MICRODIALYSIS SAMPLING FOR THE STUDY OF IN VIVO CORTISOL METABOLISM

by

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ABSTRACT

The dysregulation of cortisol metabolism in tissues has been increasingly implicated in the pathogenesis of obesity, metabolic syndrome and type 2 diabetes. Local tissue regeneration of cortisol from cortisone is regulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme. To investigate the role of 11β-HSD1 in metabolic disease development, a reliable assay to measure the tissue-specific activities of 11β-HSD1 is required.

Microdialysis sampling has been used as a complementary tool to study drug metabolisms in peripheral tissues. In this dissertation, microdialysis sampling coupled with liquid chromatography/mass spectrometry (LC/MS) was developed to observe 11β-HSD1 catalyzed conversion of stable isotope labeled (SIL) cortisone to cortisol. The substrate SIL-cortisone was delivered to the site of enzyme action via a localized infusion through the microdialysis probe. The enzyme reaction product SIL-cortisol was collected in the dialysate. The conversion rate between cortisone and cortisol was used as a biomarker of 11β-HSD1 activity. Human, dog and monkey liver microsomes were examined with respect to SIL-cortisol production. Species-specific conversion profiles from SIL-cortisone to SIL-cortisol were observed. In human liver microsomes co-incubated with a proprietary 11β-HSD1 inhibitor, the degrees of enzyme activity inhibition determined from microdialysis were found to be 42 and 85%, consistent with the values obtained with other methods. *In vivo* assay assessment was performed in Rhesus monkey adipose tissue. SIL-cortisol formation was observed when 100 to 1000 ng/mL SIL-cortisone was infused at 0.3 to 1.0 μL/min. The *in vitro* and *in vivo* experimental results indicate the feasibility of using microdialysis to study 11β-HSD1 activity in tissues of mammalian species including humans.

To further understand the parameters that influence microdialysis assay results, a mathematical model was developed to correlate the metabolite concentration in the dialysate with substrate delivery rate and tissue metabolic and capillary removal kinetics, based on the hypothesis of a well-stirred tissue compartment. The model was evaluated by estimating infusion penetration distances of cortisone, acetaminophen and

hypoxanthine. Reasonable agreement was demonstrated between the values predicted by this model and the values reported in the literature, which provided first evidence for the validity of the model and the underlying assumptions.