Diabetic Complications Consortium

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

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1. Project Accomplishments:

The global **hypothesis** of this project is that cerebrovascular dysfunction in diabetes facilitates entrapment of microemboli (ME) 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 ME in diabetes and acquire the key preliminary data on SVD and VCI development in diabetes. With the support of this DiaComp award, we initiated studies to determine the effect of cholesterol crystal ME 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) ME 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 ME leading to SVD and VCID, which was awarded. The **objective** of this follow-up study is to refine the ME model of VCID and begin preclinical testing for the prevention/treatment of SVD and VCID in diabetes.

2. Specific Aims:

Determine the impact of microemboli (ME) on development of SVD and VCI in diabetes. We proposed only one key specific aim.

To refine the ME model, we increased ME dose to 6,000 cholesterol crystals and examined the development of VCID over 16 weeks after ME injection.

Results:

ME injection aggravated cognitive impairment in diabetic animals, but not in the control animals. We hypothesized that ME injection worsened the endothelial dysfunction in diabetes and exacerbated the cognitive deficits. Male Wistar rats were treated with high fat diet and streptozotocin (STZ, 35 mg/kg) i.p. injection to induce diabetes. ME (6,000 cholesterol crystals/300 μl saline) were injected at 6 weeks after diabetes onset.

The cognitive behavioral tests were performed at baseline before ME injection and at week 8, 12, and 16 after ME injection. At baseline before ME injection, the diabetic animals had lower

recognition index (Fig. 1 A) and discrimination index (Fig. 1 B) in the novel objection recognition (NOR) test than that of control animals, while ME worsened both indices after 16 weeks only in diabetic group but not in control group (Fig. 1 A and B). The diabetic animals had subordinate performance in the Y maze test over the period of 16 weeks (Fig. 2). At the baseline, they showed less interest in entering into each arm of the maze (Fig. 2 A), had less alternations in between the arms (Fig. 2 B), and less time spent in the novel arm comparing to the control rats. By week 16 after ME injection, the diabetic animals had even worse results in the total arm entries (Fig. 2 A) and alternation in the arms (Fig. 2 B) while the control animals had recovered results similar as baseline.

Diabetic animals had worsened histopathological damage and increased perivascular space after ME injection. The vacuolization, loss of tissue elements, inflammatory cell infiltration, axonal damage, and white matter (WM) rarefaction were evident in diabetic animals with ME injection (Fig. 3 A and B). Pathology scores based on hematoxylin and eosin (HE, Fig. 3 A) and Luxol fast blue (LFB, Fig. 3 B) staining showed that diabetic animals had worse histopathological damage (Fig. 3 C) and demyelination (Fig. 3 D) as well as increased perivascular space (PVS) index (Fig. 3 E) in all brain areas than that of the control group.

Activated microglial cells in diabetic animals with ME injection. Inflammation plays an essential role in cognitive impairment and diabetes. Therefore, microglial morphology was examined in the cortical and striatal brain regions in both animal groups. To visualize the activated microglia and macrophages, the expression of ionized calcium-binding adapter molecule 1 (Iba-1) was assessed in the brain sections of control and diabetic animals with ME injection (Fig. 4 A). In diabetic animals, cell body swelling was significantly greater in all areas (Fig. 4 B), while number of protrusions (Fig. 4 C), endpoints (Fig. 4 D), and branch length (Fig. 4 E) were all decreased. Collectively, these results indicated that there was an increased activation of microglia in diabetic animals with ME injection.

3. 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.

