Cysteamine Prodrugs For an Improved Treatment of Cystinosis

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INTRODUCTION

Cysteamine is a successful treatment for cystinosis. However, the problems and side effects with its administration are well known. This project aims to develop a cysteamine prodrug treatment which will alleviate the problems and will have the following properties:

- No taste / smell
- Limited GI irritation
- Good absorption after oral administration
- Favourable pharmacokinetic parameters
- Minimal first pass metabolism, reduced halitosis
- Directed delivery of cysteamine to cystinotic cells
- Efficient depletion of cystine
- Minimal side effects overall

WHAT IS A PRODRUG?

Prodrugs can be designed to overcome a range of problems, including inefficient delivery, poor absorption, poor bioavailability and noxious taste and smell . They are inactive forms of a successful clinical drug which are activated, usually by an enzyme, once inside the body to release the active parent drug, Figure 1. Our initial work with simple prodrugs demonstrated proof of concept [1] and we are now carrying out full evaluation of a series ofpossible clinical candidates.

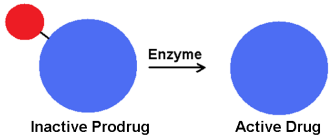


Figure 1: Cartoon showing conversion of prodrug into active parent drug

DESIGN OF PRODRUGS

- Different carbon chain length esters are being used to explore their absorption properties
- Using a thioester to mask the thiol group until the drug is absorbed from GI tract – this reduces the problem of taste and smell.
- Gamma-glutamyl transpeptidase (GGT) is a membrane-bound enzyme found on the surface of most cells. It is part of the glutathione cycle and is expressed more highly by cystinotic cells [2]. GGT is known to internalise γ-glutamyl amino acids, so a GGT targeting strategy is being employed with the aim to:
  (1) Increase bioavailability by reducing 1st pass metabolism and excretion
  (2) Reduce the necessary dose
  (3) Reduce side effects through the use of a lower dose

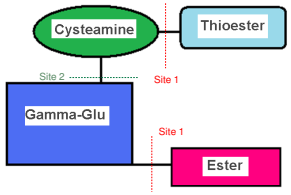
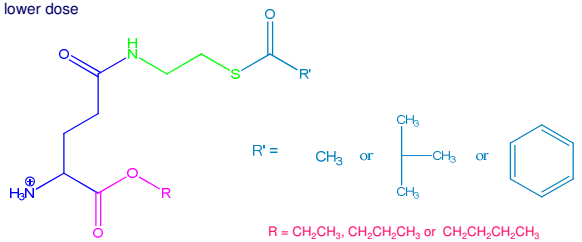


Figure 2: Diagrammatic representation of prodrugs



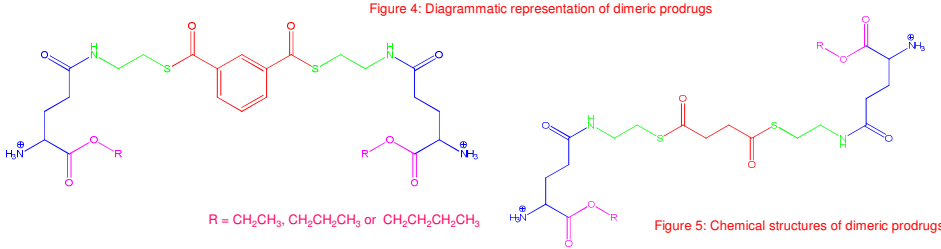
R = CH_2CH_3, $CH_2CH_2CH_3$ or $CH_2CH_2CH_2CH_3$

Figure 3: Chemical structure of prodrugs

The prodrug is broken down by two types of enzymes (Figures 2 and 3):
Sites 1: Esterases, which are commonly found throughout the body
Site 2: Gamma-glutamyl cyclotransferase, a key enzyme of the glutathione cycle inside cells
Together, these enzymes release cysteamine slowly: site 1 is broken down outside cells, for example, in the blood, to produce γ-glutamyl-cysteamine, which is transported into cells by GGT where cysteamine can be released by enzymatic action at site 2.

Improvement of bioavailability may be achieved by the delivery of two cysteamine molecules per one molecule of prodrug. It is thought that cystinotic cells are under oxidative stress [3], so the use of a disulphide cystamine dimer would be unsuitable as it could put the cell under more stress. Therefore, dimeric prodrugs have been designed which contain a linking molecule. These can be broken down by enzymes, in a similar way to the prodrugs above (Figures 4 and 5).

