Proof of Concept Success for the Targeted Prodrugs

Professors Roz Anderson and Paul Groundwater, with Pratap Suryadevara and Stephanie Cannell, University of Sunderland, UK, in collaboration with Professor William van't Hoff, Great Ormond Street Hospital, London, UK.

Cystinosis research at the University of Sunderland over the last few years, entirely funded by the Cystinosis Foundation UK, has been focussed upon the design, synthesis and testing of cysteamine prodrugs, with the aim of improving the existing treatment.

We considered the role of cysteamine in effecting cystine depletion and evaluated the key features of its chemical structure that are essential for its success in treating cystinosis. We concluded that cysteamine itself is essential for its activity and turned our sights instead to how cysteamine could be better delivered to patients in a way that reduced, or even eliminated, the unpleasant taste, smell, gastric irritation, and other side effects. Furthermore, we wanted to overcome the big problem of first pass metabolism and excretion of cysteamine, which is estimated to reduce the available dose of cysteamine to 20% of that administered; this huge loss of cysteamine is the major reason a high daily dose is required. We chose the prodrug approach for this purpose.

There are many advantages offered by the prodrug approach:

- Reduction, or even elimination, of the taste and smell;
- Reduction in first pass metabolism and excretion, resulting in more drug being available;
- Delivery of the drug to its site of action, resulting in increased efficiency;
- Increased availability and greater efficiency of drug results in a lower dose being required;
- The requirement for a lower dose is expected to result in a reduction in side effects;
- The use of a lower dose and fewer side effects leads to greater patient compliance and an improved clinical outcome.

A prodrug is an inactive form of a pharmaceutical (the 'parent drug') and remains inactive until it is activated inside the body by an identified mechanism. By careful prodrug choice and design, the prodrug can be broken down to release the parent drug in a particular site, for example, in the stomach, the small intestine, the blood, the liver, the brain, or even inside particular cells. Our goal was to design and synthesize suitable prodrugs of cysteamine with the best release characteristics to optimise the delivery of cysteamine to cystinotic cells, providing improved efficiency of this essential treatment.

We identified a target protein, gamma-glutamyl transpeptidase (GGT), that is sited on the external surface of most human cells and is part of the essential cellular glutathione cycle, which helps to maintain the correct oxidative state and normal functioning of cells. GGT is responsible for taking amino acids into cells across the cell membrane, as the gamma-

glutamyl derivative, then releasing them once inside the cell. By preparing the gamma-glutamyl derivative of cysteamine, we made a prodrug that we expected to be taken into cells by GGT and then broken down to release cysteamine by an enzyme that is already present as part of the glutathione cycle.

From the literature on cystinosis in the international scientific press, we noted that cystine accumulated in cells that had an active glutathione cycle (which is most cells) and that the cells most affected by cystine accumulation appeared to coincide with those in which the glutathione cycle is most active, such as kidney cells. This does not necessarily mean that glutathione is linked to the cause of cystinosis, but could indicate a possible link to the cells in which the highest concentrations of cystine occur. By targeting our prodrugs to GGT, we could be targeting them to the cells that most need treatment.

It was important to test a range of similar compounds to find the optimal prodrug with the best properties for treating cysteamine, so we added two variables to our gamma-glutamyl cysteamine prodrug: a set of different groups on the sulfur of cysteamine to mask the smell and taste, and another set of different groups on the gamma-glutamyl part to enhance absorption from the gastrointestinal tract after oral administration. This gave us nine related prodrugs to evaluate, all based on gamma-glutamyl cysteamine.

In order to monitor the release of cysteamine inside cells, we had to develop a reliable method that allowed us to use standard laboratory equipment. By adapting and validating a method from the scientific literature, we were able to monitor not only the release of cysteamine, but also the entry of prodrug into the cells before it was broken down, along with many other important cellular sulfur-containing molecules. In fact, our method was also used successfully by Professor Donald Cairns and his team at Robert Gordon University in Aberdeen to evaluate the efficiency of their simple, untargeted prodrugs.

The evaluation of the nine GGT-targeted prodrugs required a number of different tests to investigate their ability to deliver cysteamine where and how intended. First, we needed to know if the prodrugs could be taken into cells from the surrounding liquid; they must be absorbed through the cell membrane. Second, the prodrugs must be broken down to release cysteamine inside the cells; in cystinotic cells, this may depend upon the activity of the glutathione cycle, which is known to be altered in cystinosis. Third, the cysteamine released must efficiently deplete lysosomal cystine. Lastly, for these particular tests, the prodrugs should not be toxic to cells.

Most tests required the use of cultured kidney cells, which can be expensive and difficult to grow in the laboratory, as these cells show significant cystine accumulation in cystinosis and also a high surface concentration of GGT. Our initial tests used monkey kidney cells, which are relatively easy to culture, and indicated that the prodrugs were taken into mammalian cells, with the subsequent release of cysteamine. Subsequent tests used normal cultured human kidney cells, which confirmed that these targeted prodrugs were taken up into

normal cells and broken down to release cysteamine over a short period of time; these tests were important to establish that the GGT-targeted prodrug concept was successful with normal human kidney cells, which would support the subsequent tests with cystinotic cells.

Recently, the kind donation of cells from Dr Martijn Wilmer of Professor Elena Levtchenko's group in the Netherlands allowed us to test one of the prodrugs in a cultured cystinotic cell line, alongside its normal control cell line, when we observed good cystine depleting ability, at least comparable to cysteamine and even better over the short time course studied. Futhermore, we observed extremely low toxicity of the prodrugs in the cultured cells used for these experiments and in a range of other cell-based laboratory tests, specifically designed to evaluate cell toxicity.

These results are very encouraging – they provide proof of concept for the GGT-targeted prodrugs and support the evaluation of the remaining prodrugs to quantify their cystine-depleting ability and their toxicity through a range of carefully designed tests. They confirm that the prodrug was taken into both normal and cystinotic cells and broken down to release cysteamine. This also showed that the glutathione cycle in these cystinotic cells was sufficiently active to metabolise the prodrug, or that the part of the glutathione cycle necessary to release cysteamine is unaffected in cystinosis, and that the cysteamine released was able to enter the lysosome and react with cystine.

Our next aim is to identify the best of the nine prodrugs, after careful testing, and to provide the data for the Investigational Medicinal Product Dossier that will be required to take the optimum prodrug forward to clinical trials.

We would like to take this opportunity to thank the Cystinosis Foundation UK for supporting this work and for introducing us to new friends and colleagues across the world. With your continued support, we aspire to move towards an improved treatment for cystinosis.