32, 42, 111]. Finally, PH3 is caused by mutations of the *HOGA1* gene encoding human HOGA1 (OMIM 613616). PH3 accounts for 10% of PHs cases and is characterized by a mild phenotype, which usually does not progress to ESRD [7, 10, 80]. The molecular mechanisms explaining the reasons underlying glyoxylate accumulation in PH3 are not straightforward. It has been demonstrated that the deficit of HOGA1 leads to the accumulation of HOG, but the link between its accumulation and glyoxylate production is unclear. One of the hypothesis predicts that HOG could be transformed into glyoxylate by a cytosolic aldolase [80], while another one predicts that it could inhibit GRHPR thus indirectly preventing glyoxylate detoxification [95].

Notwithstanding the fact that the three forms of PH arise from the deficit of different liver enzymes, all result in the accumulation of cytosolic glyoxylate. When present at high concentrations, glyoxylate becomes a substrate for lactate dehydrogenase (LDH) and is oxidized to oxalate. Endogenously-produced oxalate cannot be further metabolized and has to be excreted by urine. In PH, the high urinary oxalate concentration leads to the formation of CaOx, a poorly soluble salt that very easily reaches supersaturation and precipitates.

CaOx crystals deposition is then responsible for the progressive kidney damage typical of patients [25, 48] (Fig. 1).

Therapeutic approaches available for the cure of PHs

PHs are difficult-to-treat diseases. An early diagnosis and the immediate administration of a correct therapy are crucial to preserve renal function in patients. Although the metabolic defect underlying PH is related to the liver, classical treatments have the main objective of either preventing kidney failure or restoring kidney function [11, 16, 25–27]. One of the strategies employed is the control of patient diet to decrease the intake of exogenous oxalate. This approach is not considered very helpful, because it has been claimed that dietary oxalate contributes only marginally to the pathogenesis of PH, where accumulating oxalate is of endogenous origin [107]. However, more recent studies have shown that the role of patient diet could be more significant than previously known [54]. Other first-line treatments for PH patients are directed to prevent CaOx crystallization. This is usually done by the administration of crystallization inhibitors (i.e. magnesium and potassium citrate) and by forcing hyper-hydration through a consistent water intake [48, 55]. Patients showing deposits of CaOx stones are subjected to lithotripsy, while dialysis or kidney transplantation are necessary under more severe conditions of renal failure and associated uremia [14]. Overall, classical therapies address the distal aspects of the disease and, although suitable to slow down the progression and mitigate the symptoms, they do not represent a definitive solution. Nevertheless, in the case of PH1, a minority of patients responds to the administration of pyridoxine (PN), a vitamer B6 precursor of the AGT coenzyme PLP [81]. PN is an oral drug endowed with a very good safety profile, and it has a demonstrated efficacy in reducing urinary oxalate in patients bearing the common G170R mutation on the *AGXT* gene [59].

Patients unresponsive to PN and/or to classical treatments undergo liver transplantation or, more often, a liver/kidney transplantation [24]. Liver transplantation represents a definitive treatment, because it allows to substitute the entire pool of AGT thus restoring the glyoxylate detoxification ability of the patient. However, it is very risky and invasive [24]. Although this strategy was considered an option only available for PH1, recently it has been also employed with success in PH2 [37].

New avenues in the treatment of PH

The significant progress in the understanding of the pathogenic mechanisms at the basis of PH have opened the way for the identification and implementation of new therapeutic approaches, which could overcome the limited efficacy of classical treatments and/or avoid too invasive and risky surgery procedures [11, 24–26, 48, 55]. The most promising strategy that is currently in the pipeline is a substrate-reduction approach based on the use of the RNA interference technology. The target genes encode enzymes, GO and LDH, involved in the pathway leading to oxalate production, which is common to the three forms of PH. Attempts have been also made to identify small molecules acting as GO inhibitors. Since PHs are single-gene diseases, gene therapy directed to the liver has been also considered as a possible option. Moreover, some efforts are directed to promote the intestinal metabolism of oxalate using either microorganisms or purified enzymes. In the case of PH1, the use of molecules acting as chaperones is regarded as a possible solution, based on the finding that misfolding seems to be one of the main mechanisms leading to the AGT deficit. In addition, recent reports suggest the possibility to directly administer purified AGT [16].

The basic principles of each therapeutic strategy along with their application to PH are summarized in Table 1 and described below.

Substrate reduction therapy as a common strategy for the treatment of the three forms of PH

Substrate reduction therapy (SRT) is a therapeutic approach for diseases that are caused by loss-of-function mutations in genes encoding key metabolic enzymes and result in the accumulation of potentially dangerous substrates [30]. Such overproduction can be prevented using small molecules that inhibit the enzymes working upstream of the mutated one in the same metabolic pathway [46, 105, 108]. SRT is already in use in some inborn error of metabolism such as hereditary tyrosinemia type 1, in which the deficiency of fumarylacetoacetate hydrolase leads to the accumulation of fumarylacetoacetate (FAA) that in turn is converted to the toxic succinylacetone. The drug nitisinone, by inhibiting parahydroxyphenylpyruvic acid dioxygenase, prevents FAA accumulation and decreases the hepatocellular damage in patients [36]. A similar effect is observed in the case of Miglustat and Eliglustat, two FDA-approved drugs in use for the treatment of Gaucher disease that act by inhibiting glucosylceramide synthase. Glucosylceramide synthase is the enzyme responsible for the synthesis of glucosylceramide, the substrate that accumulates due to the deficit of glucocerebrosidase in Gaucher disease [105, 108].

The application of a similar strategy to PH is based on the finding that the common point of the three forms of disease is the increased production of glyoxylate in cell cytosol [102]. Being glyoxylate the direct precursor of oxalate,

Table 1 Currently available and new therapeutic strategy for PHs

Theoretical principle	Therapeutic strategy	Applicability	Current status
Pharmacological chaperone	Pyridoxine	PH1	FDA approved
	Pyridoxamine, Pyridoxal	PH1	Developmental stage
	AOA analogues	PH1	Developmental stage
Inhibition of mitochondrial import machinery	Dequalinium chloride (DECA)	PH1	Developmental stage
Substrate reduction therapy (*HAO1* gene silencing)	Lumasiran (ALN-GO1)	PH1, PH2, PH3	Phase 2 study
	DCR-PH1	PH1, PH2, PH3	Phase 1 study
Substrate reduction therapy (*LDHA* gene silencing)	DCR-PHXC	PH1, PH2, PH3	Phase 1 study
Substrate reduction therapy (GO inhibition)	4-carboxy-5-dodecylsulfanyl-1,2,3-tiazole (CDST) analogues	PH1, PH2, PH3	Developmental stage
Enzyme replacement therapy	PEG-PGA-AGT	PH1	Developmental stage
Enzyme substitution therapy	Nephure™	PH1, PH2, PH3	Food supplement approved by FDA
	OxDC CLEC	PH1, PH2, PH3	Phase 1 study
	Oxazyme™	PH1, PH2, PH3	Phase 2 study
	ALLN-177	PH1, PH2, PH3	Phase 3 study
Metabolism of intestinal oxalate	Oxabact™	PH1, PH2, PH3	Phase 3 study

