Diabetic Complications Consortium

Application Title: Use of a Novel PTBA analog to ameliorate CKD progression after AKI in experimental diabetic nephropathy

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Accomplishments

Studies in this proposal have been completed but were hampered by a number of factors: a) the fact that we did not apreciate that *eNOS* null mice have markedly reduced viability, and that ~50% of *eNOS* null mice died after induction of diabetes before 25 weeks of age; b) the fact that none of the *eNOS* null mice with established diabetic nephropthy survied more than 24 hours after the delayed contralateral nephrectomy surgery in our IR-AKI model. These data can be made available on request. These factors resulted in considerable delays in initiating and completing the studies so that we were only able to complete the studies during the no cost extention phase. Despite this, we were able to demonstrate in a small number of mice that there was a reduction in markers of renal fibrosis as well as improved renal function in mice with established diabetic nephropathy after induction of IR-AKI with delayed contraleral nephrectomy. These data are shown in Fig. 1. Because of the low numbers, these data are not suitable for publication. However, they do provide an indication that this class of drugs maybe useful to prevent CKD progression after AKI when this occurs on a backgrpound of established diabetic nephropathy.

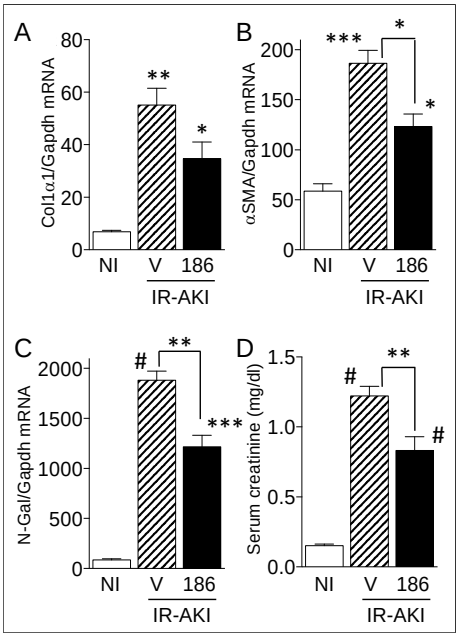

Figure 1. UPHD186 reduces injury after IR-AKI in mice with diabetic nephropathy. eNOS null mice were treated with low dose Streptozotocin to induce diabetes at 10 weeks and underwent unilateral IR injury at 26 weeks, followed by contralateral nephrectomy 8 days later. Mice were euthanized 24 hours after nephrectomy due to increased mortality. Mice were treated with 50mg/kg/day UPHD186 or vehicle control IP from day 1-7. Data as mean +/- SEM, n=3 no injury (NI) and IR-AKI treated with vehicle (V), n=4 IR-AKI treated with UPHD186. 1-way ANOVA, $p<0.05$ with post hoc analysis vs. control, or vs. vehicle treated IR-AKI (indicated by brackets): *$p<0.05$, **$p<0.01$, ***$p<0.001$, #$p<0.0001$

While studies were delayed because of these breeding and viability concerns, were were able to use this opportunity to extend some of our preliminary work on UPHD186 development, which led to the first publication of the development and analysis of UPHD186 as a therapeutic for IR-AKI and UUO in mice(1). This was follwed up by a review we wrote outlining our stragety for phenotype-driven discovery for AKI(2). In addition, buoyed by our observation that UPHD186 appears to be effective in reducing CKD progression after AKI on a background of diabetic nephropathy, we were able to leverage these preliminary data as well as our published data to develop a successful research proposal to study the effects of UPHD186 (as well as new second generation PTBA analogs that we plan to develop over the course of these studies)(1), for the prevention of CKD progression after AKI in association with diabetes, diabetic nephropathy, CKD and ageing. This work was recently recommended for funding by the Department of Defense as part of a multi-PI grant with Drs. Hukriede and Huryn at the University of Pittsburgh. These studies are expected to begin in September 2017 (PR161028: "Optimizing small molecule thereaputics for duabetic kidney disease and Acute Kidney Injury"). The specific focus of these DOD funded studies will be on these effects of PTBA analogs in causes of injury that reflect the types of injury that combat veterans and retired miltary veterans are commonly exposed to. This includes models of IR-AKI, rhabdomyolysis (crush injury) induced AKI, and cisplatin-induced AKI. However, based on our observations that diabetic *eNOS* null mice have low viability and are difficult to breed, we elected to to perform diabetes studies using

another model of diabetic nephropathy developed by Dr. Chris Kennedy from the University of Ottowa, using renin over-expressing TTRhRen transgenic mice after streptozotocin-induced diabetes(3). Like *eNOS* null mice, these mice develop hypertension, and severe diabetic nephropathy 16 weeks after induction of diabetes. However, unlike *eNOS* null mice, Dr. Kennedy has assured me that these mice do not have low viability or high attrition rates after induction of diabetes with streptozotocin. We obtained these mice recently and are starting to expland our colony. Thus we consider that this DIACOMP funded pilot project, while in some respects unsucessful, was the means by which we were able to develop and now extend our studies on the use of PTBA analogs for the prevention of diabetes, and other com-morbidity-associated CKD progression after AKI.

References Cited

1. Skrypnyk NI, Sanker S, Skvarca LB, et al. Delayed treatment with PTBA analogs reduces postinjury renal fibrosis after kidney injury. *American journal of physiology Renal physiology.* 2016;310(8):F705-F716.
2. Hukriede N, Vogt A, de Caestecker M. Drug Discovery to Halt the Progression of Acute Kidney Injury to Chronic Kidney Disease: A Case for Phenotypic Drug Discovery in Acute Kidney Injury. *Nephron.* 2017.
3. Thibodeau JF, Holterman CE, Burger D, Read NC, Reudelhuber TL, Kennedy CR. A novel mouse model of advanced diabetic kidney disease. *PloS one.* 2014;9(12):e113459.

Publications arising from this grant

Skrypnyk NI, Sanker S, Skvarca LB, et al. Delayed treatment with PTBA analogs reduces postinjury renal fibrosis after kidney injury. *American journal of physiology Renal physiology.* 2016;310(8):F705-F716. PMID: 26661656

Hukriede N, Vogt A, de Caestecker M. Drug Discovery to Halt the Progression of Acute Kidney Injury to Chronic Kidney Disease: A Case for Phenotypic Drug Discovery in Acute Kidney Injury. *Nephron.* 2017. June 15 2017. [E-pub ahead of print]. PMID: 28614822

Grants arising from these pilot project studies

Department of Defence. PR161028: "Optimizing small molecule thereaputics for diabetic kidney disease and Acute Kidney Injury". Multi-PI grant (Hukiede/Huryn and de Caestecker).

Direct costs: $3 million (9/o1/2017-8/31/2020).

The goal of these studies is to develop new drugs that will improve recovery after AKI when given to patients days after injury, and which are effective in multiple experimental models of AKI that reflect the types of AKI and associated diseases, particularly diabetes, that commonly affect combat personnel and veterans.