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IMMUNE RESPONSE TO ENZYME REPLACEMENT THERAPY IN MPS I AND GSD II PATIENTS

By

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This thesis is submitted for the degree of Doctor of Philosophy

February, 2006

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THESIS SUMMARY

Enzyme replacement therapy (ERT) has been shown to be an effective treatment strategy for a number of lysosomal storage diseases (LSD's) in both animal models and in human clinical trials. Immune response to replacement proteins however has been reported for a number of LSD and can be a potential complication for therapy. Immune responses to ERT are variable and dependent on both the inherent antigenicity of the protein being infused and the individual patient. I have investigated the immune reactivity of two lysosomal glycosidases, α-D-glucosidase and α-L-iduronidase. Functional deficiencies of these lysosomal enzymes are the cause of the LSD Glycogen storage disease type II (GSD II) and mucopolysaccharidosis type I (MPS I) respectively.

In a phase I/II clinical trial of ERT in MPS I patients, an immune response to α-L-iduronidase was observed in 50% of patients. The MPS I patients that initially had an immune response were shown in this thesis, to develop natural immune-tolerance to α-L-iduronidase after a year of ERT. This finding has positive implications for MPS I patients who are undergoing long term ERT. A specific peptide that mapped to the catalytic element of α-L-iduronidase also appeared to be important in the development and maintenance of the immune response to α-L-iduronidase. In defining sites on the replacement protein, to which antibodies react, ways to improve ERT by minimising or eliminating antibody reactivity may be engineered.

It was postulated that the conservation of structural features in the active sites of glycosidases using a retaining catalytic mechanism, might result in common antigenicity. This study demonstrated that despite limited sequence identity between α-L-iduronidase and α-D-glucosidase, conserved micro-structural features and regions of short sequence

identity could make a major contribution to common glycosidase antigenicity. This common antigenicity in related glycosidases may contribute some background immune reactivity, representative of the major antigenic sites on the missing glycosidase and may account for why immune response to ERT has proven to be less of an issue than initially postulated.

A high incidence of antibody production to the rh-α-D-glucosidase replacement protein has been reported in the literature for GSD II patients treated by ERT. Furthermore, relative to other LSD types, large doses of rh-α-D-glucosidase are required for effective ERT in GSD II patients. In this thesis, the α-D-glucosidase catalytic site was identified as one of the main antigenic regions of this lysosomal protein. It was postulated that stabilising the catalytic site of α-D-glucosidase could increase the amount of protein recovered during purification and partially mask antibody reactivity against this region. This study demonstrated that D-glucose stabilised α-D-glucosidase allowing increased expression and improved purification of the recombinant protein from an over-expressing CHO-K1 cell line. This simple strategy could accommodate the high demand of enzyme required for ERT studies. D-Glucose also enhanced the residual α-D-glucosidase protein/activity in adult-onset GSD II patient cells suggesting the development of D-glucose analogues may be beneficial as an enzyme enhancement therapy for adult-onset GSD II patients. Furthermore, D-glucose-treated rh-α-D-glucosidase had reduced antibody reactivity *in vitro* suggesting that it may also be useful as a modifier of the immune response to ERT in GSD II patients. These findings have positive implications for ERT in GSD II patients.

When considering the safety and efficacy of ERT in LSD patients, it is important to know when an immune response develops, and whether it is maintained over the course of

treatment. It is clear that some LSD patients will develop antibodies against ERT and now there is also some evidence to suggest that these could compromise the health of the patient and therapy efficacy. Therefore, characterising these immune responses becomes crucial for the management of patients receiving long term ERT. An increased understanding of antibody development in enzyme treated LSD patients will aid in engineering ways to avert the potential affects of antibody production and will assist in the delivery of a more effective and safe therapy.