whose accumulation is responsible for CaOx crystals deposition, any approach aimed at inhibiting glyoxylate formation could be an effective treatment. The protein considered a suitable target for SRT in PH is GO, because it is the enzyme mainly involved in glyoxylate production in human hepatocytes [12, 73, 101]. People bearing inherited mutations causing the deficit of GO do not display a pathological phenotype, except for an asymptomatic glycolic aciduria, a finding that strongly supports the safety of the target [41, 67, 72]. GO is a flavin-dependent enzyme belonging to the hydroxyacid oxidases family. It is encoded by the *HAO1* gene, and catalyses the conversion of (S)-2-hydroxy acids and molecular oxygen to 2-oxo acids and hydrogen peroxide [31, 73]. The crystal structure of the enzyme is already known and most of its biochemical properties have been elucidated [12]. The identification of GO inhibitors has been largely investigated, especially for their use in agriculture [84, 106]. GO agrochemical inhibitors have been obtained based on the crystal structure of the enzyme from spinach [109]. Among them, 4-carboxy-5-dodecylsulfanyl-1,2,3-tiazole (CDST) was co-crystallized and validated as inhibitor of human GO [13]. Higueras et al. have identified another compound similar to CDST, 4-carboxy-5-[(4-chlorophenyl) sulfanyl]-1,2,3-thiadiazole (CCPST). The crystal structure of the complex between CCPST and human GO has shown that the ligand interacts with five residues located at active site, a key information for its future optimization. It has been also demonstrated that CCPST inhibits GO in Agxt1-/- hepatocytes, and that the inhibition reduces oxalate production. Moreover, when the compound is orally administrated to *AGXT* knock-out mice, it reduces the excretion of urinary oxalate by 30–50%. Although the results are promising, CCPST presents some limitations, which are mainly related to the high dosage required and to the side effects occurring in some animals [72]. Therefore, a more detailed investigation of the toxicity of this putative drug would be necessary.

In the last years, another SRT approach for PH has been implemented, thanks to the use of the RNA interference (RNAi) technology. RNAi is based on ≈20 bp RNA molecules that bind and specifically degrade a target mRNA, thus suppressing the expression of the corresponding protein [1]. This innovative technique has been widely exploited in drug discovery programs, as demonstrated by the fact that more than 30 RNAi-based drugs are in clinical trials since 2004 [1]. As for PH, the development of a SRT based on the use of RNAi has been first focused on the *HAO1* gene encoding GO. Dutta et al. reported that a Dicer-substrate small interfering RNAs (DsiRNAs), delivered by lipid nanoparticles, decreases the conversion of glycolate to glyoxylate and in turn reduces urinary oxalate in mice and monkeys models of PH1 [41]. Liebow et al. tested a therapeutic RNAi (ALN-GO1) delivered by subcutaneous injection, whose administration leads to a potent, dose-dependent and durable silencing of the *HAO1* gene encoding GO, which in turn is able to reduce urinary oxalate in mice, rats and non-human primates [67]. Based on this data, Alnylam Pharmaceuticals developed Lumasiran, a drug based on ALN-GO1 conjugated to N-acetylgalactosamine, for an efficient delivery to hepatocytes. Lumasiran is currently tested in a phase 1/2 clinical trial aimed at evaluating the safety, tolerability, pharmacokinetics and pharmacodynamics of single- and multiple-ascending doses of drug in healthy volunteers and patients with PH1 (https://clinicaltrials.gov-NCT02706886). The company has also extended the trial to study

the effects of a long-term administration of ALN-GO1 in PH1 patients (https://clinicaltrials.gov-NCT03350451). Very recently, the possibility to target LDH for SRT has been also evaluated. LDH is the enzyme directly involved in glyoxylate-to-oxalate conversion in the cytosol of liver hepatocytes. Although LDH plays a crucial metabolic role in carbohydrate metabolism, cases of inherited LDH deficiency have been reported in people showing no particular adverse effects [110]. This finding supports the safety as target for RNAi approaches in PH patients. Dicerna Pharmaceuticals has developed the DCR-PHXC drug targeting the *LDHA* gene. Results described on the company website indicate that the knockdown of the *LDHA* gene decreases oxalate excretion in animal models in the absence of evident toxic effects (http://www.dicerna.com). On these bases, a phase 1 clinical trial with dose escalation of the molecule in healthy volunteers and PH patients just started (https://clinicaltrials.gov-NCT03392896). Nevertheless, deep investigation on the actual safety of LDH silencing should be done before the definitive approval of the drug.

Enzyme administration therapies

When a disease is directly due to the deficit of an enzyme, and to the consequent loss of the specific function it plays in the organism, a possible therapeutic strategy is represented by enzyme administration. Enzyme administration has the rationale of replenishing patients with the defective enzymatic activity and can be done by two different approaches named enzyme replacement therapy (ERT) and enzyme substitution therapy (EST). ERT is based on the direct administration of the non-mutated and hence functional enzyme in the purified form. FDA-approved ERT therapy are currently available for Gaucher disease, Fabry disease and other lysosomal storage diseases [2, 105]. On the other hand, EST aims at introducing a new enzyme able to degrade the accumulating metabolite generating a non-toxic product. Such an approach is one of the therapies in use for phenylketonuria, where the intravenous administration of a PEGylated form of phenylalanine ammonia lyase is able to metabolize accumulating phenylalanine [104].

Both ERT and EST approaches have been explored in PH. Attempts to develop an ERT have been limited to PH1. In 2014, Mesa-Torres et al., using a consensus-based approach, created an engineered form of AGT endowed with high catalytic activity and stability, a first step for the application of the enzyme in the biomedical field [77]. However, to be effective in playing its metabolic role upon administration, AGT should be able to penetrate into cells and to be delivered to peroxisomes. To solve this issue, our group created a fusion protein of AGT with the cell-penetrating Tat peptide at the N-terminus (Tat-AGT). The biochemical characterization of Tat-AGT demonstrated that the presence of the fused peptide does not induce functional or structural alterations [100]. The fusion protein is internalized in a mammalian cell model of PH1 and shows a cytosolic localization, but is able to detoxify endogenously-produced glyoxylate. However, it is not suitable for human delivery because of the remarkable immune response probably arising from the Tat peptide. Recently, we developed a nanoconjugate of AGT with a di-block polymer formed by a moiety of polyethylene glycol (PEG) and a moiety of polyglutammic acid (PGA) (PEG-PGA-AGT). Conjugation was achieved trough the formation of disulphide bonds between reactive pyridildithiol groups of the polymer and solvent-exposed cysteine residues of AGT. The binding to the polymer endows AGT with the ability to reach the correct peroxisomal localization and to restore glyoxylate detoxification ability in a cellular model of PH1 [99]. Moreover, PEG-PGA-AGT conjugates are hemocompatible, stable in plasma and non-immunogenic in *in vitro* assays. Although further investigations will be necessary to optimize conjugation efficiency and to implement a targeted hepatic delivery, the results obtained hold promise for a future development of an ERT for PH1.