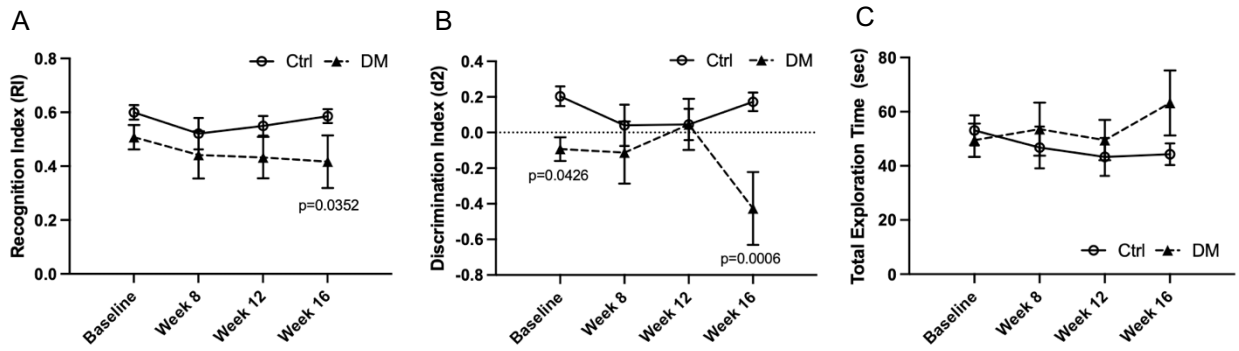

Fig. 1, Diabetic animals with ME injection had worse performance in NOR test. A: Diabetic rats had a significant lower Recognition Index in NOR test at week 16 after ME injection comparing to the control animals (p=0.0352 vs. Ctrl). B: Diabetic rats had a lower performance than control group in Discrimination Index of NOR test at baseline (p=0.0426 vs. Ctrl), which is exacerbated at week 16 after ME injection (p=0.0006 vs. Ctrl). C: The total exploration time had no significant difference in between the two groups. Data are mean ± SEM and were compared with corrected two-way ANOVA.

