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Final Research Performance Progress Report

Project Title: Evaluating the efficacy of MSI-1436 in slowing the progression and in reversing diabetic nephropathy in the BTBR *ob/ob* mouse.

Award #: 5 U24 DK115255-04
Subaward #: 32307-29

Accomplishments

What were the major goals and objectives of the project?

The Aim of this pilot study was to characterize the effects of MSI-1436 on kidney functional and morphological damage induced by diabetes in the BTBR *ob/ob* mouse. For these proof-of-concept studies, we used the BTBR *ob/ob* mouse. This mouse model has been reported to develop multiple, severe kidney abnormalities, including podocyte loss, that closely resemble human DN, and the development of these abnormalities is more rapid than for most other animal models. In addition, experimentally induced reversal of DN has been well described in BTBR *ob/ob* mice. We employed blood chemistry and detailed morphological analysis of the kidney to characterize the effect of MSI-1436 on the progression and possible reversal of DN.

What was accomplished under these goals?

These preliminary studies resulted in several beneficial effects from MSI-1436 treatment that warrant additional investigation.

1. Synthesis of new batch of MSI-1436: We had experienced an unexpected interruption to our supply chain for MSI-1436. The aminosterol MSI-1436 is very difficult and costly to synthesize. However, after great efforts, we identified a non-GMP CMO that could cost-effectively manufacture API in a timely manner and in sufficient quantity to support this project.

2. IP delivery of 1.25 mg/kg MSI-1436 improves survival: We treated 16-week old female BTBR *ob/ob* with saline or MSI-1436 at 3 doses (1.25, 2.5 and 5 mg/kg) once every 3 days via intraperitoneal microinjections. Treatment continued for a total of 6 weeks. MSI-1436 (1.25mg/kg) administration resulted in 80% survival, compared to 40% in saline control group (Figure 1). However, administration of MSI-1436 at 5.0mg/kg resulted in 100% mortality within the first 3 days of the first dose. This result was surprising given that previous work indicated that 10mg/kg was well-tolerated in a wildtype C57BL/6J mouse strain.

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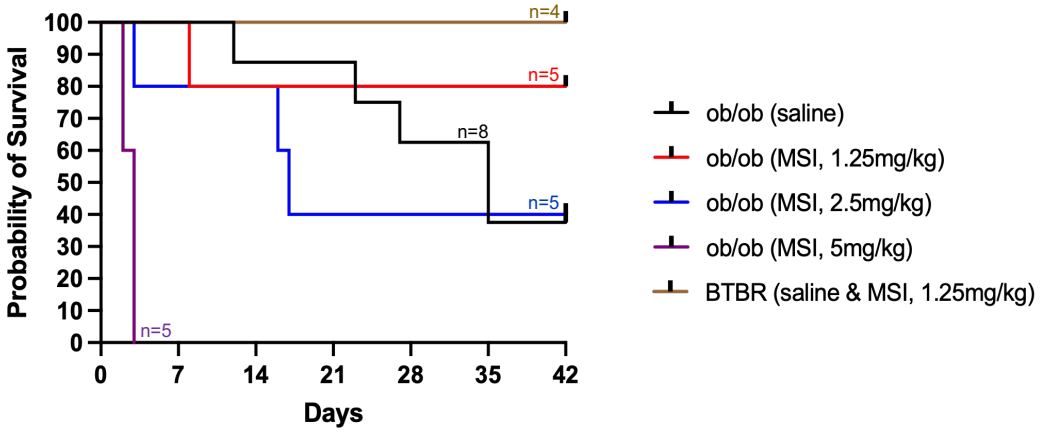

Figure 1. Survival rates in BTBR$^{+/+}$ and *ob/ob* animals.

3. Delivery of 2.5 mg/kg MSI-1436 induced weight loss in BTBR *ob/ob* animals: MSI-1436 has been shown to be a potent appetite suppressant that culminates in weight loss when administered to mammalian models. In our study, treatment with MSI-1436 at 2.5mg/kg stimulated body weight loss from 66g (baseline) to 50g after a 6-week treatment period. Treatment at a lower dose of 1.25mg/kg did not induce a significant change compared to saline control group (Figure 2). This result is consistent with the lack of changes to blood chemistry levels in *ob/ob* animals treated with the lower MSI-1436 dose (Table 1).

