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Cystinosis

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Summary: Nephropathic cystinosis is an autosomal recessive inborn error of metabolism characterized by the lysosomal storage of the disulphide amino acid cystine. It produces a variety of clinical manifestations including failure to thrive, the renal Fanconi syndrome, eye findings, and end-stage renal disease. A variety of phenotypes are known; however, the molecular defect underlying any of the forms has not yet been identified. Therapy of cystinosis with cysteamine averts the otherwise inevitable renal failure, but systemic therapy does not improve the corneal keratopathy. A number of presentations in this review detail approaches to gene identification, systemic therapy with cysteamine, measurement of cystine, and pathophysiological effects at the cellular and clinical level.

Cystinosis (McKusick 219800) has been known as a clinical entity since the early years of this century when Abderhalden noted a child dying of inanition who had curious white crystals found in the liver at autopsy (Abderhalden 1903). The pedigree revealed other children similarly affected and classical chemical analysis demonstrated that the compound found in the liver was cystine. Initially, cystinosis was confounded with the genetically unrelated condition cystinuria, and is now known as the most common cause of the renal Fanconi syndrome.

Cystinosis is a multisystemic disease (see Table 1) affecting all organ systems. The primary defect that underlies the pathophysiology is lysosomal cystine storage (Schulman et al 1969). Lysosomes of affected homozygous patients demonstrate cystine content up to 100-fold greater than that of unaffected normal individuals. The cause of the cystine storage is now known to be a defective transport system for cystine in the lysosomal membrane cystine (Gahl et al 1989). Lysosomal accumulation of cystine correlates with cell death in ways not yet understood. Cystinosis is inherited as an autosomal recessive condition, like most other inborn errors of metabolism, and heterozygotes have been shown to have approximately 50% of the cystine transport capacity in their lysosomes of that of normal individuals (Gahl et al 1984). Homozygotes demonstrate little or no cystine export. Cystine in some tissues forms crystals owing to its insolubility (approximately 112 mg/L at 25°C).

The tissue first manifesting pathological change is the retina, which has been shown to display a salt-and-pepper pattern as early as fetal life. Subsequently, the renal Fanconi syndrome develops within the first year of life, with the major clinical findings of polyuria accompanied by glucosuria, phosphaturia and aminoaciduria. Recently, Gahl's group has

Table 1 Clinical findings in cystinosis

Skin	*Kidney*	*Eye*	*Endocrine*	*General*
Fair, blond	Fanconi syndrome: ≤1 year: nephropathic	Photophobia	Hypothyroidism (8–10 years)	FTT
(Caucasians only)	ESRD: ≤10 years: nephropathic	Crystalline keratopathy		Lysosomal cystine storage
	10–20 years: intermediate	Retinopathy		Autosomal recessive inheritance
	Never: benign			

ESRD, end-stage renal disease; FTT, failure to thrive

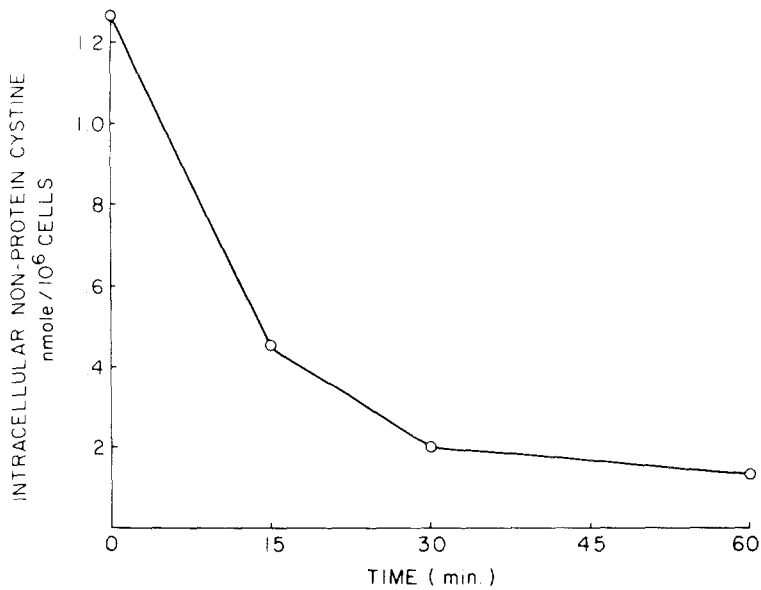

Figure 1 Cystine depletion of cystinotic fibroblasts by cysteamine. At time 0, 0.1 mmol/L cysteamine was added to confluent cultures of cystinotic fibroblasts. At the intervals indicated, fibroblasts were harvested and the cystine content was determined by a cystine-binding protein assay

shown that carnitinuria is another concomitant of the renal Fanconi syndrome and this may well correlate with the muscle weakness experienced by some patients in later years.

