Diabetic Complications Consortium

Application Title: Stroke Outcome in a New Mouse Model of Type II Diabetes: Therapeutic Intervention

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1. Project Accomplishments:

One of the major objectives of this proposal was to determine whether the NONcNSO10/LtJ (RCS10) mice could serve as a better mouse model to study the effects of Type II diabetes on stroke outcome than the *db/*db mouse, which is currently the most widely used Type II model. To this end, this project has been able to: 1.) Establish a detailed description of the onset of diabetes in the RCS10 mice when maintained on an 11% fat diet from weaning; 2.) The diabetic RCS10 mice exhibited a significantly great infarct following a Hypoxia/ Ischemic (H/I) insult –Stroke than non-diabetic RCS10 controls and NONShiLtJ, which are 85% genetically identical to the RCS10 mice and develop overt obesity but (>90%) do not develop diabetes. 3.) We were able to demonstrate that Metformin was able to restore and maintain euglycemia in diabetic RCS10 mice over a period of 4 weeks, however this restoration of euglycemia did not significantly reduce the infarct volume following H/I.

2. Specific Aims:

Specific Aim 1: To compare the effects of restoring euglycemia with the various anti-diabetic drugs with insulin on stroke outcome in the diabetic RCS10 mouse. The restoration of euglycemia elicited by the respective agents is anticipated to reduce the infarct and promote recovery in the diabetic mice, however, the extent to which this is achieved will depend on additional actions of the various agents on peripheral and cerebral metabolism. ***The hypothesis to be tested is that the TZDs, darglitazone and pioglitazone will be the most efficacious in improving stroke recovery as they will not only restore euglycemia and normalize lipid levels, but also modulate the inflammatory response***

3. Results:

When we initially undertook our studies with the RCS10 mice, we purchased them from Jackson Laboratories at 8 weeks of age and maintained them on the recommended 11% fat diet. Typically 60-70% of the mice developed hypoglycemia and 30-40% remained euglycemic with blood sugars <250 mg /dl. Thus we formulated the proposal with the view of using the non-diabetic animal as controls for our metabolic interventions. Figure 1 illustrates the effects of diabetes on stroke outcome in diabetic and non-diabetic RCS10 mice at 16 weeks. The infarct volume is

clear greater in the diabetic mice when exposed to the same hypoxia time and exposure of an additional 2 min of hypoxia was required to normalize the extent of the insult in the non-diabetic mice.

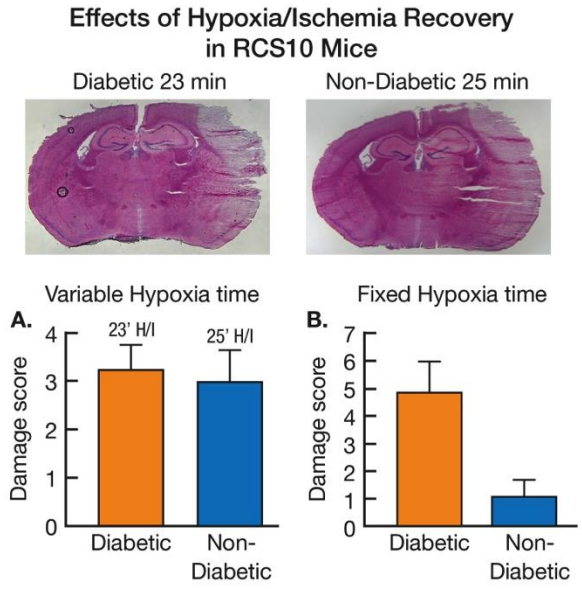

Figure 1: Upper panels illustrate typical H/E staining of diabetic and non-diabetic brains at 48 hours after H/I at a fixed hypoxia time of 23 min. (9% O2). Panel A indicates the quantification of the corresponding infarct volumes. Panel B illustrates a comparison between non- diabetic mice exposed 25 min. hypoxia (n=13) with diabetic min (n=20) exposed to 23 min. hypoxia. The infarct volumes are significantly smaller in the non-diabetic RCS10 mice and to normalize infarct volume to that of the RCS10 mice requires an additional two minutes of hypoxia.

Upon obtaining the DCC grant, we elected to breed the RCS10–see Methodologies associated with this report. However, in contrast to our previous experience, we found that the penetrance of overt hyperglycemia in RCS10 mice bred in house dramatically increased to >90% by 16 weeks of age. Figure 2 illustrates the weight gain and blood glucose levels of the RCS10 mice that are maintained on a 11% fat diet from weaning at 4 weeks until 18 weeks. There appears to be a tight relationship between attaining 40 g and the onset of overt hyperglycemia, which generally occurs between 10-14 weeks. By 18 weeks 95% of all animals were diabetic.