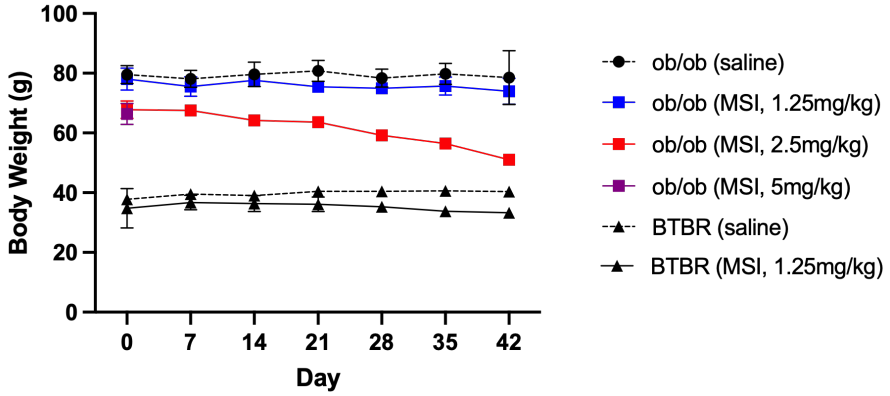

Figure 2. Changes to weekly body weight over the duration of the 6-week study.

4. BTBR *ob/ob* animals do not show overt signs of glomeruli damage: The BTBR *ob/ob* strain has been reported to be a robust and reliable model of DN. To define the structural changes to glomeruli, we performed histological studies on saline and MSI-1436 treated kidneys. However, in our hands the *ob/ob* glomeruli were indistinguishable from BTBR$^{+/+}$ as shown with Hematoxylin and eosin staining (Figure 3). Likewise, histological staining for fibrosis with Masson Trichrome did not show a striking presence of collagen deposition between BTBR$^{+/+}$ and *ob/ob* kidneys (Figure 4).

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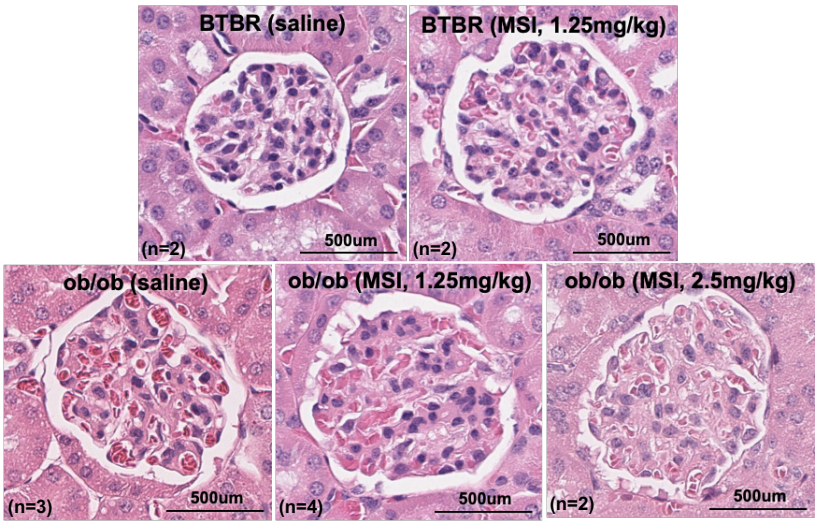

Figure 3. Hematoxylin and eosin staining of glomeruli at 22-weeks (6-weeks post-treatment).

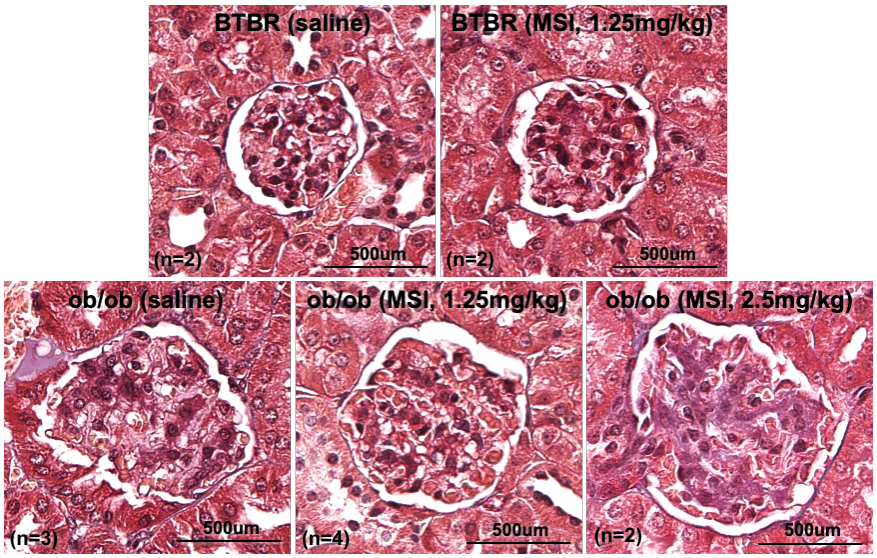

Figure 4. Masson's Trichrome staining of glomeruli at 22-weeks (6-weeks post treatment).

