Diabetic Complications Consortium

Application Title: Increased small vessel disease in brain and cognitive impairment in diabetes

Principal Investigator: Weiguo Li

1. Project Accomplishments:

The global **hypothesis** of this project is that cerebrovascular dysfunction in diabetes facilitates entrapment of microemboli leading to inflammation and accelerates the development of small vessel disease (SVD) ultimately resulting in vascular cognitive impairment (VCI) in a sex independent manner. The goal of this feasibility application is to develop the model of microemboli in diabetes and acquire the key preliminary data on SVD and VCI development in diabetes in both sexes. With the support of this DiaComp award, we initiated studies to determine the effect of cholesterol crystal microemboli injection on cognition over the course of 8-12 weeks. Behavioral tests, MRI imaging and histochemical analyses of neurodegeneration markers suggested that 1) diabetes causes white matter damage and cognitive deficits, and 2) microemboli injection worsens demyelination and cognitive decline in both male and female diabetic animals.

Based on this set of exciting data and recent clinical studies on management of endothelial dysfunction to prevent SVD and ultimately VCID, we developed a new DIACOMP proposal to test the **hypothesis** is that early endothelial dysfunction in diabetes facilitates entrapment of microemboli leading to SVD and VCID, which was awarded. The **objective** of this follow-up study is to refine the microemboli model of VCID and begin preclinical testing with isosorbide mononitrate and cilostazol for the prevention/treatment of SVD and VCID in diabetes.

A. Specific Aims:

Determine the impact of microemboli on development of SVD and VCI in diabetes. We proposed only one key specific aim and conducted 3 main studies.

Study 1. Diabetes was induced by a high fat diet and low dose streptozotocin (35 mg/kg, IP) injection in male Wistar rats. Cholesterol crystal microemboli [40-70 µm, 3000] were injected 8 weeks later through internal carotid artery. Cognitive function was monitored by the novel object recognition (NOR) test. White matter injury was assessed by Luxol fast blue (LFB) and hematoxylin/eosin (HE) staining. ***While there was no difference in sensorimotor functions, Novel Object Recognition (NOR) test results indicated differences in cognition*** **(Fig 1).** Discrimination index (DI), a well-accepted measure of for working memory, was calculated as [time spent at novel object- time spent at familiar object/total time]. A low score indicates

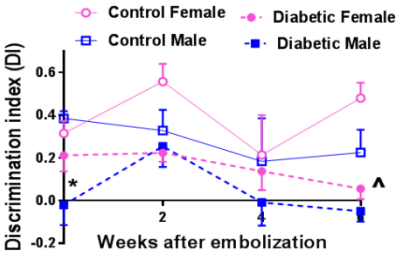

Fig 1. Diabetes worsens cognition and working memory. Diabetic male animals have lower DI scores as compared to control (*p<0.05) at baseline. Diabetic female animals show a greater decline by week 6 after embolization (^p=0.02), n=6.

the inability of the animal to recognize and discriminate between novel and familiar objects. Diabetic male animals showed baseline cognitive deficits as demonstrated by lower DI scores. By week 6 after microemboli injection, DI scored declined further. Diabetic animals exhibited cerebrovascular dysfunction prior to embolization (not shown).

At the end of 8 weeks, histopathological analyses were conducted to assess tissue damage in different brain regions including cortex, striatum and corpus callosum. Additional animals included sham operated control and diabetic rats. Hematoxylin/eosin (H&E) staining demonstrated greater pathology score (diffuse vacuolization and pyknotic nuclei) in diabetic animals which was amplified in the emboli injected group as compared to control emboli or sham diabetic groups. (Fig 2). Control emboli group also showed tissue injury as compared to control sham group, but this was not significant. Microinfarcts were not observed. Luxol fast blue staining for myelination showed similar results (Fig 2). Collectively, this data set provides proof of concept that microemboli injection initiates white and gray matter injury to a greater extent in diabetic male animals that also start showing signs of cognitive impairment. However, only one emboli injection paradigm was used, and tissue injury was evaluated at one time point. In order to refine the model with respect to number of injected microemboli and temporal development of tissue injury, another study was conducted.