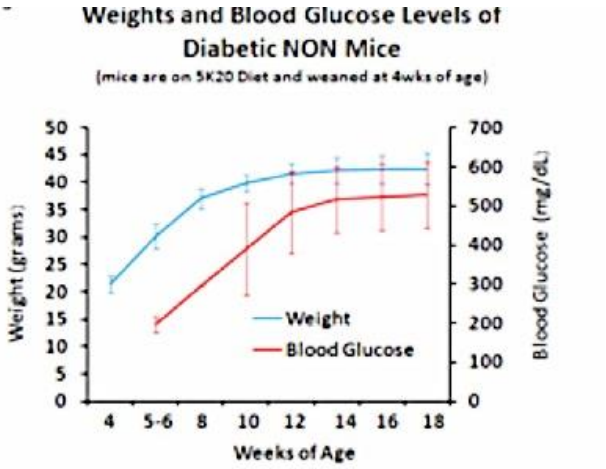

Figure 2 illustrates the relationship between the weight of the RCS10 mice and the onset of overt diabetes. Upon attaining a weight of 40g, the mice all become diabetic which occurs between 10 and 14 weeks of age.

The current recommendations from both NIDDK and NINDS suggest that to test the effects of stroke and diabetes, the animals should be older (> 8 weeks) and have had diabetes for at least 1 month. In attempting to comply with the recommendations of the respective Institutes, it became apparent that we would be unable to obtain sufficient non-diabetic RCS10 mice to serve as

controls and an alternative control would be required. The closest strain to the RCS10 mice are the NON/ShiLtJ mice which are 85% genetically identical to the RCS10 mice and develop overt obesity but when bred in house (>90%) do not develop diabetes. Figure 3 illustrates the comparison of the stroke outcomes between the diabetic RCS10 mice and the non-diabetic NON/ShiLtJ.

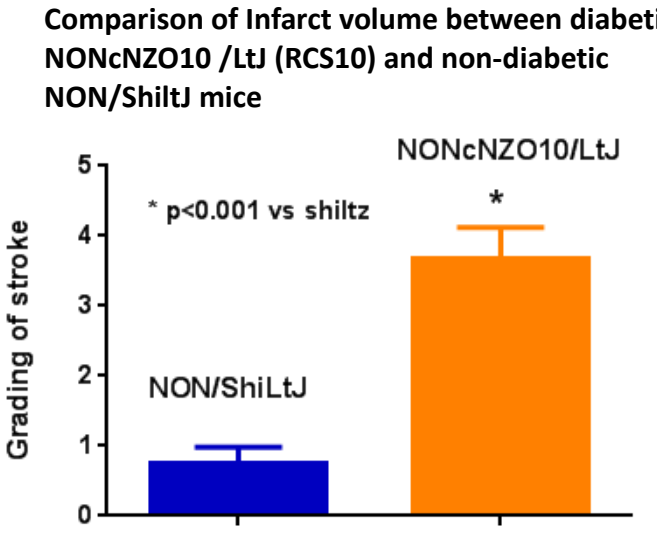

Figure 3. Comparison of Infarct volume between diabetic NONcNZO10/LtJ (RCS10) and non-diabetic NON/ShiltJ mice. This illustrates the infarct volumes that were determined by H&E in twenty week old RCS10 (n=23) and NONShiLtJ (n=28) mice that were exposed to 23 min of hypoxia /ischemia and allowed to recover for 48 h. The infarct volume is significantly smaller in the non- diabetic mice, which share 85% genetic identity with the diabetic RCS10 mice (Jackson Laboratories).

The original Specific Aim was to ascertain the relative efficacy of different anti-diabetic agents that are used to treat Type II diabetes and we elected to examine the effects of the most widely used agent Metformin on glucose homeostasis and subsequent stroke outcome. Metformin was administered to the mice in their water to which 0.15% saccharin was added to ensure that mice consumed sufficient metformin/water to normalize their blood sugar (0.8-1.3 g/kg/D). The saccharin levels in the water of the control mice that remained diabetic, was diluted to normalize saccharin consumption between the two groups. The effects of the Metformin and glucose homeostasis are illustrated in Figure 4