Over the course of the first decade of life there is a quasilinear decline in renal function such that, by age 10 years, renal function is absent and patients must be maintained on dialysis or receive a renal transplant.

Thoene and colleagues demonstrated that addition of cysteamine to cultures of cystinotic fibroblasts produced rapid cystine depletion (Thoene et al 1976; see Fig. 1). Subsequent studies in several laboratories have demonstrated that the mechanism of action involves a disulphide exchange reaction between cysteamine and cystine followed by export of the product of the reaction (this is termed the mixed disulphide of cysteamine

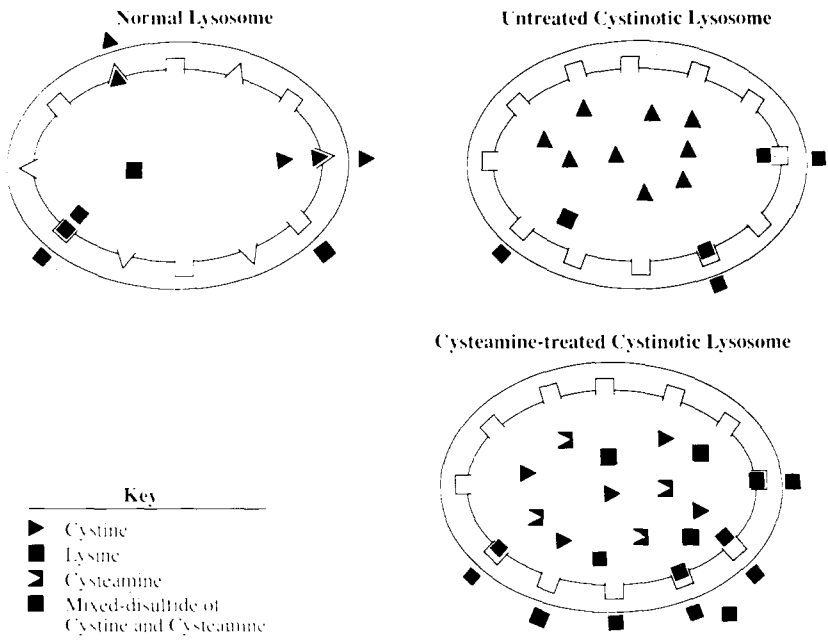

Figure 2 The mechanism of cystine depletion by cysteamine. Cysteamine reacts with cystine to form the mixed disulphide of cysteamine and cystine. This compound is an analogue of lysine and is recognized by the intact lysine porter in the lysosomal membrane, leading to cystine depletion

and cystine) on a normally functioning lysine porter not defective in cystinotic lysosomes (see Fig. 2).

Based on the promising biochemical evidence described above, it seemed reasonable to determine whether cysteamine administration would lead to long-term systemic cystine depletion and whether this might correlate with stabilized or improved renal function. Several long-term clinical trials of cysteamine in patients with cystinosis have been conducted. Cysteamine administration does stabilize renal function and improves linear growth (Gahl et al 1989). Subsequent studies in the United Kingdom and a second trial in the United States confirmed these initial observations, leading to new drug approval for cysteamine for the treatment of cystinosis in the United States on 15 August 1994.

Significant unanswered questions remain with respect to nephropathic cystinosis and its milder forms, benign and intermediate. The first question is 'What is the molecular basis of cystinosis?' Attempts to define both the chromosomal location of the gene and the gene sequence itself are proceeding in the author's laboratory at the University of Michigan and with Dr van't Hoff at Guy's Hospital in London in conjunction with Dr Gahl's laboratory at the National Institutes of Health. The groups have taken separate approaches. Thoene and co-workers are attempting to define the gene by phenotype rescue. In that technique, cystinotic fibroblasts are transfected with a human fibroblast cDNA library in a convenient plasmid vector (pcDNA III). Following transfection, the cells are placed in selective medium containing cystine dimethyl ester (2 mmol/L for 20 min). It has been found (Pisoni et al 1992)

that this exposure is toxic to cystinotic but not to normal fibroblasts. Following exposure to cystine dimethyl ester, the cells are then placed in g418 medium to select for the presence of the neomycin resistance gene, which is a component of the vector. Cells that emerge from this dual selection system are then expanded and the DNA is subjected to PCR using primers derived from the vector regions flanking the insert to recover the portion of the cDNA library that codes for the cystine transport gene. A number of candidate cDNAs have been obtained, some of which show homology to other transporters, which offers some encouragement that the identity of the gene will be discoverable by this technology.

