Primary hyperoxaluria type 1: clinical, genetic and biochemical studies

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CHAPTER 10  Summary

This thesis comprises clinical, genetic and biochemical studies on primary hyperoxaluria type 1 (PH1).
In this chapter, an overview is presented of the various studies performed.

In CHAPTER 01, the historical developments in the field of research on primary hyperoxaluria in the twentieth century are described, starting with the description of the first patient with primary hyperoxaluria in 1925, followed by the elucidation of the AGT enzyme deficiency in 1986 and the characterization of the corresponding AGXT gene in 1990, with attempts to unravel genotype-phenotype relationships at the end of the twentieth century. An overview of the standard approach for diagnosis and treatment is included. A list of reference ranges for clinical metabolites based on extensive studies in control individuals is provided in the Appendix.

CHAPTER 02 presents a study in which we assessed the need for acidification of 24-hour collection of urine at home, instead of postponing acidification until arrival at the laboratory. Previously, it has been established that the oxalate concentration in a non-acidified urine sample decreases over time as a result of precipitation with calcium. Therefore, postponement of acidification of the urine sample may lead to false negative results with respect to oxalate analysis. On the other hand, false positive results also have been reported, caused by the in vitro conversion of ascorbate to oxalate at alkaline pH. Therefore, acidification of urine is usually recommended in home settings. Since this exposes patients and their caretakers to the hazards of hydrochloric acid spill or inhalation, we investigated whether acidification of urine could be postponed for 24 hours. The study showed that urinary oxalate levels were not different if acidification was postponed as compared to immediate acidification. Therefore, we suggest that acidification should no longer be performed at home.

In CHAPTER 03, we present the assembly of the first Dutch cohort of patients with primary hyperoxaluria type 1. In order to detect all patients with primary hyperoxaluria type 1, all Dutch centers for adult and pediatric nephrology were actively approached. The epidemiologic data present information on clinical presentation of patients, and the outcome with special regard to kidney function. The predictive value of five clinically relevant parameters with respect to the development of renal insufficiency was investigated, including levels of urinary oxalate, levels of residual activity of AGT, responsiveness to pyridoxine therapy, nephrocalcinosis and age at first symptoms. A total number of
57 patients could be included in this study, resulting in a prevalence of nearly 3 per million. This prevalence is markedly higher than assessed by studies in other countries (France: 1 per million). Symptoms were remarkably heterogeneous, ranging from a single stone at age 30, to development of end-stage renal disease, extensive nephrocalcinosis and systemic calcium oxalate depositions before the first year of age. A high percentage of patients was diagnosed in end-stage renal disease (33%). Most patients diagnosed at adult age (59%) were diagnosed in end-stage renal disease, compared to 23% of those diagnosed in childhood. It was previously assumed that the outcome in PH1 was worse in patients with a younger presentation. Interestingly, in our cohort, age at initial symptoms was not a predictor of outcome. Of the other investigated potential prognostic parameters, absence of responsiveness to pyridoxine therapy and presence of nephrocalcinosis were indicative of development of renal insufficiency.

In CHAPTER 04, we describe the genotype-phenotype analysis for the Dutch PH1 cohort. Of 46 living patients, clinically described in Chapter 3, 33 were available for mutation-analysis. We found a high prevalence (44%) of the most common mutation associated with the so-called minor allele, leading to the amino acid substitution Gly170Arg. The Phe152Ile substitution appeared to be the second most common substitution in our cohort, probably being a founder mutation. All patients who were homozygous for the Gly170Arg or the Phe152Ile mutation showed full responsiveness to pyridoxine treatment with a significant decrease of urinary oxalate levels and preservation of renal function. This also explained the favorable outcome of Gly170Arg or Phe152Ile patients who received kidney transplantation without liver transplantation. Apparently, all patients who are responsive to pyridoxine therapy preserve renal function if treated timely. A rather poor outcome was observed for the c.33_34insC mutation and a novel Gly82Arg substitution. Four patients were heterozygous for one mutation only, but were AGT deficient, confirming the diagnosis of PH1. Detection of a heterozygous AGXT mutation in the diagnostic work-up can therefore not exclude PH1. In general, renal insufficiency was observed in all genotypes if diagnosis and treatment were delayed, and preservation of renal function was best achieved in patients with early diagnosis and treatment. This illustrates the importance of environmental and potentially other factors in the outcome of patients. It implicates early diagnostic screening of patients in whom primary hyperoxaluria is suspected in order to preserve renal function.

