

# Glycolate Oxidase Inhibition and the Treatment of Primary Hyperoxaluria Type 1

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## Background

Primary hyperoxaluria type 1 (PH1) results from a deficiency of peroxisomal alanine:glyoxylate aminotransferase (AGT) activity in hepatocytes. The liver is unable to effectively remove glyoxylate and it is converted to oxalate. The primary source of this glyoxylate is the enzyme, glycolate oxidase (GO), which similar to AGT, is localized in peroxisomes. A logical strategy to limit the excessive oxalate synthesis that occurs in PH1 is to block GO activity. The pharmaceutical company, Merck Sharpe and Dohme 3 decades ago, and more recently, the agrochemical division of Astra-Zeneca, have invested significant resources in developing drugs that will inhibit GO activity. Astra-Zeneca has provided some of its more potent drugs for evaluation in blocking GO activity. Preliminary data indicated that a compound we termed CDST, was the most potent in inhibiting GO activity in vitro.

## Research Objectives

Three specific aims were formulated for this project. The first specific aim was to characterize the effects of CDST in inhibiting GO activity in cultured cells. The second specific aim was to develop an assay to measure the concentration of CDST in plasma and urine. The third specific aim was to examine the efficacy of CDST in inhibiting urinary oxalate excretion in an animal model of PH1.

## Results

1. *Inhibition of GO activity in cultured cells.* COS cells, a line of cells derived from the kidney of an African green monkey, were transiently transfected with a cDNA encoding human GO. These cells efficiently converted glycolate added to the medium to oxalate. The amount of oxalate produced was dependent on the amount of cDNA transfected, the amount of glycolate added to the culture medium, and the time of incubation with glycolate. The concentration of CDST producing a 50% inhibition of oxalate was approximately 45  $\mu\text{M}$ . These results indicated that cultured cells could take up CDST and that it was quite potent in inhibiting GO activity. The inhibition, however, was about 5 times less than that observed in liver tissue homogenates or in cultured cell extracts, suggesting either that significant amounts of the drug were sequestered by cell membranes or that cellular permeability to the drug was impaired. The drug has a lipophilic tail, which is thought to be important in anchoring it to a cleft in the enzyme that runs through the center of the protein from the active site.

2. *Assay of CDST.* A method was developed for assaying CDST using LC/MS/MS. We relied on the lipophilic properties of the drug and first extracted samples using a Bligh and Dyer extraction procedure. A structurally similar compound to CDST that was supplied by Astra-Zeneca was used as an internal standard (IS) and added to the sample before extraction. A calibration curve was constructed by varying the concentration of CDST while keeping the IS concentration constant. The linear range of the assay was established and it was shown to be both reproducible with a coefficient of variation of < 5% and accurate by the recovery of > 90% of added compound to samples.

3. *Effect of CDST in the AGT KO mouse.* In collaboration with Dr. Eduardo Salido of Laguna University, Spain, we have examined the effect of CDST on the hyperoxaluria observed in the AGT KO mouse. Baseline urine collections were obtained for 6 days. Mice were then dosed by gavage for 7 days with 0.5 – 2 mg/g body weight of CDST dispersed in an aqueous solution. Dose dependent decreases in urinary oxalate excretion were observed. With the highest dose, 2 mg/g, the mean decrease was 58%. At sacrifice, blood and tissue samples were obtained. Less than 10% of the ingested dose was recovered in blood and urine samples, indicating that either the drug was poorly absorbed or that it was rapidly metabolized. The storage of drug in adipose and other tissues is yet to be assessed. It is possible that the lipophilic tail on the drug adversely affects its absorption and promotes its accumulation in adipose tissue.

## **CONCLUSIONS**

The inhibition of GO activity is a suitable therapeutic strategy for decreasing endogenous oxalate synthesis in individuals with PH1. To overcome its apparent limited absorption in animals and decreased efficacy in cultured cells, the lipophilic tail should either be shortened or a hydrophilic group added to enhance its solubility. An alternative strategy to examine is to use other means of drug delivery that are more efficacious for this type of compound. Such approaches could include liposome encapsulation of the drug or its incorporation in patches.