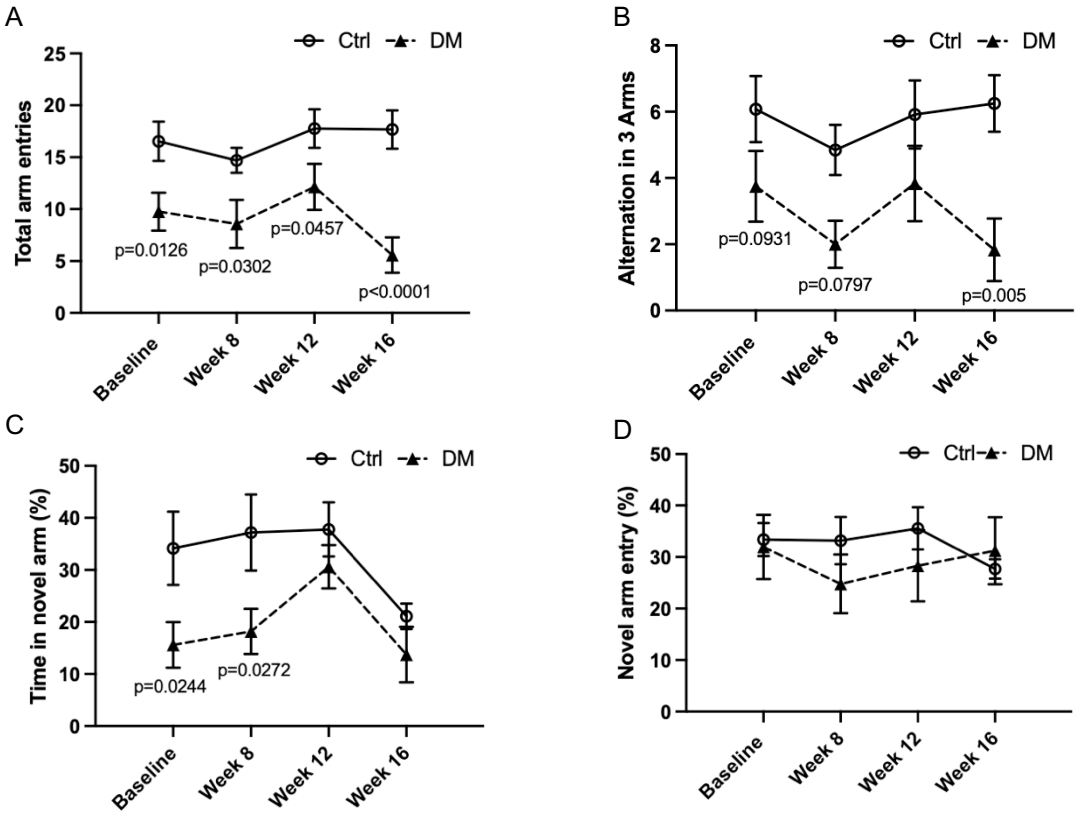

Fig. 2, Diabetic animals with ME injection had worse performance in Y maze test. A: Diabetic rats had an overall less Total arm entries than control animals which is exacerbated at week 16 after ME injection (p=0.0126, 0.0302, 0.0457, <0.0001 vs. Ctrl, respectively). B: There was a trend for diabetic animals had less Alternations than controls at baseline and week 8 (p=0.0931, p=0.0797 vs. Ctrl, respectively), while it was significant at week 16 after ME injection (p=0.005 vs. Ctrl). C: Diabetic animals spent less time in the novel arm than the control group at baseline and week 8 after ME injection (p=0.0244 and 0.0272 vs. Ctrl, respectively). Data are mean ± SEM and were compared with corrected two-way ANOVA.