As for the EST approach, the strategy employed is based on the common theme of the three forms of PH, i.e. oxalate accumulation, and relies on the use of oxalate degrading-enzymes [90]. Oxalate-degrading enzymes are non-human proteins able to convert oxalate in products that are safe for human beings [90]. They can be orally administered and degrade intestinal oxalate. Although this approach has been developed to reduce exogenous oxalate intake in oxalosis and secondary hyperoxaluria, a possible application to PH cannot be excluded. In fact, it has been demonstrated that the intestine could have a sink effect to promote oxalate clearance from blood, thus decreasing the endogenous levels [51]. In fact, small intestine can participate to oxalate excretion thanks to the presence of the anion exchanger SLC26A6 [49]. The protein currently employed is oxalate decarboxylase (OxDC), a Mn-dependent enzyme that catalyses the conversion of oxalate to formate and carbon dioxide [65]. Different OxDC formulations are available such as Nephure™, OxDC CLEC, Oxazyme, and ALLN-177. Nephure™ is OxDC from *S. elongatus* used as a food ingredient (www-nephure.com), while Oxazyme is the commercial name of OxDC from *Bacillus subtilis* formulated as a dietary additive for secondary hyperoxaluria [83]. A phase 1 clinical trial for subjects with enteric hyperoxaluria after Roux-en-Y Gastric Bypass or with idiopathic hyperoxaluria revealed a significant reduction of urinary oxalate only in the first group (https://clinicaltrials.gov-NCT01127087). OxDC-CLEC is a crystalline form of OxDC from *Bacillus subtilis* proven to be effective in degrading oxalate and reducing kidney damage in hyperoxaluric mice [45]. This formulation has also shown some effectiveness in a

swine model of nephrocalcinosis [61]. ALLN-177 is derived from OxDC-CLEC and has been recently tested in a phase 1 clinical trial, showing the ability to degrade oxalate in the gastrointestinal tract of healthy volunteers with hyperoxaluria induced by ingestion of a high oxalate, low calcium diet [64]. Recently, it has received orphan drug designation for primary hyperoxaluria and for pediatric hyperoxaluria and a phase 2 clinical trial in adult and paediatric patients with enteric or primary hyperoxaluria and hyperoxalemia is currently ongoing (https://clinicaltrials.gov-NCT03391804). Overall, these results and observations confirm the hypothesis that reduction of intestinal oxalate absorption could influence endogenous oxalate concentration and hence reduce urinary oxalate excretion. However, it should be taken into account that OxDC displays an optimum pH around 4, with approximately 2% residual activity at pH 7 [65]. Thus, attempts to increase the catalytic efficiency at neutral pH should be undertaken.

Among oxalate degrading-enzymes, a potential alternative to OxDC is represented by oxalate oxidase (OxOx), a Mn-dependent enzyme that catalyses the conversion of oxalate to carbon dioxide and hydrogen peroxide [90]. Dahiya and Pundir showed that OxOx from *Bougainvillea glabra* immobilized with ethylene maleic anhydride displays a residual activity of 70% at pH 7. Interestingly, in a liposome-encapsulated form, the enzyme degrades oxalate in a rat model of hyperoxaluria [33].

Promotion of intestinal oxalate degradation

In humans, oxalate is eliminated from the body mainly through the kidneys and partly through the intestine [97]. *Oxalobacter formigenes* is a bacterium part of the intestinal flora, which relies on oxalate as energy source [3]. Since the elimination of intestinal oxalate could induce a concentration-dependent transport into the intestinal lumen, the possibility that the intestine colonization with *O. formigenes* could represent a treatment option in PH1 has been tested. In animal models, the oral administration of *O. formigenes* leads to a net reduction of the levels of oxalate in urine and plasma, due to the complete colonization of the intestinal tract that effectively induces a progressive excretion of endogenous oxalate [49, 50, 57]. In this regard, it has been demonstrated that the treatment of intestinal epithelial cells with bioactive factors produced by the bacterium actually promotes oxalate transport [5]. Oral formulations of *O. formigenes* have a good safety profile and lead to a reduction in urinary oxalate levels, as shown in a Phase 1 trial in PH patients [56]. In 2011, Hoppe et al. have also reported the effectiveness of the same treatment in two patients affected by infantile oxalosis [57]. A lyophilized form of *O. formigenes* with the name of Oxabact (produced by OxThera, Sweden) has been granted as orphan drug in USA and Europe for the treatment of PH. Unfortunately, a randomized Phase I/II trial has not completely confirmed the results obtained in animal models. Although the treatment is well-tolerated, no significant differences between control and treated group have been observed. This is probably due to the clinical heterogeneity of the patients in the two groups and to the influence that the baseline kidney function can have on patient responsiveness [58, 78]. Therefore, an optimization of the procedure and a better stratification of the patients will be required for future studies.

In the same field, two studies in animal models of hyperoxaluria have reported that gut colonization with other bacterial strains, such as *Bifidobacterium animalis* subsp. *lactis* and *Lactobacillus plantarum*, reduces urinary oxalate concentration [62, 115]. These approaches have been mainly regarded as treatments for dietary hyperoxaluria. Two probiotics analysed in a double-blind, placebo-controlled study failed to reduce urinary oxalate in mild hyperoxaluric patients [68]. However, a possible application to PH cannot be excluded. Nevertheless, the importance of determining the intraluminal factors affecting colonization and how colonization with beneficial oxalate degraders is established and maintained, are essential knowledges for the development of therapeutic approach that utilized probiotics [113].

Use of gene and cell therapy for the treatment of PH1

Single-gene inherited diseases can be treated by gene therapy, which aims at replacing the mutated gene with a normal copy, thus restoring protein function. All three forms of PH are single-gene disease suitable for the development of gene therapy approaches. This strategy has been particularly explored in PH1, because it would represent a good alternative to liver transplantation. *AGXT* gene transfer has been achieved by two different research groups. In a first study, the administration of two adeno-associated virus serotypes (AAV5 and AAV8) in mice knock-out for the *AGXT* gene has been found to reduce urinary oxalate excretion without significant untoward effects [103]. More recently, using helper-dependent adenoviral vectors, a reduction of oxalate excretion has been observed in a mice model of PH1 [15]. Although these results are promising, high doses of vectors are required to induce a complete phenotypic response of hepatocytes, a condition that decreases the safety of the treatment and prompts for the identification of new viral vectors showing an enhanced tropism for the liver and/or an increased transduction efficiency.

In the past, liver cell transplantation has been also used in a young patient affected by infantile oxalosis who showed a very poor clinical condition [8]. The therapy has resulted

successful as a bridge to whole liver transplant, although improvements of the protocols and methodology have been recommended by the authors. In particular, optimal treatment conditions to stimulate liver recolonization requires detailed studies, in particular to give a mitotic advantage to transfused cells with respect to patient cells.

The role of protein misfolding in PH1 and the use of pharmacological chaperones

Proteostasis represents an ideal condition in which the homeostasis of the entire pool of cellular proteins is maintained. Proteostasis depends on an important network of biologic processes that controls biogenesis, folding, and trafficking of the pool of proteins present inside the cell [52]. During the process of folding, the polypeptide chain must rapidly self-assemble into a highly structured native conformation, which is essential for biological function. In many cases, the folding process requires the presence of molecular chaperones, as well as of other proteins that assist and protect the nascent protein during folding. Molecular chaperones are components of the cellular protein quality control system that can act either by preventing aggregation of folding intermediates or unfolded proteins, or by helping misfolded proteins to achieve the native conformation. In addition, it has been reported that some chaperones act as disaggregating agents, able to breaking up protein aggregates [23].

The perturbation of the proteostasis network, due to environmental stresses or to the accumulation of misfolded or partially folded intermediates, could generate the onset of a pathological state. Protein misfolding diseases are disorders whose underlying mechanism is related to the inability of a protein to achieve or maintain its native conformation [52, 93]. Misfolded or misassembled proteins have two potential fates: (i) they could be degraded by the ubiquitin–proteasome degrading pathway (UPS), thus leading to the loss of the biological activity of the protein involved (loss-of-function), or (ii) they can self-assemble generating aggregates of misfolded proteins, which in some cases form amyloid fibrils that are toxic for the cell and cause tissue damage (gain-of-function disease) [22].

