

**Design and Synthesis of Novel
Prodrugs for the Treatment of
Cystinosis.**

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Background

Nephropathic cystinosis is a rare autosomal recessive disease characterised by raised intracellular levels of cystine. Symptoms include renal Fanconi syndrome and growth retardation. If untreated, cystinosis results in death from renal failure by the second decade of life. The only treatment for cystinosis is administration of cysteamine, an aminothioliol with an offensive taste and smell, which is excreted in breath and sweat causing halitosis and body odour as well as gastric irritation. As a result, patient compliance may be poor.

The purpose of this investigation was the synthesis, characterization and biological evaluation of a series of odourless and tasteless pro-drug forms of cysteamine. It is envisaged that these compounds will improve compliance among cystinotic patients and lead to an increase in patient treatment success and quality of life.

The detailed aims of this project were: -

- (i) The synthesis, purification and characterisation of a number of pro-drug derivatives of cysteamine and cystamine that will reduce the cystine burden within cells.
- (ii) Evaluation of the *in vivo* activity of synthesized prodrugs using a 'knock-out' mouse model. This work will be carried out in collaboration with Dr C. Antignac, Hôpital Necker-Enfants Malades, Paris, France.

Results

This project required the synthesis of a number of odourless, tasteless and orally active pro-drug derivatives of cysteamine and cystamine, development of a specific assay for thiol containing compounds in cell culture samples and the determination of the ability of test compounds to deplete the high levels of cystine found in cultured cystinotic cells. This work was described recently [McCaughan et al, *Bioorganic & Medicinal Chemistry Letters* **18** (2008) 1716–1719] See Appendix 1.

Since the publication of this paper, studies undertaken have concentrated on optimising the published assay. Two variables have been examined:

- The use of fluorimetry instead of absorbance to determine intracellular thiol content. The use of fluorescence should increase the specificity of the assay (since not all compounds fluoresce) and offers the possibility of increasing the sensitivity of the assay by a factor of 10 – 1000x
- The determination of levels of cystine at shorter time intervals than 72 hours.

Fluorescence Evaluation

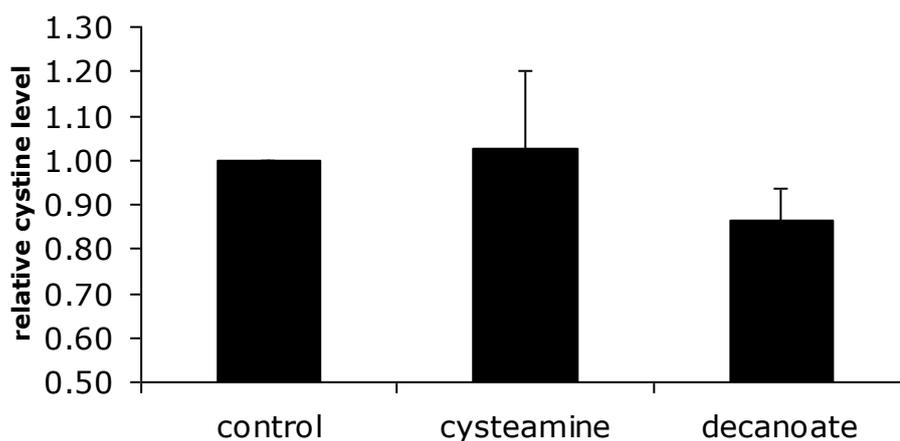
The quantitative and facile conversion of 2-chloro-1-methylquinolinium tetrafluoroborate to 2-methoxy-1-methylquinolinium tetrafluoroborate as an experimental fluorescent, thiol specific tag was evaluated. The

fluorescent quantum yield and pH stability of the experimental tag were evaluated and compared to results obtained with 2-chloro-1-methylquinolinium tetrafluoroborate. The results of this work are in preparation for submission to an analytical chemistry journal and are not detailed here; a copy of any resulting publications will be forwarded to CRN on acceptance.

Assay Evaluation

The published assay used an end point of 72 hours for the determination of cystine levels in cultured fibroblasts. In an attempt to simplify the assay and increase the throughput of samples, various time courses of 12, 24 and 48 hours were evaluated. The results of the 48h evaluation are presented below

Efficacy of cysteamine and cysteamine decanoate in lysosomal cystine reduction in cystinotic fibroblasts: (48h)



The results from the 48h assay clearly show that the level of cystine in lysosomes isolated from cystinotic fibroblasts was significantly reduced ($p=0.001$ Student's t test) in the presence of our prodrug, cystamine decanoate. Absorbance values are measured at 595 nm as previously described (McCaughan *et al*, 2008).

These data support the values obtained for 72 h and confirm the efficacy of cystamine decanoate as an effective reducer of levels of intracellular cystine. Studies using 12 and 24h end points are ongoing and will be reported in the next report.

