β-AMINOTHIOLS AND THE REGULATION OF HEPATIC OXALATE PRODUCTION

A Thesis submitted by

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ABSTRACT

Of the numerous risk factors for calcium oxalate urolithiasis, oxalate is the most important as small reductions in urinary oxalate excretion can markedly reduce the risk of calcium oxalate urolithiasis. The investigations in this thesis centre on the use of β-aminothiols (cysteine, cysteamine and penicillamine) to decrease endogenous oxalate production by forming adducts with glyoxylate, the immediate precursor of oxalate.

The investigations include, the preparation, characterisation and metabolism of the cysteine-glyoxylate adduct (Chapter 3), the ability of β-aminothiols to decrease urinary oxalate excretion under normo- and hyperoxaluric settings (Chapter 4) and *in vitro* studies with hepatocytes to establish the mechanism by which β-aminothiols regulate hepatic oxalate production and hence urinary oxalate excretion (Chapter 5). The latter includes an examination of the pathways regulating endogenous oxalate production.

Different diastereomeric ratios, i.e. *cis*- and *trans*-, of the cysteine-glyoxylate adduct were prepared and characterised. At pH 7.4 the ratio of *cis*- to *trans*- was 51% to 49% while at pH 1.4 the preparation was found to be 100% *trans*-. Although *in vivo* studies (i.p. injection of adduct) indicated that considerable adduct metabolism occurs, only 2% of the metabolised dose appeared in the urine as oxalate.

The ability of orally administered (L)-cysteine, (D)-penicillamine and (L)-2-oxothiazolidine-4-carboxylic acid (OTC, an intracellular (L)-cysteine delivery drug) to decrease urinary oxalate excretion in rats under normo- and hyperoxaluric conditions (ethylene glycol or glycollate induced) was investigated.

Under normooxaluric conditions, OTC significantly decreased urinary oxalate, 30% (long term administration, $p<0.05$) and 10% (short term administration, $p<0.05$); but (L)-cysteine did not, 9% (short term administration, $p=0.22$). In contrast, (D)-penicillamine significantly increase urinary oxalate excretion, 68% (short term administration, $p<0.05$); in association with decreased plasma alanine, 34% ($p<0.01$); and aspartate, 23% ($p<0.001$); aminotransferase activity. *In vitro* studies indicate that (D)-penicillamine exerts its effect through inhibition of glyoxylate:alanine aminotransferase via interference with pyridoxal phosphate, the result being increased availability of glyoxylate for oxidation to oxalate.

Under mild hyperoxaluric conditions (glycollate induced, urinary oxalate 3× normal) short term administration of both OTC and (L)-cysteine was shown to be effective in reducing urinary oxalate, 39% ($p<0.001$) and 31% ($p<0.001$) respectively. After extensive investigation (including the use of gas chromatography/mass spectroscopy) evidence for the presence of adduct in the urine of these rats could not be found. This was consistent with earlier studies which indicated that extensive metabolism of adduct would occur at the levels expected to be produced endogenously.

OTC was also investigated under more severe hyperoxaluric conditions (ethylene glycol induced, urinary oxalate 18× normal). Significant reductions in urinary oxalate were not observed and this was attributed to the 5 fold difference in the dose of ethylene glycol and OTC. Hence OTC could not supply sufficient cysteine for adduct formation under these conditions.

Isolated rat hepatocytes were used to further investigate the effect of β-aminothiols on oxalate production from glycollate, which is metabolised within the cell to glyoxylate and oxalate. Addition of extracellular glyoxylate was inappropriate in this setting due to the potential for extracellular formation of adduct. Adduct formation was shown to be proportional to the decrease in oxalate production, the first confirmation of the postulated mechanism by which β-aminothiols reduce oxalate production and excretion.

Further investigation of the roles of glycollate oxidase and lactate dehydrogenase using isolated hepatocytes and a reconstituted enzyme systems indicate that hepatic oxalate production is dependant on compartmentalisation between peroxisomal (glycollate oxidase) and cytosolic (lactate dehydrogenase) metabolism.

Evidence was prevented to suggest that at physiological substrate levels lactate dehydrogenase is the enzyme responsible for oxidation of exogenous glyoxylate to oxalate while glycollate oxidase is responsible for the two step oxidation of glycollate to oxalate. Hence, *in vivo* glycollate oxidase may be the enzyme responsible for the major portion of oxalate production.

These studies indicate that cysteine delivery drugs like OTC have the potential to aid in management of calcium oxalate stone disease presumably through formation of the (L)-cysteine-glyoxylate adduct, thereby reducing endogenous oxalate production and urinary oxalate excretion.