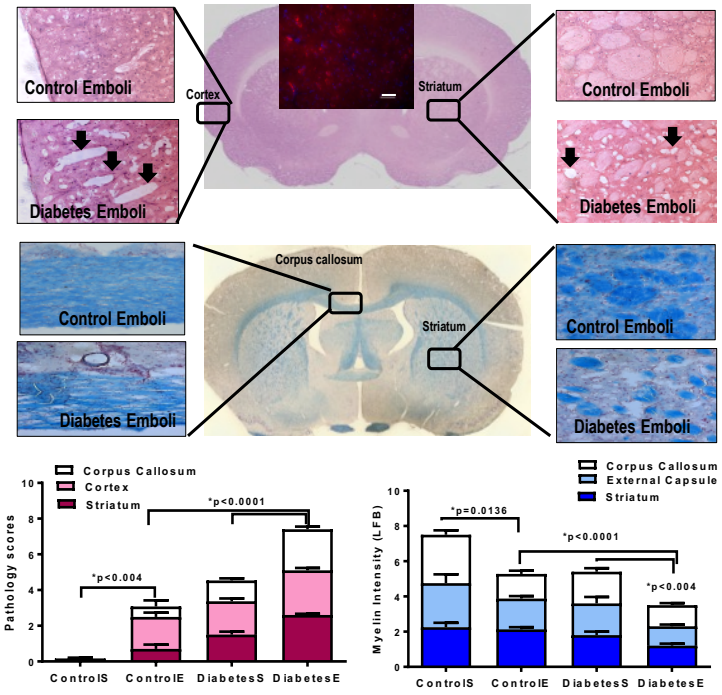

Fig 2. Diabetic animals show greater tissue damage and demyelination 8 weeks after microemboli injection, n=5 in emboli (E), 2 in sham (S) control, and 5 in S diabetes groups. Only male animals are shown. In a separate set of animals, fluorescently labeled crystals were injected[20] and brain sections were imaged on Day 3. A representative image from cortex in a diabetic rat, shown on top of the HE section, demonstrates some of the labeled crystals are retained within the brain. Scale bar is 100 µm.

Study 2: The results shown above are all conducted in the male animals, it remains unclear if the female animals have the same pathological mechanism. In a subset group of female Wistar animals, diabetes was induced and microemboli was injected as described above. The same endpoints including DI in NOR, LFB and HE stain in brain sections were assessed as mentioned above. While there was no difference in baseline scores between control and diabetic female rats, microemboli injection reduced DI scores to a great extent in diabetic females suggesting that this high-risk group is more prone to cognitive decline (Fig 1). Diabetes female animals showed significant worsened pathological score, which is assessed with HE staining (Fig 3), demyelination also showed similar pattern with LFB staining. These results suggest that microemboli injection triggered the brain injury even in young female animals with diabetes.

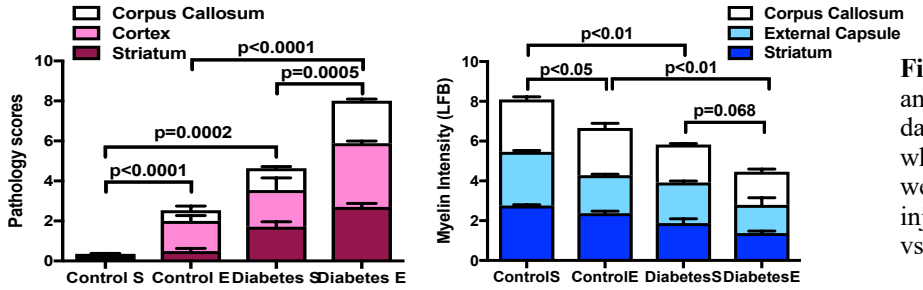

Fig 3. Female diabetic animals showed pathological damage and demyelination, which is exacerbated at 8 weeks after microemboli injection. *p<0.05, **p<0.01 vs. Control.