Figure 4: Diagrammatic representation of dimeric prodrugs



R = CH_2CH_3, $CH_2CH_2CH_3$ or $CH_2CH_2CH_2CH_3$

Figure 5: Chemical structures of dimeric prodrugs

RESULTS

To evaluate the success of the synthesized prodrugs, the key processes to be monitored are:

- Internalisation of prodrugs into cells
- Breakdown of prodrug intracellularly to release cysteamine
- Cysteine depletion by the released cysteamine

The majority of the chemical species involved at each stage in the processes are not detectable by normal laboratory methods, *e.g.* HPLC with UV detection. In order to detect the thiol species, it is possible to use a molecule called CMQT [4]. The CMQT molecule reacts specifically with thiols to produce a compound that is visible with UV and fluorescence detection, Figure 6.

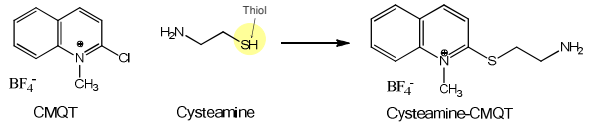


Figure 6: Reaction of CMQT with cysteamine to make a UV and fluorescence visible derivative

HPLC VALIDATION

In order to have absolute confidence in the results, it is necessary to validate the methodology for these compounds. HPLC validation involves testing various parameters to ensure that the method is precise, linear and repeatable.

SPECIFICITY – the first parameter involves testing the method for its suitability. A variety of related substances, particularly cellular thiols, were reacted with CMQT, to test the methodology. The chosen method achieves good separation of cellular thiols, allowing accurate identification and quantification within cells.

PRECISION – Reproducibility of the method is crucial for reliable results. An assay of the method showed good precision (%RSD 0.37).

LINEARITY – The relationship between response and concentration of thiols was continuous and reproducible. Calibration curves of standards were linear with R^2 values close to 0.999.

LOD and LOQ – the limits of detection (LOD) and quantification (LOQ). Optimisation of the HPLC method development has enabled much lower limits of detection and quantification than were previously available (<1 µmol and between 2 - 4 µmol, respectively), Figure 7. This means that *in vitro* biological testing can be carried out at concentrations of prodrug and cysteamine which are comparable to systemic values.

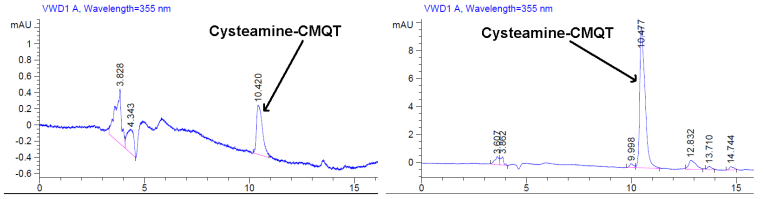


Figure 7: Chromatograms showing cysteamine-CMQT at 1µmol and 4 µmol, respectively

PRELIMINARY PRODRUG FINDINGS

- We have observed that two of the prodrugs (initially at 1mM) were successfully internalised into cells and released cysteamine ; this was detected by a marked increase in cellular cysteamine levels, which was delayed in prodrug treated cells compared to cysteamine treated control cells, suggesting a step-wise breakdown of prodrug.
- Ester and thioester groups were hydrolysed before cysteamine was released: γ-glutamylcysteamine (Figure 8) was observed in cell lysate after incubation with prodrug and was characterised by LC-MS.
- A decrease in cystine / cysteine levels have been observed in cystinotic cells

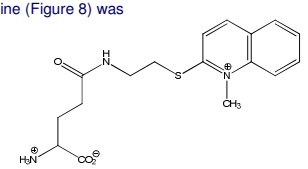


Figure 8: Chemical structure of γ-Glutamylcysteamine

FUTURE WORK

- Evaluate remaining monomer prodrugs
- Evaluate dimeric prodrugs
- Use range of (lower) concentrations
- Measure log P values
- Assay enzymic / acidic stability
- Carry out basic cell toxicity experiments
- Identify optimal prodrugs for further evaluation

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