One of the approaches under development in the last decades for the treatment of protein misfolding diseases, in particular of those displaying a loss-of-function mechanism, is the use of chemical or pharmacological chaperones, small molecules able to improve the folding and restore the activity of misfolded proteins. This strategy has been successfully applied to treat lysosomal storage disorders, phenylketonuria, tyrosine hydroxylase deficiency, cystic fibrosis, nephrogenic diabetes insipidus and others [93]. The term chemical chaperones usually refers to low molecular weight molecules that non-specifically stabilize a misfolded protein without directly interact with it. The term pharmacological chaperones (PCs) instead refers to small molecules able to specifically bind a misfolded protein and promote the attainment of its correct structure, by determining a thermodynamic stabilization of the polypeptide chain [96]. When a disease is due to an enzymatic deficit, compounds acting as PCs are either vitamin derivatives functioning as coenzymes, or competitive inhibitors of the enzyme involved. Both classes of molecules are usually endowed with a high binding affinity and specificity, two features that allow them to be effective at very low concentration [96].

As mentioned above, PH1 is the most frequent and most severe form of PH and it is due to the deficit of liver peroxisomal AGT [87]. The *AGXT* gene encoding AGT is present in humans as two polymorphic alleles named *major allele* (encoding AGT-Ma) and *minor allele* (encoding AGT-Mi). The *minor allele* has a mean frequency of 20% in the European population and is characterized by a 74-bp duplication in intron 1 and by the two amino acid substitutions P11L and I340M [34, 94]. The frequency of the minor allele increases to 50% in PH1 patients, because the P11L mutation makes AGT more susceptible to the effect of pathogenic missense mutations associated with PH1 [18, 19, 21, 69]. Moreover, AGT-Mi shows a 5% mistargeting to mitochondria, due to the P11L mutation that creates a putative mitochondrial targeting sequence (MTS) at the N-terminus of AGT [34].

PH1 is a very heterogeneous disease from a genetic, enzymatic and clinical point of view. Up to now, more than 150 different pathogenic mutations on the *AGXT* gene have been identified, and the most common ones are missense mutations [114]. Studies on the molecular and cellular features of the variants have indicated that the large majority of mutations do not affect the intrinsic AGT catalytic activity but rather interfere with the proper folding pathway of the protein, thus increasing its aggregation and/or degradation propensity, as well as decreasing the overall kinetic stability of the protein and promoting the binding with molecular chaperones [74, 75, 91, 92, 102]. In the case of variants on the minor allele, mitochondrial mistargeting is also often observed. A complex equilibrium during the folding pathway, in which the achievement of the correct structure and the consequent peroxisomal import compete with the population of partly-folded species prone to be degraded, to aggregate, or to be imported into mitochondria probably exists [76, 82]. Thus, researchers have paid attention to the discovery of small molecules that could positively interfere with this equilibrium promoting the correct folding of the protein [75, 79, 88].

Some research groups have analysed the action of chemical chaperones. Phenylbutyric acid (PBA), betaine, glycerol and trimethylamine N-oxide (TMAO) have been tested. However, only glycerol and betaine were found to exert a stabilizing effect of the pathogenic variants F152I-Mi, G170R-Mi, and I244T-Mi [28, 29].

Based on the finding that in some variants the folding defect only or mainly affects the apo-form, and that this is also true for mutations responsive to Vitamin B6 at clinical level [20, 59, 81], the possible chaperone role of the coenzyme has been evaluated. Studies carried out on purified proteins and in a cellular model of PH1 have revealed that PLP not only shifts the equilibrium from the less stable apo-form to the more stable holo-form of AGT, but also binds apomonomeric and apodimeric folding intermediates promoting the acquirement and the maintenance of the dimeric structure that is crucial for functionality [18, 19, 21, 39, 74, 75, 92]. PLP also exerts a stabilizing effect on native AGT and prevents its aggregation mediated by electrostatic forces [38]. As a confirmation of the chaperone role of PLP, it has been recently reported that mutations located at the AGT monomer–monomer interface that cause structural alterations on the apo-form but not on the holo-form, are responsive to PN administration in vitro. Interestingly, the responsiveness seems to inversely correlate with the degree of conformational alteration of each variant, thus implying that a kind of threshold could exist, above which the coenzyme is not able to rescue for the folding defect of a variant [40]. Another parameter that could influence B6 responsiveness is the type of vitamer. In fact, even though PN is the only vitamer used in clinics, also pyridoxal (PL) and pyridoxamine (PM), can be internalized by cells [112]. The comparison of the effectiveness of the three B6 vitamers in a cellular model of PH1 has revealed that PN and PM are more effective than PN in the rescue of folding-defective variants of AGT [86]. The reason underlying this difference is that PM and PL administration, differently from PN, avoid the intracellular accumulation of pyridoxine phosphate (PNP) that competes with PLP for AGT binding and inhibits catalytic activity. Considering the safety of vitamins administration, and the fact that PM is already in the market for the treatment of diabetes Type II, these data hold promise for a possible future improvement of the therapy with Vitamin B6, which could completely or at least partly relief disease symptoms, provided that responsive mutations are identified and that the more effective vitamer is administered.

In an attempt to identify competitive inhibitors acting as PCs for AGT, different approaches have been undertaken in the last few years. Aminooxyacetic acid (AOA), a well-known ligand of the protein [4], has been analysed as candidate. AOA behaves as a slow, tight-binding inhibitor and plays a chaperone role in the rescue of the most common mutations leading to PH1. To overcome the low specificity of AOA, which makes it unsuitable for clinical use, a preliminary structure activity relationship analysis has been performed. This study allowed to identify a number of AOA derivatives among which 2-aminooxy-3-phenylpropionic acid was found to work as PC [89].

Other researchers have focused their efforts on the correction of the molecular defect of the common G170R-Mi variant, which is expressed by 30% of PH1 patients and is mistargeted to mitochondria [47]. A high-throughput phenotypic assay based on the evaluation of the change in the AGT subcellular localization has been implemented [71]. The first pilot screening has identified three active molecules that do not completely redirect AGT to peroxisomes, but could undergo future optimization programs. In addition, Miyata and colleagues have tried to prevent the aberrant targeting using FDA-approved molecules acting as inhibitors of the mitochondrial import machinery [79]. They identified dequalinium chloride (DECA) as a molecule able to redirect AGT into peroxisomes and to partially restore the glyoxylate detoxification capability of the cells. Unfortunately, DECA is approved by FDA only as topical medication, and its parental administration resulted in toxic effects in mice [43].

Overall, many progresses have been made in the last years for the identification of compounds effective in correcting AGT folding by acting as PCs on either the apo- or the holo-form of the protein, or in promoting the peroxisomal import. Future studies will be necessary to design more specific and more effective molecules as well as to test possible additive or synergic effects of their combined administration.

Conclusions

PHs are a group of diseases due to defects on the hepatic glyoxylate metabolism and leading to a wide plethora of clinical manifestations ranging from mild nephrolithiasis to severe renal damage and systemic oxalosis. In the last years, the advancements in the knowledge of the molecular and cellular aspects of the disease have driven the setup of new therapeutic strategies. Some of them are already in clinical trial for PH patients and look promising for a final approval in the near future. Others are still at the developmental stage, waiting for optimization studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they do not have any conflicts of interest to declare.