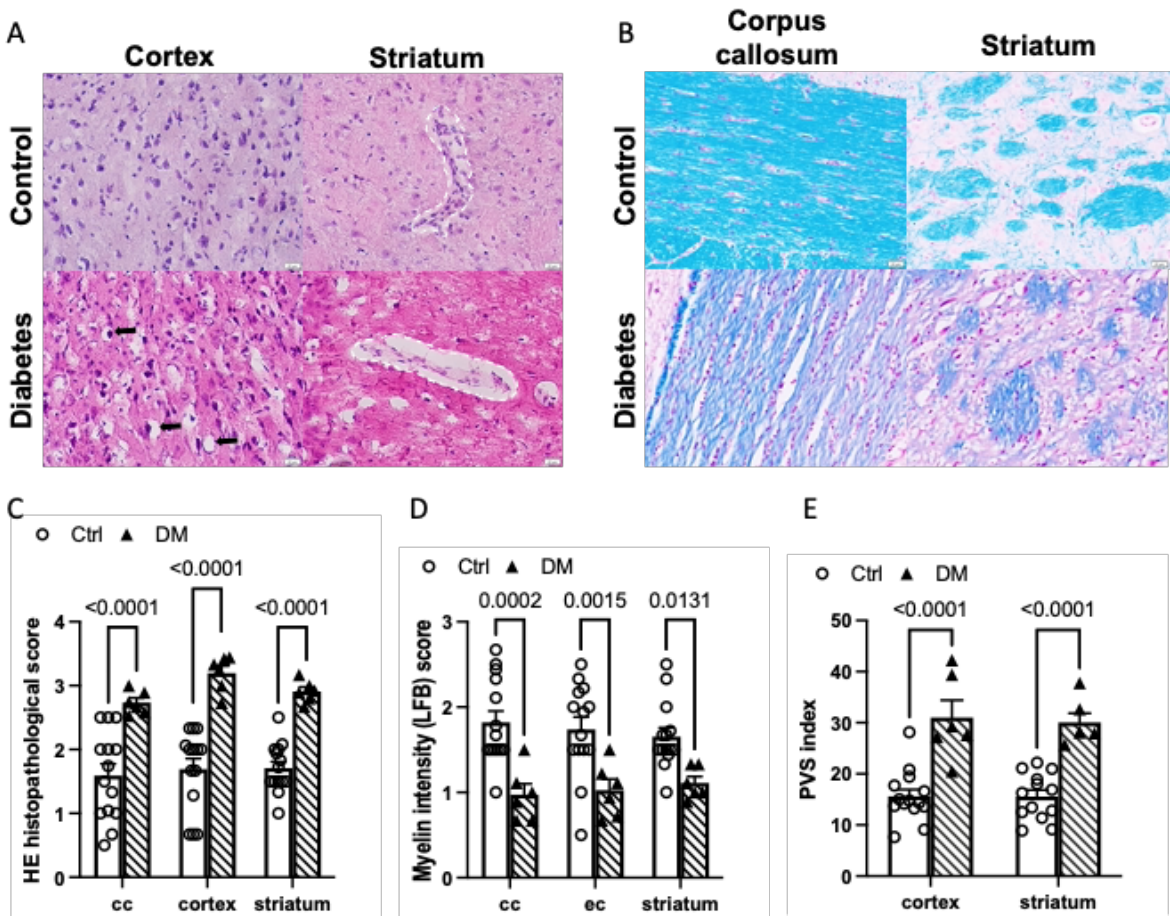

Fig. 3, Worsened histopathological damage after ME injection in diabetic animals. A: Representative images of HE staining showing histopathological damage in control and diabetic animals with ME injection, with more vacuole formation (black arrow) and increased perivascular space (dash line) in diabetes. B: Representative images of LFB staining showing increased demyelination, white matter rarefaction, and inflammatory cells immersing in diabetes. C to F: Histopathological score, Myelin intensity score, and PVS index showed significantly worsened histopathological damage in diabetic animals with ME injection. Data are mean ± SEM and were compared with corrected two-way ANOVA.

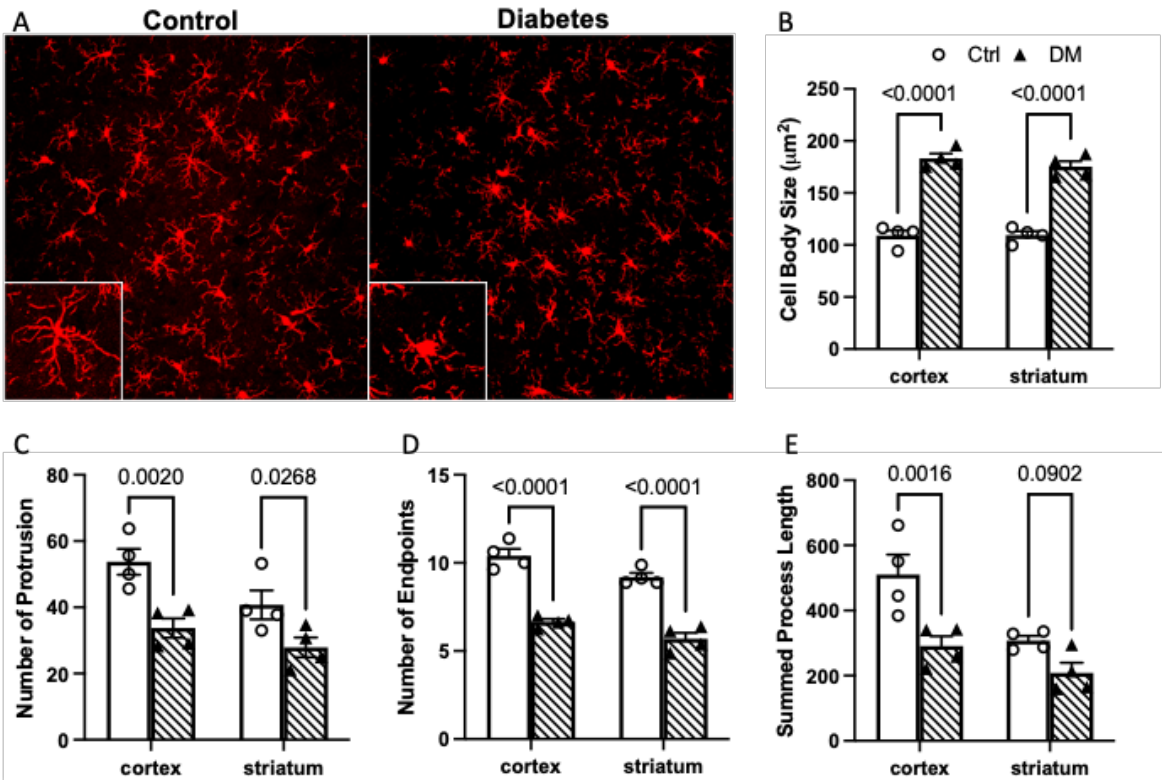

Fig. 4, ME augmented microglial activation in diabetic animals. A: Representative images of Iba-1 positive microglial cells in control and diabetic animals with ME injection. Representative single cells were showed in the inserts. B to E: Increased cell body swelling, and decreased number of protrusions, endpoints, and branch length were seen in diabetic group, which indicating increases of activated microglia. Data are mean ± SEM and were compared with corrected two-way ANOVA.