

A. Specific Aims

Acute kidney injury (AKI) is a major cause for morbidity and mortality in hospitalized patients, developing in about 5-7% of patients and impairing recovery of about 15-25% of intensive care unit (ICU) patients (1-3). Despite major advances in renal replacement therapy, the mortality in patients with AKI remains largely unchanged (2, 4-11) and can be as high as 80% in ICU patients (5, 7, 11). Additionally, AKI is now linked to the subsequent development of chronic kidney disease (CKD)(1, 10, 12-16). Numerous therapeutic interventions have been evaluated in clinical trials, with none proven successful. General supportive care and dialysis remain the primary treatment modalities. The overall goal of this project is to elucidate the biologic basis for the cytoprotective effects of heme oxygenase-1 (HO-1) in AKI, and to generate relevant and feasible therapeutic strategies based on upregulation of HO-1.

During the previous funding period (2008-present), we focused on the mechanisms underlying the protective effects of heme oxygenase-1 (HO-1) and the regulation of HO-1 gene expression in AKI. HO-1 is a 32-kD microsomal enzyme that catalyzes the breakdown of heme, releasing iron, carbon monoxide (CO) and biliverdin (17-19). Using pharmacological and genetic approaches, we and others have demonstrated that the induction of HO-1 is a protective response in renal and non-renal settings of ischemia-reperfusion injury (20-29), nephrotoxin-induced renal injury (30-34), organ transplantation (35-39), acute glomerulonephritis (40, 41) and rhabdomyolysis (42, 43). **This proposal is based on our discovery that the protective effects of HO-1 in AKI result from the action of HO-1 expression and activity in cells of both renal and hematopoietic origin.** This conclusion arises from the following lines of evidence: (i) HO-1 expression regulates autophagy (44) and its expression confers protection in AKI [reviewed in (19)], (ii) Overexpression of HO-1 in a “humanized” bacterial artificial chromosome (hBAC-HO-1) transgenic mouse confers protection against AKI (45); and (iii) HO-1 regulates inflammation and trafficking of myeloid cells in AKI and influences the AKI to CKD transition (46). Therefore, either selective or global manipulation of HO-1 expression could be an important therapeutic strategy for prevention or treatment of AKI. *Our **central hypothesis** is that HO-1 expression regulates cross-talk between the renal proximal tubules and myeloid cells, and HO-1 upregulation either in tubules or myeloid cells will not only protect against AKI, but also the subsequent evolution of CKD.*

Aim 1: To test the hypothesis that proximal tubule (PT) HO-1 expression affects differentiation, infiltration and trafficking of myeloid cells in AKI. Using Cre-lox technology, we have generated mice in which HO-1 expression can be manipulated within the renal proximal tubules. By selective overexpression or deletion, we will determine how proximal tubule HO-1 expression modulates renal myeloid cells (mainly macrophages and dendritic cells) following AKI, and their functional differentiation, infiltration and release of local mediators such as colony stimulating factor-1 (CSF-1) and interleukin-6 (IL-6).

Aim 2: To test the hypothesis that myeloid cell HO-1 expression modulates inflammation in AKI and the transition of AKI to CKD. Based on our findings that myeloid-specific HO-1 deficiency results in increased renal fibrosis and impaired recovery after AKI, this aim will elucidate the mechanisms by which HO-1 protects against these effects. We will focus on inflammatory cell trafficking, inter-cellular communication, renal function and structure in AKI as well as the transition to CKD.

Aim 3: To test the hypothesis that HO-1 induction using small molecules will provide protection against AKI and the transition to CKD. We discovered a novel enhancer sequence that regulates human HO-1 gene expression via chromatin looping in renal epithelial cells (47) and in vivo in “humanized” BAC transgenic mice (45). A high-throughput screen of >150,000 compounds resulted in X small molecules from three chemically distinct series of compounds that specifically activate this enhancer in renal epithelial cells. Initial structure activity-relationships were determined by testing commercial analogs of the hits identified via a high-throughput screen. ***Aim 3a:*** A hypothesis driven medicinal chemistry effort will be used to identify novel, potent HO-1 inducers with the appropriate pharmacokinetic properties including metabolic stability, solubility, permeability and bioavailability. ***Aim 3b:*** Compounds will be selected for in vivo testing in models of AKI, focusing specifically on their effects in the tubular and myeloid compartments.

This project incorporates a highly interdisciplinary team with a collective history of successful collaborations in basic and translational science, including a medicinal chemistry expert, Dr. Mark J. Suto, Vice President, Drug Discovery Division, Southern Research, which is situated adjacent to the UAB campus.

Successful completion of the studies in this renewal application will (i) provide key information on protective mechanisms in AKI, (ii) highlight the role for HO-1 in orchestrating the cross-talk between the renal tubules and myeloid cells, and (iii) provide novel candidate agents to be potentially utilized in the prevention and treatment of AKI, thereby paving the way for a new therapeutic approach in this disease.