References

1. Alagia A, Eritja R (2016) siRNA and RNAi optimization Wiley. Interdiscip Rev RNA 7:316–329. https://doi.org/10.1002/wrna.1337

2. Alegra T, Vairo F, de Souza MV, Krug BC, Schwartz IV (2012) Enzyme replacement therapy for Fabry disease: a systematic review and meta-analysis. Genet Mol Biol 35:947–954
3. Allison MJ, Dawson KA, Mayberry WR, Foss JG (1985) Oxalobacter formigenes gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. Arch Microbiol 141:1–7
4. Andy V, Horvath P, Wanders RJ (1995) Aminooxy acetic acid: a selective inhibitor of alanine:glyoxylate aminotransferase and its use in the diagnosis of primary hyperoxaluria type I Clinica chimica acta. Int J Clin Chem 243:105–114
5. Arvans D et al (2017) Oxalobacter formigenes-derived bioactive factors stimulate oxalate transport by intestinal epithelial cells. J Am Soc Nephrol JASN 28:876–887. https://doi.org/10.1681/ASN.2016020132
6. Asplin JR (2002) Hyperoxaluric calcium nephrolithiasis Endocrinology. and metabolism clinics of North America 31:927–949
7. Beck BB et al (2013) Novel findings in patients with primary hyperoxaluria type III and implications for advanced molecular testing strategies European. J Hum Genet EJHG 21:162–172. https://doi.org/10.1038/ejhg.2012.139
8. Beck BB et al (2012) Liver cell transplantation in severe infantile oxalosis–a potential bridging procedure to orthotopic liver transplantation? Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association. Eur Renal Assoc 27:2984–2989. https://doi.org/10.1093/ndt/gfr776
9. Beck BB, Hoyer-Kuhn H, Gobel H, Habbig S, Hoppe B (2013) Hyperoxaluria and systemic oxalosis: an update on current therapy and future directions Expert opinion on investigational. Drugs 22:117–129 https://doi.org/10.1517/13543784.2013.741587
10. Belostotsky R, Pitt JJ, Frishberg Y (2012) Primary hyperoxaluria type III–a model for studying perturbations in glyoxylate metabolism. J Mol Med 90:1497–1504. https://doi.org/10.1007/s00109-012-0930-z
11. Ben-Shalom E, Frishberg Y (2015) Primary hyperoxalurias: diagnosis and treatment Pediatric Nephrology 30:1781–1791 https://doi.org/10.1007/s00467-014-3030-1
12. Booth MP, Conners R, Rumsby G, Brady RL (2006) Structural basis of substrate specificity in human glyoxylate reductase/hydroxypyruvate reductase. J Mol Biol 360:178–189. https://doi.org/10.1016/j.jmb.2006.05.018
13. Bourhis JM, Vignaud C, Pietrancosta N, Gueritte F, Guenard D, Lederer F, Lindqvist Y (2009) Structure of human glycolate oxidase in complex with the inhibitor 4-carboxy-5-[(4-chlorophenyl)sulfanyl]-1,2,3-thiadiazole Acta crystallographica Section F. Struct Biol Cryst Commun 65:1246–1253. https://doi.org/10.1107/S1744309109041670
14. Carrasco A Jr, Granberg CF, Gettman MT, Milliner DS, Krambeck AE (2015) Surgical management of stone disease in patients with primary hyperoxaluria Urology 85:522–526. https://doi.org/10.1016/j.urology.2014.11.018
15. Castello R, Borzone R, D'Aria S, Annunziata P, Piccolo P, Brunetti-Pierri N (2016) Helper-dependent adenoviral vectors for liver-directed gene therapy of primary hyperoxaluria type 1. Gene therapy 23:129–134. https://doi.org/10.1038/gt.2015.107
16. Cellini B (2017) Treatment options in primary hyperoxaluria Type I Expert Opin Orphan Drugs 5:11. https://doi.org/10.1080/21678707.2017.1298439
17. Cellini B, Bertoldi M, Montioli R, Paiardini A, Borri Voltattorni C (2007) Human wild-type alanine:glyoxylate aminotransferase and its naturally occurring G82E variant: functional properties and physiological implications. Biochem J 408:39–50. https://doi.org/10.1042/BJ20070637
18. Cellini B et al (2010) Molecular defects of the glycine 41 variants of alanine glyoxylate aminotransferase associated with primary hyperoxaluria type I Proc Natl Acad Sci U S A 107:2896–2901. https://doi.org/10.1073/pnas.0908565107
19. Cellini B, Montioli R, Paiardini A, Lorenzetto A, Voltattorni CB (2009) Molecular insight into the synergism between the minor allele of human liver peroxisomal alanine:glyoxylate aminotransferase and the F152I mutation. J Biol Chem 284:8349–8358. https://doi.org/10.1074/jbc.M808965200
20. Cellini B, Montioli R, Voltattorni CB (2011) Human liver peroxisomal alanine:glyoxylate aminotransferase: characterization of the two allelic forms and their pathogenic variants Biochimica. et biophysica acta 1814:1577–1584. https://doi.org/10.1016/j.bbapap.2010.12.005
21. Cellini B, Oppici E, Paiardini A, Montioli R (2012) Molecular insights into primary hyperoxaluria type 1 pathogenesis. Front Biosci 17:621–634
22. Chiti F, Dobson CM (2017) Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade annual. Rev Biochem 86:27–68. https://doi.org/10.1146/annurev-biochem-061516-045115
23. Ciechanover A, Kwon YT (2017) Protein quality control by molecular chaperones in neurodegeneration Front Neurosci 11:185 https://doi.org/10.3389/fnins.2017.00185
24. Cochat P, Fargue S, Harambat J (2010) Primary hyperoxaluria type 1: strategy for organ transplantation Curr Opin Organ Transpl 15:590–593. https://doi.org/10.1097/MOT.0b013e32833e35f5
25. Cochat P, Groothoff J (2013) Primary hyperoxaluria type 1: practical and ethical issues Pediatric Nephrol 28:2273–2281 https://doi.org/10.1007/s00467-013-2444-5
26. Cochat P et al (2012) Primary hyperoxaluria Type 1: indications for screening and guidance for diagnosis and treatment Nephrol Dial Transpl 27:1729–1736. https://doi.org/10.1093/ndt/gfs078
27. Cochat P, Rumsby G (2013) Primary hyperoxaluria N Engl J Med 369:649–658. https://doi.org/10.1056/NEJMra1301564
28. Coulter-Mackie MB, Lian Q (2006) Consequences of missense mutations for dimerization and turnover of alanine:glyoxylate aminotransferase: study of a spectrum of mutations Mol Genet Metab 89:349–359. https://doi.org/10.1016/j.ymgme.2006.07.013
29. Coulter-Mackie MB, Lian Q (2008) Partial trypsin digestion as an indicator of mis-folding of mutant alanine:glyoxylate aminotransferase and chaperone effects of specific ligands. Study of a spectrum of missense mutants Mol Genet Metab 94:368–374. https://doi.org/10.1016/j.ymgme.2008.03.010
30. Coutinho MF, Santos JI, Alves S (2016) Less is more: substrate reduction therapy for lysosomal storage disorders. Int J Mol Sci 17, 1065 https://doi.org/10.3390/ijms18010178
31. Cramer SD, Ferree PM, Lin K, Milliner DS, Holmes RP (1999) The gene encoding hydroxypyruvate reductase (GRHPR) is mutated in patients with primary hyperoxaluria type II. Hum Mol Genet 8:2063–2069
32. Cregeen DP, Williams EL, Hulton S, Rumsby G (2003) Molecular analysis of the glyoxylate reductase (GRHPR) gene and description of mutations underlying primary hyperoxaluria type 2. Hum Mutat 22:497. https://doi.org/10.1002/humu.9200
33. Dahiya T, Pundir CS (2013) In vivo oxalate degradation by liposome encapsulated oxalate oxidase in rat model of hyperoxaluria. Indian J Med Res 137:136–141
34. Danpure CJ (2006) Primary hyperoxaluria type 1: AGT mistargeting highlights the fundamental differences between the peroxisomal and mitochondrial protein import pathways Biochimica. et biophysica acta 1763:1776–1784. https://doi.org/10.1016/j.bbamcr.2006.08.021