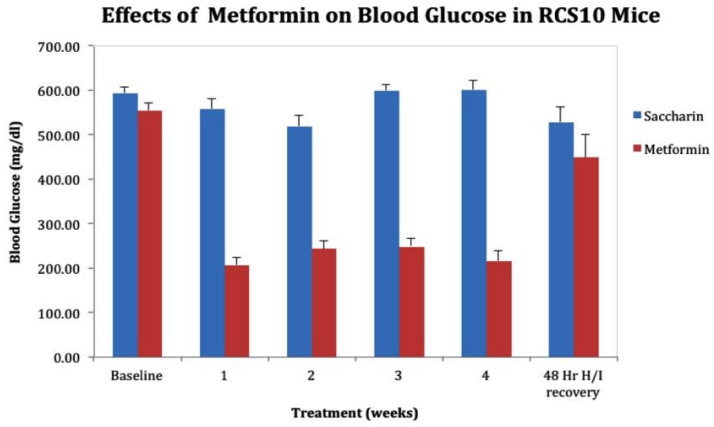

Figure 4. Time course for the normalization of blood glucose in the RCS10 mice
Metformin administered to the RCS10 mice in the water (100-120 mg/ml) normalized blood glucose within first week and maintained euglycemia over a period of 4 weeks.

The RCS10 mice were 20 weeks old when Metformin treatment was initiated and had been diabetic for at least 4 weeks To maintain euglycemia the Metformin concentration in the drinking water was increased from 100 -120 mg/ml over the course of the 4 weeks. 0.15% saccharin was added to mask Metformin and the concentrations were adjusted to ensure equal saccharin intake in the control RCS10 mice. The Metformin was unable to maintain euglycemia following the stroke presumably due to the increased insulin resistance. The effects of the normalized glycemia on stroke outcome are illustrated in Figure 5.

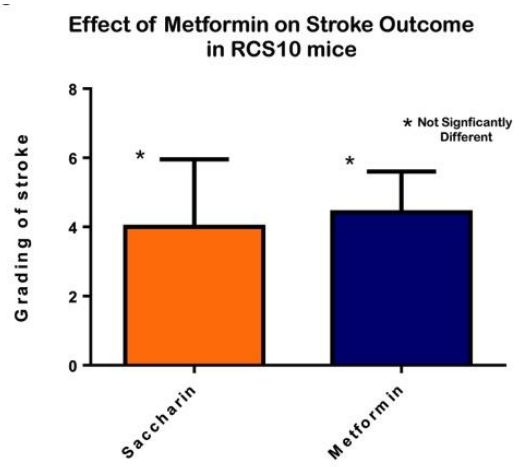

Figure 5. Effect of Metformin on Stroke Outcome in RCS10 mice.
the effects of maintaining RCS10 mice euglycemic for four weeks on the subsequent outcome following stroke in the mice maintained as described in Figure 4. Both sets of animals were exposed to 22 min of 9% oxygen. The extent of the stroke damage was determined at 48 h post stroke infarct from H&E sections as described in methods. Saccharin control mice (n=8) and Metformin mice (n=15). There was no significant difference in the extent of the insult between the diabetic and euglycemic mice.

These results in some respects were disappointing as our expectations were that the metformin would elicit a comparable effect to that we had obtained when euglycemia was induced in the *ob/ob* mouse with darglitazone, a *ppar* γ agonist (Kumari et al.,2010). In that study, darglitazone not only normalized the blood glucose levels it dramatically reduced the infarct volume in both diabetic and non-diabetic animals. The data described in Figure 5 is comparable to that obtained by (Tureyen et al., 2007) who found that Metformin treatment in the *db/db* mouse had no effect on stoke outcome. However, in those studies the *db/db* mice were too insulin-resistant to fully restore euglycemia and thus the observations were always considered equivocal. It should also be noted that the levels of Metformin used in this study and that of Tureyen et al. is significantly higher on a mg/kg basis than used in patients, suggesting marked difference in sensitivity between rodents and humans. This represents a very important observation that will alter the scope of our RO1 resubmission, which included an investigation into the mechanistic aspects of metformin actions on stroke recovery. However, we have clearly demonstrated the glycemic state in RCS10 mouse can be modulated, unlike *db/db* mouse, and is therefore a more amenable model for the study the complications of Type II diabetes.

References

Kumari, R, Willing L, Patel S D, Krady J K, Gibbs E M, and Simpson I A (2010). The PPAR-gamma agonist, darglitazone, restores acute inflammatory responses to cerebral hypoxia-ischemia in the diabetic ob/ob mouse. J Cereb Blood Flow Metab, 2010. **30**(2): p. 352-60.

Tureyen K, Kapadia R, Bowen KK, Satriotomo I, Liang J, Feinstein DL, Vemuganti R. (2007) Peroxisome proliferator activated receptor-gamma agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents. *J Neurochem* 101:41-56