Effects of Renal Ischemia-Reperfusion Injury on Cytochrome P450 Activity

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Effects of renal ischemia-reperfusion injury on cytochrome P450 activity

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Introduction

Ischemia (reduced blood and oxygen flow) and reperfusion (restoration of blood flow) (I/R) injury is an inherent part of the kidney transplantation surgery. However, kidney I/R injury causes remote organ damage, especially in the liver. The liver is the most important site of drug metabolism and clearance1. Cytochrome P450 enzymes (CYP450) are the largest family of drug metabolizing enzymes. Changes in CYP450 activity can alter liver metabolism and the clearance of drugs, leading to sub-therapeutic or toxic effects. Approximately 2 million people worldwide are in need of kidney transplantation1, thus it is necessary to study the relationship between kidney I/R injury and CYP450 activity. The objective of this study is to determine the effect of kidney I/R injury on CYP450 activity in the liver.

Methods

• Bilateral kidney I/R injury: Male Sprague Dawley rats (Charles River, MA) weighing between 170 – 210 gm were maintained in a laminar-flow, specific-pathogen-free atmosphere at the Central Research Facilities, RI Hospital, Providence, RI. All I/R surgeries were performed by a single surgeon, who was blinded to treatment.

• Microsome fractions containing CYP450 enzymes were isolated from liver tissue obtained from male rats that were subjected to bilateral kidney I/R injury.

• Animal groups include: sham or I/R-injury.

• CYP450 activity was tested by incubating microsomes with testosterone (TST), a well-known CYP450 substrate.

Isolation of microsomes from liver to test CYP450 activity

Analysis of microsomal CYP450 activity

The formation of testosterone (TST) metabolites: 2α and 16α, and 6β-hydroxytestosterone (–OH TST) serve as substrates for CYP2C11 and CYP3A2 activity, respectively. Experimental conditions for incubations included: 0.4 mg/mL of isolated microsomal protein, 100 μM TST, 10 mM MgCl2, and 100 mM phosphate buffer, pH 7.4. These parameters were optimized in order for microsome incubations, shown in Figure 2. Samples were pre-incubated in a shaking water bath at 37 °C for 5 mins before addition of 5 mM NADPH and terminated with ice-cold methanol. Levels of 2α-, 6β-, 6α-OH TST, androstenedione and TST were individually measured by UPLC with detection set at 245 nm (Waters Acquity, Milford, MA).

Results

Figure 2. Hepatic CYP450 activity was determined using microsomes prepared from rat liver tissue. Parameters were optimized to carry out incubation in the linear working range using control animal sample: (A) substrate (TST), (B) protein concentration, and (C) time of incubation. Each incubation included microsomal protein, 10 mM MgCl2, and 0.1 mM phosphate buffer, pH7.4. Samples were pre-incubated in a shaking water bath at 37 °C for 5 minutes before addition of 1 mM NADPH. Reactions were terminated with addition of ice-cold methanol and samples were placed on ice. Samples were centrifuged at 15,000 g for 15 minutes and analyzed immediately.

Figure 3. Standard concentration curves of (A) TST and (B) 6β-OH TST were prepared to quantify the concentration of parent drug, TST, and metabolite 6β-OH TST relative to their respective areas under the curve. These concentrations were then used to compare the percentage of TST lost and the percent formation of metabolite in various animal groups.

Figure 4. Separation of TST and metabolites on a C18 column (Waters, Milford, MA). Detection was obtained by UV absorption at 210 nm. TST was eluted at 7.60 minutes after 4 major metabolites: 6α, 6β, 16α, and 16β TST (D).

Figure 5. Comparison of peak area under the curve (AUC) values amongst sham and I/R injury animal groups. Peak AUC were measured and integrated using an UPLC. CYP3A4 activity is analyzed by the metabolite formation of 6β-OH (A), and CYP2C11 is analyzed by the formation of metabolites 16α-OH TST (B), Androstenedione (C), and 2α-OH TST (D).

Discussion

Previous animal studies show that kidney I/R injury causes liver dysfunction, as shown by:
• elevated levels of serum biomarkers of liver function3
• oxidative stress that triggers an inflammatory response due to elevated cytokines3

However, limited data exists on the effects of kidney ischemia/reperfusion injury on CYP450 enzyme activity in the liver. Since the pro-inflammatory response associated with I/R injury can alter the expression and activity of CYP450 enzymes in the liver4, we hypothesized that rats subjected to bilateral kidney I/R injury would show a decrease in CYP450 activity.

Our preliminary results indicate approximately a 50% reduction in CYP450-mediated metabolism in the liver at 24 hours post-reperfusion injury, therefore we accept our hypothesis. We expected to see a decrease in CYP450-mediated metabolism in kidney I/R injured animals and we saw a reduction in activity with I/R injury compared to sham (Figure 5). Formation of testosterone (TST) metabolites: androstenedione, 2α- and 16α-, and 6β-hydroxytestosterone (–OH TST) correlate with CYP2C11 and CYP3A2 activity, respectively5. Garley et al.6 study results supports these results as they found a significant decrease in CYP3A and CYP2E1 in an animal model of 1 and 3 hours post-renal I/R injury, but did not test longer times post-reperfusion, i.e. 24 hours.

Conclusions:
The results of this project are important in the field of biomedical and pharmaceutical research. A decrease in liver CYP450 activity can lead to toxic effects or adverse reactions of medications. Thus, we propose to expand this research to ensure patient safety by:
• Expanding our study to conduct additional experimental time points of reperfusion in our laboratory
• Presentation of preliminary results, further examination of the mechanisms by which renal I/R injury effects hepatic CYP450-mediated metabolism in progress.

References


Acknowledgements

• Sponsor: Nisanne Ghonem, PharmD, PhD

• Lab Members: Joyce Hsu, Adam Aurichi, Mawen Ding, and Gina Gallucci

Office of Undergraduate Research and Innovation ((URI)

• Research reported in this poster is supported in part by an Institutional Development Award from the National Institute of General Medical Sciences of the National Institutes of Health (NIGMS, 1-U47-GM111877), which funds Advance Clinical and Translational Research (Advance-CTR).

The content presented is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.