35. Danpure CJ, Jennings PR (1986) Peroxisomal alanine:glyoxylate aminotransferase deficiency in primary hyperoxaluria type I. FEBS Lett 201:20–24
36. Das AM (2017) Clinical utility of nitisinone for the treatment of hereditary tyrosinemia type-1 (HT-1) Appl Clin Genet 10:43–48 https://doi.org/10.2147/TACG.S113310
37. Dhondup T, Lorenz EC, Milliner DS, Lieske JC (2018) Combined liver-kidney transplantation for primary hyperoxaluria type 2: a case report Am J Transpl 18:253–257. https://doi.org/10.1111/ajt.14418
38. Dindo M, Conter C, Cellini B (2017) Electrostatic interactions drive native-like aggregation of human alanine:glyoxylate aminostransferase. The FEBS J 284:3739–3764. https://doi.org/10.1111/febs.14269
39. Dindo M, Montioli R, Busato M, Giorgetti A, Cellini B, Borri Voltattorni C (2016) Effects of interface mutations on the dimerization of alanine glyoxylate aminotransferase and implications in the mistargeting of the pathogenic variants F152I and I244T Biochimie 131:137–148 https://doi.org/10.1016/j.biochi.2016.10.001
40. Dindo M, Oppici E, Dell'Orco D, Montone R, Cellini B (2018) Correlation between the molecular effects of mutations at the dimer interface of alanine-glyoxylate aminotransferase leading to primary hyperoxaluria type I and the cellular response to vitamin B6. J Inherit Metab Dis 41:263–275. https://doi.org/10.1007/s10545-017-0105-8
41. Dutta C et al (2016) Inhibition of glycolate oxidase with dicer-substrate siRNA reduces calcium oxalate deposition in a mouse model of primary hyperoxaluria type 1 Mol Ther J Am Soc Gene Ther 24:770–778. https://doi.org/10.1038/mt.2016.4
42. Fu Y, Rope R, Fargue S, Cohen HT, Holmes RP, Cohen DM (2015) A mutation creating an out-of-frame alternative translation initiation site in the GRHPR 5′UTR causing primary hyperoxaluria type II Clin Genet 88:494–498. https://doi.org/10.1111/cge.12541
43. Gamboa-Vujicic G, Emma DA, Liao SY, Fuchtner C, Manetta A (1993) Toxicity of the mitochondrial poison dequalinium chloride in a murine model system. Journal of pharmaceutical sciences 82:231–235
44. Giafi CF, Rumsby G (1998) Kinetic analysis and tissue distribution of human D-glycerate dehydrogenase/glyoxylate reductase and its relevance to the diagnosis of primary hyperoxaluria type 2 Ann Clin Biochem 35 (Pt 1):104–109 https://doi.org/10.1177/000456329803500114
45. Grujic D et al (2009) Hyperoxaluria is reduced and nephrocalcinosis prevented with an oxalate-degrading enzyme in mice with hyperoxaluria. Am J Nephrol 29:86–93. https://doi.org/10.1159/000151395
46. Guerard N et al (2018) Lucerastat, an iminosugar for substrate reduction therapy: tolerability, pharmacodynamics, and pharmacokinetics in patients with fabry disease on enzyme replacement. Clin Pharmacol Ther 103:703–711. https://doi.org/10.1002/cpt.790
47. Harambat J et al (2010) Genotype-phenotype correlation in primary hyperoxaluria type 1: the p.Gly170Arg AGXT mutation is associated with a better outcome. Kidney Int 77:443–449. https://doi.org/10.1038/ki.2009.435
48. Harambat J, Fargue S, Bacchetta J, Acquaviva C, Cochat P (2011) Primary hyperoxaluria Int J Nephrol 2011:864580. https://doi.org/10.4061/2011/864580
49. Hatch M, Freel RW (2008) The roles and mechanisms of intestinal oxalate transport in oxalate homeostasis. Semin Nephrol 28:143–151. https://doi.org/10.1016/j.semnephrol.2008.01.007
50. Hatch M, Freel RW (2013) A human strain of Oxalobacter (HC-1) promotes enteric oxalate secretion in the small intestine of mice and reduces urinary oxalate excretion Urolithiasis 41:379–384. https://doi.org/10.1007/s00240-013-0601-8
51. Hatch M, Gjymishka A, Salido EC, Allison MJ, Freel RW (2011) Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with *Oxalobacter* Am J Physiol Gastrointest Liver Physiol 300:G461-469 https://doi.org/10.1152/ajpgi.00434.2010
52. Hipp MS, Park SH, Hartl FU (2014) Proteostasis impairment in protein-misfolding and -aggregation diseases Trends Cell Biol 24:506–514. https://doi.org/10.1016/j.tcb.2014.05.003
53. Holmes RP, Assimos DG (1998) Glyoxylate synthesis, and its modulation and influence on oxalate synthesis. J Urol 160:1617–1624
54. Holmes RP, Assimos DG, Wilson DM, Milliner DS (2001) (L)-2-oxothiazolidine-4-carboxylate in the treatment of primary hyperoxaluria type 1. BJU Int 88:858–862
55. Hoppe B (2012) An update on primary hyperoxaluria. Nature reviews Nephrology 8:467–475. https://doi.org/10.1038/nrneph.2012.113
56. Hoppe B et al (2006) Oxalobacter formigenes: a potential tool for the treatment of primary hyperoxaluria type 1. Kidney Int 70:1305–1311. https://doi.org/10.1038/sj.ki.5001707
57. Hoppe B, Dittlich K, Fehrenbach H, Plum G, Beck BB (2011) Reduction of plasma oxalate levels by oral application of Oxalobacter formigenes in 2 patients with infantile oxalosis. Am J Kidney Dis 58:453–455. https://doi.org/10.1053/j.ajkd.2011.05.012
58. Hoppe B et al (2011) Efficacy and safety of Oxalobacter formigenes to reduce urinary oxalate in primary hyperoxaluria Nephrol Dial Transpl 26:3609–3615. https://doi.org/10.1093/ndt/gfr107
59. Hoyer-Kuhn H, Kohbrok S, Volland R, Franklin J, Hero B, Beck BB, Hoppe B (2014) Vitamin B6 in primary hyperoxaluria I: first prospective trial after 40 years of practice. Clin J Am Soc Nephrol CJASN 9:468–477. https://doi.org/10.2215/CJN.06820613
60. Karaolanis G, Lionaki S, Moris D, Palla VV, Vernadakis S (2014) Secondary hyperoxaluria: a risk factor for kidney stone formation and renal failure in native kidneys and renal grafts Transpl Rev 28:182–187 https://doi.org/10.1016/j.trre.2014.05.004
61. Katerina Pierzynowska K 1D, Stefan G. Pierzynowski 1,2,3(ACHF), Paper O, Liudamyla Lozinska 1 D, Sara Jarmakiewicz 5(DEFG), Paulina Świeboda 1(BCDE), Olexandr Fedkiv 1(BC) KSB, Jose Louis Valverde Piedra 3(BC), Filip R (2017) The influence of oxalate decarboxylase on the urinary oxalate excretion in swine model of nephrocalcinosis induced by hydroxyproline. Eur J Clin Exp Med 15:11. https://doi.org/10.15584/ejcem.2017.3.4
62. Klimesova K, Whittamore JM, Hatch M (2015) Bifidobacterium animalis subsp. lactis decreases urinary oxalate excretion in a mouse model of primary hyperoxaluria Urolithiasis 43:107–117. https://doi.org/10.1007/s00240-014-0728-2
63. Knight J, Jiang J, Assimos DG, Holmes RP (2006) Hydroxyproline ingestion and urinary oxalate and glycolate excretion. Kidney Int 70:1929–1934. https://doi.org/10.1038/sj.ki.5001906
64. Langman CB, Grujic D, Pease RM, Easter L, Nezzer J, Margolin A, Brettman L (2016) A double-blind, placebo controlled, randomized phase 1 cross-over study with ALLN-177, an orally administered oxalate degrading enzyme. Am J Nephrol 44:150–158. https://doi.org/10.1159/000448766
65. Lee E, Jeong BC, Park YH, Kim HH (2014) Expression of the gene encoding oxalate decarboxylase from Bacillus subtilis and characterization of the recombinant enzyme. BMC Res Notes 7:598. https://doi.org/10.1186/1756-0500-7-598
66. Li X et al (2016) Metabolism of (13)C5-hydroxyproline in mouse models of Primary Hyperoxaluria and its inhibition by RNAi therapeutics targeting liver glycolate oxidase and hydroxyproline

