Towards the pharmacological treatment of primary hyperoxaluria: multiply-transformed CHO cells as a model system (July 2009 – December 2011)

PI: Chris Danpure, University College London.

Using the model system of Chinese Hamster Ovary (CHO) cells stably expressing glycolate oxidase (GO) and various normal and mutant forms of alanine:glyoxylate aminotransferase (AGT), we have made significant discoveries in three main areas:

1) Our previous studies have shown that the Pro11Leu amino acid replacement found in the polymorphic form of AGT encoded by the minor *AGXT* allele (AGT-mi) generates a cryptic mitochondrial targeting sequence (MTS). The additional presence of a Gly170Arg replacement, which is the most common mutation in PH1, leads to the exposure of this polymorphic MTS and the wholesale peroxisome-to-mitochondrion mistargeting of AGT. We have now shown that a number of other PH1-specific mutations expressed on the background of the minor allele (i.e. Gly41Arg, Phe152Ile and Ile244Thr) do the same in stably transformed CHO cells. In addition, the Gly170Arg, Phe152Ile and Ile244Thr mutants were all catalytically active when expressed in the same CHO cell system. It is well known that PH1 patients expressing the Gly170Arg and Phe152Ile mutant forms of AGT are responsive to pyridoxine, an observation that appears to be related to the presence of the Pro11Leu polymorphism and AGT mistargeting. Whether patients with other mutations, such as Ile244Thr, which also have the potential to be mitochondrially mistargeted, are also responsive remains to be seen.

2) Although pyridoxine is metabolised to pyridoxal phosphate, which is the essential cofactor for AGT, its exact mechanism of action in pyridoxine-responsive PH1 patients has remained elusive. Other workers have shown that pyridoxal phosphate stabilises purified recombinant AGT in cell-free systems, but how this extrapolates to the intracellular environment is unclear. We have now demonstrated in our stably transformed CHO cell system that pyridoxine enhances the expression, increases the catalytic activity and increases targeting to peroxisomes, not only of the Gly170Arg mutant form of AGT, but also other mutant forms, such as Phe152Ile and Ile244Thr, all on the background of the minor allele (i.e. in the presence of the Pro11Leu polymorphism). The Gly41Arg mutant form remained inactive and aggregated even in the presence of large concentrations of pyridoxine. These results provide an explanation for the effectiveness of pharmacological doses of pyridoxine in some PH1 patients as well as opening up the possibility that patients expressing other mutant forms of AGT, such as Ile244Thr might also be responsive. However, a word of caution is necessary following the finding that increased pyridoxine concentrations actually decreased the catalytic activities of normal AGT (encoded by both the major and minor alleles), possibly due to its increased metabolism to inhibitory derivatives.

3) Finally, we have developed our stably transformed CHO cell system to a level that it can be used to identify compounds that might have therapeutic use in PH1. Using our previously published indirect glycolate toxicity test, we have shown that normal AGT (encoded by both the major and minor alleles) can protect the cells, but the Gly170Arg mutant form can only partially protect the cells. Increasing the pyridoxine concentration was able to improve the protection offered by the Gly170Arg mutant form of AGT. As predicted from the effect on catalytic activity (see 2 above), increased concentrations of pyridoxine decreased the protective efficiency of the normal AGT.

These studies using stably transformed CHO cells have not only given us insights into the effects of mutations on the intracellular behaviour of AGT and how it is influenced by pyridoxine, but also they have allowed us to develop a novel cell-based system with the potential to identify the drugs of the future for the pharmacotherapeutic treatment of PH1.