van't Hoff and colleagues (1994b) have undertaken a linkage study to map the gene for cystinosis. They employ 250 evenly distributed markers spaced at approximately 20 centimorgan intervals. The microsatellite markers are fluorescently labelled and automated analysis allows multiple markers to be run on a single gel. Fifteen families have been typed with 168 microsatellite markers covering approximately 65% of the genome. The LOD scores for 8 chromosomes have been negative. Work continues to define the chromosomal location of the lysosomal cystine gene.

Schneider and colleagues (1995) report on the clinical trials that lead to US FDA new drug approval for cysteamine treatment of nephropathic cystinosis (Cystagon®, Mylan Pharmaceuticals, Inc). In particular, they describe a technique that smooths the data on renal function to permit a clear delineation between the effects of cysteamine treatment and non-treatment on the course of renal failure in cystinosis.

Henderson and Evans (1994) reported a comparison of leukocyte cystine analysis by GC-MS and competitive binding protein assays. A leukocyte pellet was prepared by the precipitation of erythrocytes with acid citrate dextran. The pellet was sonicated and then frozen. Deuterated cystine, the internal standard, was added prior to assay. Cystine was reduced to the monomer cysteine by mixing with tributylphosphine. *t*-Butyldimethylsilyl esters were formed using the reagent MTBSTFA. This was necessary because the cystine dimer cannot form silyl derivatives directly. Cysteine and deuterated cysteine were quantified by selected-ion monitoring at 378 and 380 amu. The assay was linear up to a cystine concentration of 100 μmol/L, and was found to have good precision with aqueous standards. However, it gave very high results with little or no discrimination between controls (6.2–29.2 nmol ½cys/mg protein, $n=32$) and cystinotic patients (12.2–45.2 nmol ½cys/mg protein, $n=9$). They concluded that the GC-MS assay as described did not give meaningful results and that overstimulation of cystine might be due to measurement of cysteine residues from sources other than the cystine dimer. The results of the competitive cystine binding assay for cystine in their hands paralleled the published experience for sensitivity and the ability to discriminate between homozygotes and normal individuals.

Skovby and Hertel (1994) reported on the use of growth hormone in cystinosis. Growth hormone was supplied in conjunction with cystamine and standard electrolyte replacement therapy. They found that urinary levels of growth hormone were several thousandfold elevated and serum levels of IGF1 and mean concentrations of growth hormone in urine were low compared to normal control children. They suggest that the growth retardation seen in cystinosis could be partly due to the excessive loss of growth hormone in the urine. As reported by other authors, they found that treatment with growth hormone dramatically increased linear growth. They caution that this also may hasten the onset of relative renal failure, as previously suggested (Andersson et al 1992).

Pintos-Morell and colleagues (1994) noted that linkage between lysosomal cystine and the pathophysiology that occurs in nephropathic cystinosis is not yet understood, and that an altered oxidative metabolism in polymorphonuclear cells and monocytes as well as changes in oxygen consumption and reabsorption capacity of the renal tubular cells had been suggested previously. Since cysteine is a component of the sulphidopeptide-leukotrienes LTC_4, LTD_4 and LTE_4, which have potent vasoactive actions and induce contractions of renal mesangial cells, they studied leukotriene production in isolated polymorphonuclear leukocytes stimulated with the ionophore A23187 in cystinotic children, in heterozygotes and controls. They found that the polymorphic leukocytes from untreated cystinotic children produced an increased amount of LTC_4 (417 ± 70 vs 177 ± 30.9 pmol per 10^7 cells compared to controls, $p < 0.1$). Concomitantly, LTB_4 production was decreased compared to controls, but the total amount of LTA_4 was normal. PMNs from cystinotic children treated with cysteamine produced lower amounts of LTC_4 compared to untreated patients. Cessation of cysteamine treatment for 3–4 days resulted in an increase in LTC_4 production. They conclude that the findings suggest that the metabolic abnormalities in cystinosis may result from the biosynthesis of LTC_4 from polymorphonuclear leukocytes.