dehydrogenase Biochimica. et biophysica acta 1862:233–239. https://doi.org/10.1016/j.bbadis.2015.12.001
67. Liebow A et al (2016) An investigational RNAi therapeutic targeting glycolate oxidase reduces oxalate production in models of primary hyperoxaluria. J Am Soc Nephrol JASN. https://doi.org/10.1681/ASN.2016030338
68. Lieske JC, Tremaine WJ, De Simone C, O'Connor HM, Li X, Bergstralh EJ, Goldfarb DS (2010) Diet, but not oral probiotics, effectively reduces urinary oxalate excretion and calcium oxalate supersaturation. Kidney Int 78:1178–1185. https://doi.org/10.1038/ki.2010.310
69. Lumb MJ, Danpure CJ (2000) Functional synergism between the most common polymorphism in human alanine:glyoxylate aminotransferase and four of the most common disease-causing mutations. J Biol Chem 275:36415–36422. https://doi.org/10.1074/jbc.M006693200
70. M'Dimegh S et al (2017) HOGA1 gene mutations of primary hyperoxaluria Type 3 in Tunisian patients J Clini Lab Anal 31 https://doi.org/10.1002/jcla.22053
71. Madoux F et al (2015) Development of a phenotypic high-content assay to identify pharmacoperone drugs for the treatment of primary hyperoxaluria type 1 by high-throughput screening. Assay Drug Dev Technol 13:16–24. https://doi.org/10.1089/adt.2014.627
72. Martin-Higueras C, Luis-Lima S, Salido E (2016) Glycolate oxidase is a safe and efficient target for substrate reduction therapy in a mouse model of primary Hyperoxaluria Type I Mol Ther J Am Soc Gene Ther 24:719–725. https://doi.org/10.1038/mt.2015.224
73. Mdluli K, Booth MP, Brady RL, Rumsby G (2005) A preliminary account of the properties of recombinant human Glyoxylate reductase (GRHPR), LDHA and LDHB with glyoxylate, and their potential roles in its metabolism Biochimica. et Biophysica Acta 1753:209–216. https://doi.org/10.1016/j.bbapap.2005.08.004
74. Mesa-Torres N, Fabelo-Rosa I, Riverol D, Yunta C, Albert A, Salido E, Pey AL (2013) The role of protein denaturation energetics and molecular chaperones in the aggregation and mistargeting of mutants causing primary hyperoxaluria type I PloS One 8:e71963. https://doi.org/10.1371/journal.pone.0071963
75. Mesa-Torres N, Salido E, Pey AL (2014) The lower limits for protein stability and foldability in primary hyperoxaluria type I Biochimica. et biophysica Acta 1844:2355–2365. https://doi.org/10.1016/j.bbapap.2014.10.010
76. Mesa-Torres N, Tomic N, Albert A, Salido E, Pey AL (2015) Molecular recognition of PTS-1 cargo proteins by Pex5p: implications for protein mistargeting in primary hyperoxaluria. Biomolecules 5:121–141. https://doi.org/10.3390/biom5010121
77. Mesa-Torres N et al (2014) The consensus-based approach for gene/enzyme replacement therapies and crystallization strategies: the case of human alanine-glyoxylate aminotransferase. Biochem J 462:453–463. https://doi.org/10.1042/BJ20140250
78. Milliner D, Hoppe B, Groothoff J (2017) A randomised Phase II/III study to evaluate the efficacy and safety of orally administered Oxalobacter formigenes to treat primary hyperoxaluria Urolithiasis https://doi.org/10.1007/s00240-017-0998-6
79. Miyata N, Steffen J, Johnson ME, Fargue S, Danpure CJ, Koehler CM (2014) Pharmacologic rescue of an enzyme-trafficking defect in primary hyperoxaluria 1. Proc Natl Acad Sci USA 111:14406–14411. https://doi.org/10.1073/pnas.1408401111
80. Monico CG et al (2011) Primary hyperoxaluria type III gene HOGA1 (formerly DHDPSL) as a possible risk factor for idiopathic calcium oxalate urolithiasis. Clin J Am Soc Nephrol CJASN 6:2289–2295. https://doi.org/10.2215/CJN.02760311
81. Monico CG, Rossetti S, Olson JB, Milliner DS (2005) Pyridoxine effect in type I primary hyperoxaluria is associated with the most common mutant allele. Kidney Int 67:1704–1709. https://doi.org/10.1111/j.1523-1755.2005.00267.x
82. Montioli R, Oppici E, Dindo M, Roncador A, Gotte G, Cellini B, Borri Voltattorni C (2015) Misfolding caused by the pathogenic mutation G47R on the minor allele of alanine:glyoxylate aminotransferase and chaperoning activity of pyridoxine Biochimica. et biophysica acta 1854:1280–1289. https://doi.org/10.1016/j.bbapap.2015.07.002
83. Mufarrij PW, Lange JN, Knight J, Assimos DG, Holmes RP (2013) The effects of Oxazyme on oxalate degradation: results and implications of in vitro experiments. J Endourol 27:284–287. https://doi.org/10.1089/end.2012.0214
84. Murray MS, Holmes RP, Lowther WT (2008) Active site and loop 4 movements within human glycolate oxidase: implications for substrate specificity and drug design Biochemistry 47:2439–2449. https://doi.org/10.1021/bi701710r
85. Nazzal L, Puri S, Goldfarb DS (2016) Enteric hyperoxaluria: an important cause of end-stage kidney disease Nephrol Dial Transpl 31:375–382. https://doi.org/10.1093/ndt/gfv005
86. Oppici E, Fargue S, Reid ES, Mills PB, Clayton PT, Danpure CJ, Cellini B (2015) Pyridoxamine and pyridoxal are more effective than pyridoxine in rescuing folding-defective variants of human alanine:glyoxylate aminotransferase causing primary hyperoxaluria type I. Hum Mol Genet 24:5500–5511. https://doi.org/10.1093/hmg/ddv276
87. Oppici E, Montioli R, Cellini B (2015) Liver peroxisomal alanine:glyoxylate aminotransferase and the effects of mutations associated with Primary Hyperoxaluria Type I: an overview Biochimica. et biophysica acta 1854:1212–1219. https://doi.org/10.1016/j.bbapap.2014.12.029
88. Oppici E, Montioli R, Dindo M, Cellini B (2016) Natural and unnatural compounds rescue folding defects of human alanine:glyoxylate aminotransferase leading to Primary Hyperoxaluria Type I Curr Drug Targets 17:1482–1491
89. Oppici E et al (2015) The chaperoning activity of aminooxyacetic acid on folding-defective variants of human alanine:glyoxylate aminotransferase causing primary hyperoxaluria type I. ACS Chem Biol 10:2227–2236. https://doi.org/10.1021/acschembio.5b00480
90. Peck AB, Canales BK, Nguyen CQ (2016) Oxalate-degrading microorganisms or oxalate-degrading enzymes: which is the future therapy for enzymatic dissolution of calcium-oxalate uroliths in recurrent stone disease? Urolithiasis 44:45–50. https://doi.org/10.1007/s00240-015-0845-6
91. Pey AL, Albert A, Salido E (2013) Protein homeostasis defects of alanine-glyoxylate aminotransferase: new therapeutic strategies in primary hyperoxaluria type I BioMed research international 2013:687658 https://doi.org/10.1155/2013/687658
92. Pey AL, Salido E, Sanchez-Ruiz JM (2011) Role of low native state kinetic stability and interaction of partially unfolded states with molecular chaperones in the mitochondrial protein mistargeting associated with primary hyperoxaluria. Amino Acids 41:1233–1245. https://doi.org/10.1007/s00726-010-0801-2
93. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE (2009) Biological and chemical approaches to diseases of proteostasis deficiency Ann Rev Biochem 78:959–991. https://doi.org/10.1146/annurev.biochem.052308.114844
94. Purdue PE, Lumb MJ, Allsop J, Danpure CJ (1991) An intronic duplication in the alanine: glyoxylate aminotransferase gene facilitates identification of mutations in compound heterozygote patients with primary hyperoxaluria type 1. Hum Genet 87:394–396
95. Riedel TJ, Knight J, Murray MS, Milliner DS, Holmes RP, Lowther WT (2012) 4-Hydroxy-2-oxoglutarate aldolase inactivity in primary hyperoxaluria type 3 and glyoxylate reductase