5. MSI-1436 treatment did not alter blood chemistry indicators of DN: In addition to histological studies, we also performed blood chemistry measurements to identify changes to biomarkers of diabetes. Terminal blood draws were taken and analyzed for all animals that survived the 6-week treatment. The inclusion of $BTBR^{+/+}$ animals served as an additional

control for the progression of DN. From the subset of biomarkers analyzed, we noted that serum glucose, SGPT, SGOT, and glucose levels were elevated in BTBR *ob/ob* when compared to $BTBR^{+/+}$. While treatment with MSI-1436 (2.5mg/kg) induced reductions in these levels, the limited sample size (due to animal death) did not enable statistical analyses (Table 1). Biological variability in our studies was higher than anticipated, based on published results with this animal model. It is possible that with a larger sampling size, these blood markers could be statistically significant from $BTBR^{+/+}$, and thus, be indicative of DN.

	(n=3)	(n=4)	(n=2)	(n=2)	(n=2)
Parameter	ob/ob (saline)	ob/ob (MSI, 1.25mg/kg)	ob/ob (MSI, 2.5mg/kg)	BTBR (saline)	BTBR (MSI, 1.25mg/kg)
BUN (mg/dL)	28.3 ± 4.7	24 ± 2.1	33 ± 1.0	16.5 ± 1.5	16.5 ± 1.5
Creatinine (ug/dL)	23.3 ± 3.3	17.5 ± 6.3	25 ± 5.0	20 ± 0	20 ± 0
Albumin (mg/dL)	3000 ± 173.2	2850 ± 259.8	2900 ± 0	2700 ± 0	3000 ± 0
Potassium (mEq/L)	5.3 ± 0.8	4.35 ± 0.3	5.35 ± 0.05	4.2 ± 0.3	4.35 ± 0.5
Alk Phos (IU/L)	43 ± 9.4	50.25 ± 5.9	43.5 ± 2.5	46.5 ± 1.5	43.5 ± 4.5
Glucose (mg/dL)	499.7 ± 98.5	459.75 ± 60.9	287 ± 14	228 ± 9	249 ± 15
SGOT (AST) (IU/L)	103.7 ± 18.4	87.75 ± 8.5	82 ± 5	46.5 ± 7.5	31.5 ± 4.5
SGPT (ALT) (IU/L)	61 ± 8.2	74.25 ± 23.6	49 ± 3	19.5 ± 1.5	18 ± 3
Cholesterol (mg/dL)	253 ± 41.9	212.3 ± 30.3	167 ± 5	112.5 ± 10.5	111 ± 9

Table 1. Biomarker assessment of DN.

Project Outcomes/Summary

Diabetic nephropathy (DN) is a major cause of morbidity and mortality in type 1 diabetics and is becoming an increasingly serious problem in type 2 diabetics. DN is characterized by progressive loss of glomerular function due to fibrosis, capillary damage and loss of podocytes and is the primary cause of chronic and end-stage renal disease in the world. Therapies aimed at inducing the regeneration of lost tissues and cells have been proposed as therapeutic strategies for slowing and reversing diabetic glomerular damage.

MSI-1436 is a readily synthesized aminosterol that exhibits pro-regenerative qualities in numerous animal models of tissue injury. The molecule inhibits the tyrosine phosphatase PTP1B and was tested by Genaera Corp. in 2007 in Phase 1 and 1b clinical trials for treatment of obesity and type 2 diabetes. Metabolic changes consistent with inhibition of PTP1B were reported and patients showed no adverse reactions to the drug. Importantly, the stimulatory effects on tissue repair and regeneration we observe in animal models occur at doses 5- and 50-times lower than the maximum dose shown previously to be safe in humans.

We postulated that the molecule will stimulate the regeneration of diverse cell types in the diabetic kidney. The overall Aim of this pilot project application is to carry out a proof-of-concept study to test the efficacy of MSI-1436 in slowing and reversing glomerular damage in the BTBR *ob/ob* mouse.

In these studies, histological studies of the glomeruli from $BTBR^{+/+}$ and *ob/ob* animals did not reveal overt diabetes-induced kidney damage in the BTBR *ob/ob* model, as previously reported. Biological variance and low animal numbers could have contributed to these observations. However, these pilot studies did demonstrate MSI-1436 administration reduced serum glucose levels, improved survival and lowered body weight during a 6-week treatment when compared to vehicle. These trends in MSI-1436 effects, however, did not achieve statistical significance. To better understand MSI-1436 effects on the diabetic state and kidney damage, future studies will require a larger sample size and possibly additional DN mouse models.

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