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Supplemental Material: Effects of Renal Ischemia-Reperfusion Injury on Cytochrome P450 Activity

The main outcome of my project was to identify the effect of kidney ischemia-reperfusion (I/R) injury on cytochrome P450 (CYP450) activity in the liver. We hypothesized that rats subjected to renal I/R injury would show a decrease in CYP450 activity. In order to test this hypothesis, we isolated liver microsomes from rat liver tissue previously collected to test CYP450 activity.

Figure 1 outlines the procedure of microsomal isolation. First, 200-300 mg of liver tissue was homogenized and centrifuged at 10,000 x g in order to collect the S9 portion of the liver, which contains the cytosol and microsome fractions. The S9 portion was centrifuged further to separate the cytosol and microsomal fractions at 100,000 x g for 65 minutes. The liver microsomes were then incubated under physiological conditions with testosterone, a substrate for CYP2C11 and 3A isoforms. The predetermined incubation characteristics are shown in Figure 2, with an optimization of time (40 minutes), protein concentration (0.4 mg/mL), and substrate concentration (100 μM) in respect to the linear range. Standard curves for testosterone and 6β - are shown in Figure 3.

The formation of testosterone metabolites after a predetermined incubation period were analyzed using Ultra Performance Liquid Chromatography (UPLC). Figure 4 identifies the peak elution times of the four metabolites investigated and the parent substrate, testosterone. The formation of 6β is regulated by the CYP3A isoform, while 16α, 2α, and Androstenedione are mediated by CYP2C11. A decrease in activity of these isoforms would correspond to a decrease in metabolite formation. Thus, a comparison of the metabolites formed between sham, and I/R injured animals was used to explore the relationship between kidney I/R injury and CYP450 activity.

Figure 5 demonstrates the comparison of metabolite formation in a 24 hour sham group versus I/R injured. In each case (A-D), there is a decrease in the formation of metabolite as indicated by a reduction in the area under the curve (y-axis). Our preliminary results indicate that there is a reduction in CYP-450 mediated metabolism in the liver at 24 hours post-renal I/R injury, as we hypothesized. It is important to further research in this area to ensure patient safety by continuing research in our lab at different post-reperfusion time points, and presenting preliminary results for further examination of the mechanisms by which renal I/R injury effects hepatic CYP450-mediated metabolism.