inhibition Biochimica. et Biophysica Acta 1822:1544–1552. https://doi.org/10.1016/j.bbadis.2012.06.014
96. Ringe D, Petsko GA (2009) What are pharmacological chaperones and why are they interesting? J Biol 8:80. https://doi.org/10.1186/jbiol186
97. Robijn S, Hoppe B, Vervaet BA, D'Haese PC, Verhulst A (2011) Hyperoxaluria: a gut-kidney axis? Kidney Int 80:1146–1158. https://doi.org/10.1038/ki.2011.287
98. Rokka A et al (2009) Pxmp2 is a channel-forming protein in Mammalian peroxisomal membrane PloS One 4:e5090 https://doi.org/10.1371/journal.pone.0005090
99. Roncador A et al (2016) Use of polymer conjugates for the intraperoxisomal delivery of engineered human alanine:glyoxylate aminotransferase as a protein therapy for primary hyperoxaluria type I Nanomedicine https://doi.org/10.1016/j.nano.2016.12.011
100. Roncador A, Oppici E, Montioli R Maset F, Cellini B (2013) TAT-mediated delivery of human alanine:glyoxylate aminotransferase in a cellular model of primary hyperoxaluria type I Int J Pept Res Ther 19:10. https://doi.org/10.1007/s10989-012-9333-9
101. Rumsby G, Cregeen DP (1999) Identification and expression of a cDNA for human hydroxypyruvate/glyoxylate reductase Biochimica. et biophysica acta 1446:383–388
102. Salido E, Pey AL, Rodriguez R, Lorenzo V (2012) Primary hyperoxalurias: disorders of glyoxylate detoxification Biochimica. et biophysica acta 1822:1453–1464. https://doi.org/10.1016/j.bbadis.2012.03.004
103. Salido E, Rodriguez-Pena M, Santana A, Beattie SG, Petry H, Torres A (2011) Phenotypic correction of a mouse model for primary hyperoxaluria with adeno-associated virus gene transfer Mol Ther J Am Soc Gene Ther 19:870–875. https://doi.org/10.1038/mt.2010.270
104. Sarkissian CN, Gamez A (2005) Phenylalanine ammonia lyase, enzyme substitution therapy for phenylketonuria, where are we now? Mol Gen Metab 86 Suppl 1:S22–26 https://doi.org/10.1016/j.ymgme.2005.06.016
105. Shemesh E, Deroma L, Bembi B, Deegan P, Hollak C, Weinreb NJ, Cox TM (2015) Enzyme replacement and substrate reduction therapy for Gaucher disease Cochrane Database of Syst Rev:CD010324 https://doi.org/10.1002/14651858.CD010324.pub2
106. Shirfule AL, Sangamwar AT, Khobragade CN (2011) Exploring glycolate oxidase (GOX) as an antiurolithic drug target: molecular modeling and in vitro inhibitor study. Int J Biol Macromol 49:62–70. https://doi.org/10.1016/j.ijbiomac.2011.03.016
107. Sikora P, Bienias B, Wawrzyszuk M, Zajaczkowska M (2008) 24-hour urinary oxalate excretion in healthy children and in children with calcium urolithiasis Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego 24 Suppl 4:76–79
108. Smid BE et al (2016) Biochemical response to substrate reduction therapy versus enzyme replacement therapy in Gaucher disease type 1 patients. Orphanet J Rare Dis 11:28. https://doi.org/10.1186/s13023-016-0413-3
109. Stenberg K, Lindqvist Y (1997) Three-dimensional structures of glycolate oxidase with bound active-site inhibitors Protein Sci 6:1009–1015. https://doi.org/10.1002/pro.5560060506
110. Takahashi Y, Miyajima H, Kaneko E (1995) Genetic analysis of a family of lactate dehydrogenase A subunit deficiency. Internal Med 34:326–329
111. Takayama T et al (2014) Ethnic differences in GRHPR mutations in patients with primary hyperoxaluria type 2. Clin Genet 86:342–348. https://doi.org/10.1111/cge.12292
112. Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF (2015) Direct and functional biomarkers of vitamin B6 status Ann Rev Nutr 35:33–70. https://doi.org/10.1146/annurev-nutr-071714-034330
113. Whittamore JM, Hatch M (2017) The role of intestinal oxalate transport in hyperoxaluria and the formation of kidney stones in animals and man Urolithiasis 45:89–108. https://doi.org/10.1007/s00240-016-0952-z
114. Williams EL et al (2009) Primary hyperoxaluria type 1: update and additional mutation analysis of the AGXT gene. Hum Mutat 30:910–917. https://doi.org/10.1002/humu.21021
115. Zhao C et al (2018) Oxalate-Degrading enzyme recombined lactic acid bacteria strains reduce hyperoxaluria Urology 113:253 e251–253 e257 https://doi.org/10.1016/j.urology.2017.11.038