van't Hoff and colleagues (1994a) studied the effect of storage of whole blood on leukocyte cystine concentration in patients with cystinosis. The diagnosis of many rare diseases is hampered by the need to analyse fresh samples, which, given the geographic dispersion of the patients involved, leads to logistical difficulties. Currently, the state-of-the-art method for measuring cystine is to use the cystine-binding protein assay (see above); however, this requires preparation of a mixed leukocyte pellet that is then sent on dry ice to the referral laboratory for measurement. Not all clinical laboratories have the ability to produce such a pellet in usable fashion. van't Hoff and associates determined that storage of whole blood for 24 or 48 h in either acid citrate dextran (ACD) or lithium heparin tubes had no statistically significant effect on the leukocyte cystine concentration compared to control samples isolated immediately by the usual protocol. After 24 h they found a mean cystine content of 2.35 nmol/mg protein for the controls compared to 2.62 for ACD and 2.23 for heparin. After 48 h, the controls were 2.17, ACD 2.34, and heparin 2.47. They conclude that sites unable to prepare leukocyte pellets can therefore send whole blood, anticoagulated by either method, to a specialized laboratory for assay of leukocyte cystine.

The elevation in serum cholesterol in patients with cystinosis was studied by Hulton and colleagues (1994). They measured total serum cholesterol using an enzymatic method in 25 children with cystinosis over a period of 4 years. An approximately equal male and female distribution was found with an age range of 1.8–15.9 years, all of whom were predialysis and not yet transplanted. Fifteen children (60%) had serum cholesterol concentrations greater than two standard deviations above the mean, using age-related reference data. They found no correlation between cholesterol and age or sex but did find that the higher cholesterol concentrations occurred in those who were progressing more rapidly to end-stage renal disease.

van't Hoff and colleagues (1994c) reported on the development of a newly encapsulated form of cysteamine for the treatment of cystinosis. Cysteamine, a free thiol, has a noxious odour and taste, making it intolerable in approximately 14% of patients to whom it is

administered, even though it is understood to be the only effective means of avoiding end-stage renal disease in cystinosis. Aqueous cysteamine hydrochloride is particularly odoriferous and thus encapsulation is needed. Dr van't Hoff's group undertook a single-dose study of a cysteamine capsule in six patients aged 6–19 years. Three had had renal transplant, but stable renal function, none had had cysteamine for seven days prior to the study. The capsule represented a cysteamine dose of 15 mg/kg in a poly(ethylene glycol) base and was given on an empty stomach. Plasma cysteamine and leukocyte cystine concentrations were determined over the subsequent 24 h. The cysteamine odour was noted in all patients post-dose, but it was felt to be mild and there were no other adverse side-effects. The mean leukocyte cystine fell from a pre-dose level of 6.03 to a minimum of 1.43 at 3 hours and remained low at 24 h (3.03 ± 1.61 nmol ½ cystine/mg protein). Plasma cysteamine determined in four patients showed a biphasic absorption profile with a mean peak of 29 μmol/L at 30 min, and a second peak of 23 μmol/L at 90 min. They conclude that the systemic bioavailability of the capsule is 50%.

Vilaseca and colleagues (1994) reported that a biochemical response does not always correlate with clinical benefit in late-treated nephropathic cystinosis. They reported on three patients, a 3-year-old boy and his 1-year-old sister, and an unrelated 2-year-old girl diagnosed with nephropathic cystinosis on the basis of elevated leukocyte cystine content. They were begun on therapy after renal failure had intervened with a GFR of 19.4 and 25.3 ml/min per 1.73 m^2 in the first two cases. The third patient did not have renal failure but had tubular nephropathy. Carnitine was found to be low in all three children. Cysteamine treatment of 50–60 mg/kg per day of free base and carnitine was started one year after diagnosis. The mean cystine depletion from leukocytes was 85% for two years and plasma carnitine was normalized. They note, however, that proteinuria and the excretion of other compounds due to the renal Fanconi syndrome were not altered. Growth improved and remained around the mean in the third case. They conclude that genetic heterogeneity may determine not only the severity of the disease, but also the therapeutic response.

On the other hand, the American experience has been that after serum creatinine rises above approximately 2–3 mg/dl, cysteamine is unable to rescue the affected kidney. Thus, we would have predicted that the children described in this abstract would not have shown a favourable response to cysteamine either in terms of the tubular function or in preserving the glomerular function.

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