CHAPTER 05 shows the results of a separate cohort study. Since we suspected underdiagnosis of primary hyperoxaluria in patients with renal involvement as discussed in CHAPTER 03 and 04, we reinvestigated 32 hyperoxaluric patients out of 150 in whom urinalysis for oxalate had
been performed. After analysis of fresh urines from 25 of the 32 patients who could be reinvestigated, we detected one PH1 patient, who was responsive to pyridoxine treatment, thus confirming the need for an active search for PH1 patients. As an additional result, four other patients had markedly elevated levels of urinary oxalate because of malabsorption syndromes due to Crohn’s disease and short bowel syndrome. They showed considerable co-morbidity due to frequent passage of renal stones and even renal insufficiency, leading to end-stage renal disease in one patient. Conservative treatment did not comprise citrate prescription, though its clinical benefits have been demonstrated in various clinical studies. We suggest that the treatment of patients with secondary hyperoxaluria should be optimized.

A cohort of patients with Zellweger spectrum disorders (ZSDs) was screened for the presence of hyperoxaluria and hyperglycolic aciduria, and the results obtained are reported in CHAPTER 06. The impairment of peroxisome biogenesis is the biochemical hallmark in patients with generalized peroxisomal disorders, such as the Zellweger spectrum disorders. These patients present with neurological impairment. At the cellular level, many peroxisomal functions are lost. However, some peroxisomal enzymes remain stable in the cytosol, including AGT. A very high incidence of hyperoxaluria and hyperglycolic aciduria was observed in a group of 19 out of 23 (83%) ZSD patients. Urolithiasis and hyperoxaluria were seen in five patients, with development of end-stage renal disease in one of them. Levels of oxalate showed a wide range with apparently low levels in patients with mild neurological deficits, and high levels in patients with severe neurological involvement. The wide range of oxalate levels may be caused by residual functional AGT activity outside the peroxisomes or some residual AGT import in peroxisomes in mildly affected patients. Since absence of hyperoxaluria is only seen in ZSD patients with a very mild neurological impairment and survival into adulthood, a potential role for peroxisome mosaics is suggested. Follow-up of patients with ZSDs should include renal ultrasound studies to observe development of urolithiasis. We advise prevention of renal involvement by adequate fluid intake, if necessary via a nasogastric tube.

CHAPTER 07, we studied the rate of appearance of oxalate in three patients with PH1 and three control subjects in order to develop a strategy that more accurately estimates the endogenous production of oxalate. There was a good correlation between the rate of appearance of oxalate in plasma and the calculated daily urinary excretion of oxalate. The rate of appearance of oxalate could be used in patients with anuria to assess whether endogenous oxalate production declines upon treatment with novel pharmacological agents. The applicability of this method should be further studied in patients with renal insufficiency.
In **CHAPTER 08**, the results of metabolic investigations in hepatocytes isolated from the liver of a recently generated *Agxt* /- mouse are described. This knock-out mouse serves as a model for primary hyperoxaluria type 1. The aim was to identify precursors of oxalate synthesis by incubation of hepatocytes with different potential precursors. None of the tested substrates induced significant oxalate production, apart from glyoxylate, and to a minor extent glycolate. An apparent repair in AGT metabolism seems to take place in cells treated with alanine during the incubation with substrates. Since wild type mice have AGT activity in peroxisomes and mitochondria, our results suggest an alternative, potential mitochondrial route for glyoxylate detoxification. The apparent rescuing effect of alanine in *Agxt* /- hepatocytes deserves further investigation *in vivo*.

Based on the conclusions from our cohort studies, we revised the diagnosis and treatment protocols for primary hyperoxaluria type 1, as presented in **CHAPTER 09**. The major gain in the diagnostic approach is the high diagnostic yield of DNA analysis. We recommend urine collection in dry containers for diagnostic purposes. For treatment, the decision to perform kidney transplantation alone is advised if pyridoxine responsiveness is expected by demonstration of the Gly170Arg or the Phe152Ile substitution, or by a decrease in the levels of oxalate or glycolate in urine or plasma upon treatment with pharmacological doses of pyridoxine. Two flow charts present these recommendations.