Diabetic Complications Consortium

Application Title: Testing of a selective integrin inhibitor in a type 1 model of diabetic nephropathy
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1. Project Accomplishments:

The overall goal of this study was to determine the contribution of integrin EPGN809, a major collagen binding receptor upregulated in injury, to the progression of diabetic nephropathy (DN). Based on the finding that 1) integrin α2β1 contributes to kidney injury and fibrosis by positively regulating collagen production and 2) genetic and pharmacological inhibition of this receptor in mice ameliorates kidney fibrosis following adriamycin-mediated injury (5), the goal of this preclinical testing grant was to determine the efficacy of a tool small-molecule inhibitor of integrin α2β1 in slowing and ideally preventing diabetes-mediated kidney injury in a mouse model of type 1 diabetes. This grant had the potential to be innovative and significant, as to date no integrin α2β1 inhibitors have been tested in any preclinical models of diabetic nephropathy.

Methods

Male mice C57/B6 eNOS-null mice (24 gr body weight ~ 8 weeks of age) were rendered diabetic with low-dose streptozotocin (STZ) injections. Mice received 50mg/kg STZ daily for 5 consecutive days at week 0 and then again at week 7. At week 8 mice were divided into two random groups: untreated (i.p. PBS) or treated (injected i.p. with the integrin inhibitor EPGN809 at 10 mg/Kg in PBS). Mice received a single i.p. injection every other day for a total of 8 weeks. Mice were then sacrificed at different time points (up to 16 weeks) after STZ injection.

The parameters evaluated were: 1) body weight (measure every two weeks); 2) glycemia (every two weeks); 3) glomerular filtration rate measured by sinistrin clearance (GFR, at weeks 4, 8, 12, 16) albumin/creatinine ratio measured by standard ELISA kits (ACR, at weeks 4, 8, 12, 16).

2. Specific Aims:

To determine the efficacy of a small-molecule inhibitor of integrin α2β1 in slowing and ideally preventing the progression of DN in a mouse model of type 1 diabetes

Results

All mice receiving STZ developed hyperglycemia (~ 500 mg/dL) within two weeks from injection which persisted high throughout the duration of the experiment (**Fig 1A**). Mice maintained an average body weight of ~ 24 gr throughout the duration of the experiment (**Fig. 1B**). As expected diabetic mice developed proteinuria within 4 weeks from injection (**Fig. 1C**). Most of the diabetic mice had to be sacrificed around 16 weeks after STZ injection due to lack of thrive and lack of response to external stimuli. Unfortunately, mice treated with the integrin α2β1 inhibitor EPGN809 did not perform better than untreated diabetic mice and also had to be sacrificed at 16 weeks from STZ injection. EPGN809-treated mice showed glycemia, body weight, GFR and ACR similar to non-treated mice (**Figs. 1A-D**). More than 10 mice were analyzed at each time point. We are in the process of receiving kidney histology for evaluation.

Conclusions

Despite the observation that in a model of adriamycin-induced kidney injury administration of a selective integrin α2β1 inhibitor ameliorated proteinuria and overall fibrosis, administration of the integrin α2β1 inhibitor EPGN809 starting at 8 weeks from induction of diabetes up to 16 weeks did not ameliorate ACR and had no beneficial effects on GFR. Although we do not have histology data yet, it is very unlikely that this inhibitor might be beneficial in the reverting and/or stopping diabetes-mediated kidney fibrosis. Unfortunately, from this experiment we cannot determine if the integrin inhibitor was able to inhibit integrin-mediated function and/or collagen binding. At present we have no information about exposure and it is possible that constant rather than intermitted blockage of the receptor is required.

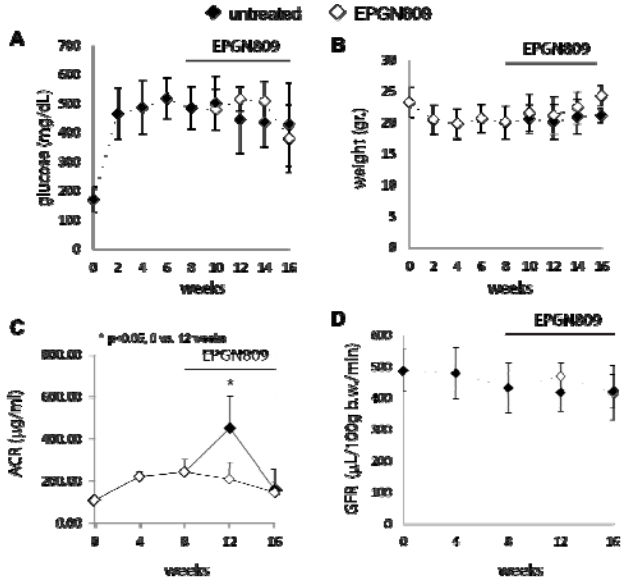

Figure 1. Analysis of blood glucose (**A**), weight (**B**), ACR (**C**) and GFR (**D**) in eNOS(-/-) mice rendered diabetic with streptozotocin (STZ). At 8 weeks after STZ injection mice were divided in groups of PBS treated (untreated) or EPGN809-treated mice. Mice were sacrificed at 16 weeks. Values are the mean +/- SD of n>10 mice in each group.

Future directions

One possibility we are considering is to increase the dose of this inhibitor and use mini-pumps for delivery. A second possibility is to use a less aggressive model of DN such as the Akita mouse model. We found a high degree of mortality among diabetic eNOS(-/-) mice that dramatically slowed the progress of this project. Another possibility we are contemplating is to devise novel selective integrin α2β1 inhibitors that can be delivered orally to the mice.

3. Publications:

N/A.