Future work

Now that *in vitro* efficacy has been demonstrated for the first of the synthesised prodrugs, we wish to extend the work to evaluate the ability of the compounds to deplete levels of cystine in cystinotic animals. To undertake this work we now need to complete the following;

1. **Prodrug:protein interaction:** prodrugs will be added to plasma at a range of concentrations to determine the pharmacodynamic and pharmacokinetic properties of the drug. The level of available prodrug will be determined by HPLC and from this the half-life of the prodrug will be determined.
2. ***In vivo* toxicity:** The target prodrugs, cysteamine, cystamine and vehicle control will be administered intravenously to healthy mice (6 per group) and the cardiac output will be recorded (heart rate and blood pressure). A blood sample will be removed prior to terminal anaesthesia and the level of the respective prodrug will be determined.

Expertise exists within the School of Pharmacy and Life Sciences to carry out this work. When this work has been completed we will be in a position to allow the prodrugs to be administered to cystinotic mice in collaboration with Dr C. Antignac. Planning is well established to enable these studies to be undertaken in a timely and successful manner.

We envisage that these important studies will be supported by payment of the 3rd tranche of the initial \$97,928 grant awarded in 2005.

Conclusions

The project '***Design and Synthesis of Novel Prodrugs for the Treatment of Cystinosis***', funded by CRN in 2005 continues to achieve solid progress. As discussed in the 2007 report, the chemistry section of the work has been very successful and a library of > 50 compounds has been developed.

The formulation and testing of these prodrugs and the exploration of novel routes of administration for cysteamine, cystamine and prodrugs is ongoing. These studies are supported by several other sources and are included in this report to provide a complete view of the work at RGU. The synthesis, assay and *in vitro* evaluation of several of the prodrugs was published (McCaughan *et al*) and was well received by the cystinosis scientific community. In addition three posters describing some of the work detailed in this report were presented at the International Cystinosis Conference in Dublin, Ireland in 2008. Furthermore oral and poster presentations were given at the Young Researchers conference in Loughborough, UK in summer 2008. The Loughborough podium presentation received the prize for best presentation for Dr McCaughan and since the conference was not restricted to cystinosis, achieved our desired aim of increasing the awareness of cystinosis to the widest possible audience. Financial support from CRN was acknowledged in all of these publications and abstracts of the work are included in the Appendix of this report.

The prodrug work is now at a stage where we have demonstrated the synthesis, evaluation and *in vitro* ability of the compounds to deplete cells of cystine and efforts are underway to secure additional support from a number of sources to extend the scope of the project and build on the success achieved.

It remains our intention to investigate the *in vivo* activity of these compounds in collaboration with Dr Antignac in Paris but we appreciate that preliminary toxicity studies on healthy mice must be undertaken first. These studies will be started soon and will utilise the in house facilities of The Robert Gordon University.

Appendix 1

Refereed publications arising (in whole or in part) from this work

Kay G, Cairns D, McCaughan B, Warasiha, B. *Journal of Pharmacy and Pharmacology, Supplement*. The design, synthesis and biological evaluation of novel prodrugs for the treatment of cystinosis. 2007; 59 (4). p. 7.

Cairns D, Benylles A, McCaughan B, Kay G. *Journal of Pharmacy and Pharmacology, Supplement*. Molecular modelling of the interactions between cysteamine and cystamine prodrugs and β cyclodextrin. 2007; 59(4). p. 49.

McCaughan, B.; Kay, G.; Knott, R. M.; Cairns, D. *Bioorganic & Medicinal Chemistry Letters*. A potential new prodrug for the treatment of cystinosis: Design, synthesis and in-vitro evaluation. 2008, 18, 1716.

B. E. Buchan, G. Kay, K. H. Matthews and D. Cairns. *Journal of Pharmacy and Pharmacology, Supplement*. Formulation and evaluation of novel dosage forms of cysteamine for the potential treatment of cystinosis. 2008; 139. p. 55.

R.M. Knott, E Hector, B. McCaughan, G. Kay, & D. Cairns
Lysosomal Depletion of Cystine in Cystinotic Fibroblasts with a novel Prodrug *Poster presented at the International Cystinosis Conference, Dublin, 2008*

B. McCaughan, G. Kay, R.M. Knott and D. Cairns
A potential new treatment for Cystinosis: design, synthesis and *in-vitro* evaluation
Poster presented at the International Cystinosis Conference, Dublin, 2008

G. Kay, B. Buchan, K.H. Matthews, and D. Cairns.
Formulation and Evaluation of Novel Dosage Forms of Cysteamine for the Potential Treatment of Cystinosis.
Poster presented at the International Cystinosis Conference, Dublin, 2008

B. Mc Caughan
A potential new Prodrug for the treatment of Cystinosis: Design, synthesis and *in-vitro* evaluation.
Poster and Podium presentation at APSGB, Loughborough, 2008