Study 3: This study was conducted to refine the model with respect to number of injected microemboli and temporal development of tissue injury. In this study, 6000 pieces of microemboli were injected into the male control and diabetes animal through the carotid artery as mentioned above, and the follow up period extended to 12 weeks after the injection. Cognitive function was monitored by the NOR test as in Study 1. Cerebral blood flow (CBF), white matter hyperintensities (T2 map), microbleeds (T2 asterisk), and microinfarcts (T2 W) were assessed by MRI at week 4, 8, and 12 after injection. The results showed that the male diabetic animals had decreased CBF but no difference on microinfarcts or microbleeds. These data suggest that white matter degeneration starts in the early stages of diabetes, and that microemboli exacerbate these pathological changes even before the appearance of any neurobehavioral deficits.

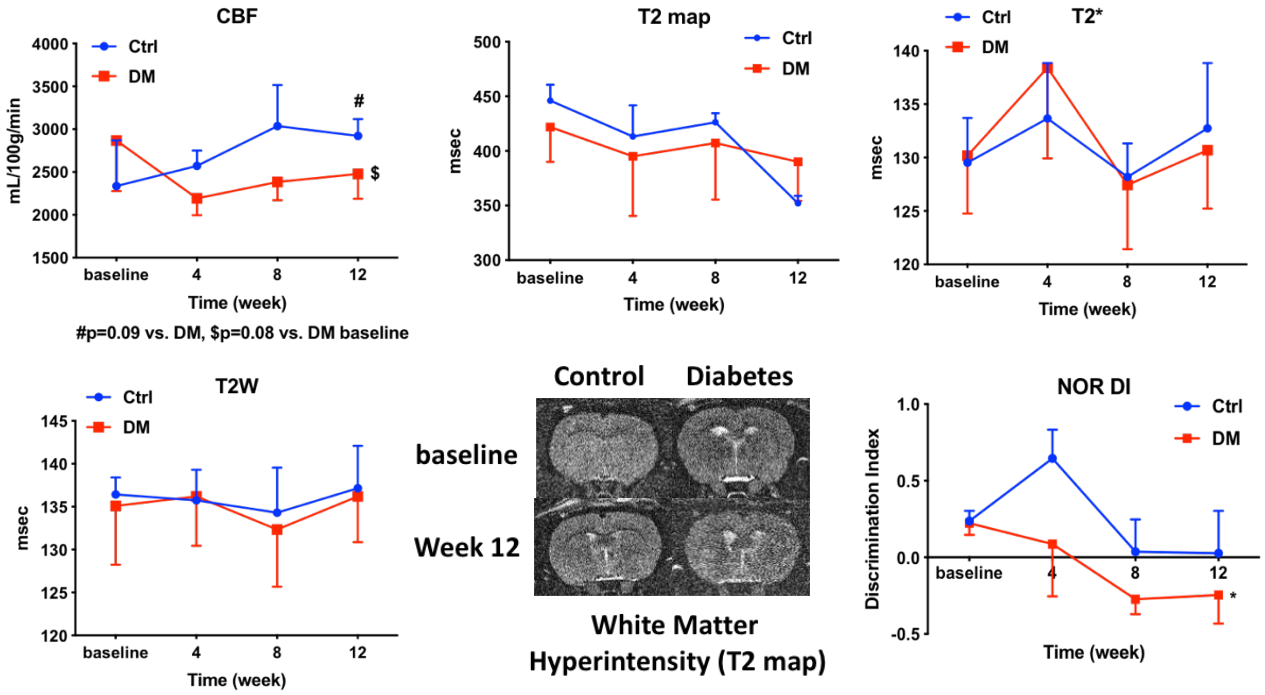

Fig 4. MRI and NOR results of the animals with higher dose microemboli injection. CBF showed exacerbated in diabetes group, but not in controls. While T2 map showed worsened demyelination in both control and diabetes animals, there was no microbleed (T2*) and microinfarcts (T2W) difference in the two groups. Representative images are showing the T2 map from baseline to week 12 after microemboli injection. DI in NOR showed significant worsened cognitive function in diabetic animals at 12 weeks after microemboli injection.

2. Publications:

Poster Presentation:
Diabetic but not Control Rats Develop White Matter Injury and Cognitive Deficits in a Microemboli Injection Model of VCID. Weiguo Li, Heba Ahmed, Ping-Chang Lin, Guangkuo Dong, Roxan Ara, Ali Arbab, Adviye Ergul. International Stroke Conference 2019, Honolulu, HI, Feb 2019.