Diabetic Complications Consortium

Application Title: Diabetes Treatment for the Prevention of Stroke and Vascular Dementia

Principal Investigator: M. Paul Murphy

1. Project Accomplishments:

Obesity and type 2 diabetes mellitus (T2DM) confer an increased risk of multiple forms of dementia, including Alzheimer's disease (AD), vascular dementia, and strokes. Few viable models currently exist in which to explore the connection between these diseases. To this end, we have created a unique murine model that takes advantage of existing models of both diabetes (the *db/db* mouse) and AD (APP^{ANL}/ $PS1^{P264L}$ knock-in). The resulting mice (*db/AD*) are obese and diabetic and exhibit impairments in learning and memory. Though these mice have significant parenchymal amyloid deposition, one of their key characteristics is a profound increase in the instance of vascular pathologies, including strokes. The purpose of this proposal was to determine whether pharmaceutical intervention of the diabetes phenotype beginning at middle-age could ameliorate or prevent their cognitive impairment and/or stroke phenotype later in life. This is particularly interesting since this treatment mirrors the most common clinical intervention for human diabetics.

We now have data from several *db/AD* mice that have completed this study, with additional cohorts ongoing (including control genotypes (*wild-type, AD, db*)). Though the cohort that has completed this study is small, we have promising initial data. Using the metformin dose that was originally proposed (200 mg/kg/day), we were able to reduce the hyperglycemia that is the primary symptom of T2DM (**Figure 1**). We are still processing the Morris water maze (MWM) and MRI data obtained from these mice. We do know that metformin-treated mice still have strokes (**Figure 2**) and perform poorly on the MWM (*not shown*), though we will need additional study animals to detect any subtle improvements. Regardless of the outcome of these analyses, the fact that we can reduce the hyperglycemias in this model is exciting. While our model is novel and unique, it is reflective of uncontrolled diabetes- a clinically unusual phenomenon. Metformin, on the other hand, is the most-often prescribed anti-diabetic drug in humans and metformin-treated db/AD mice may provide us with a useful, clinically-relevant model of mixed dementia. Having a model of controlled diabetes in the presence of strokes and dementia will be a useful tool for picking apart the mechanisms underlying these pathologies and investigating other therapeutic approaches. We, therefore, plan to pursue this avenue of investigation, using this pilot data as the basis for more extensive studies. We are thus cautiously optimistic.

2. Specific Aims:

Specific Aim 1: Does metformin treatment beginning in mid-life improve stroke pathology and cognition in db/AD mice? We will treat middle-aged mice with metformin and determine the effect on strokes and vascular dementia using MRI and Morris water maze testing.

Middle-aged (6 months) *db/AD* mice were treated with metformin (200 mg/kg/day) for six months or used as untreated controls. Prior to the start of treatment and again prior to euthanasia, the mice were monitored for changes in fasting blood glucose and glucose handling using a glucose tolerance test. Though the effect is not significant due to a small *N*, it appears this dose of metformin modestly reduced fasting blood glucose levels and improved glucose tolerance (**Figure 1**). Since the mice tolerate the drug well, we are continuing these studies using a larger dose (400 mg/kg/day). Prior to euthanasia, the mice were subjected to testing by the Morris water maze and T2*MRI. We are still analyzing this data and are awaiting data from additional animals, but it is clear that metformin-treated animals still exhibit both cognitive impairment and stroke pathology (**Figure 2)**. Whether metformin treatment leads to subtle improvements in either measure is still unclear, but may become so as we obtain more data. Finally, tissue was collected for biochemical and histological analyses, and will be processed in the near future.

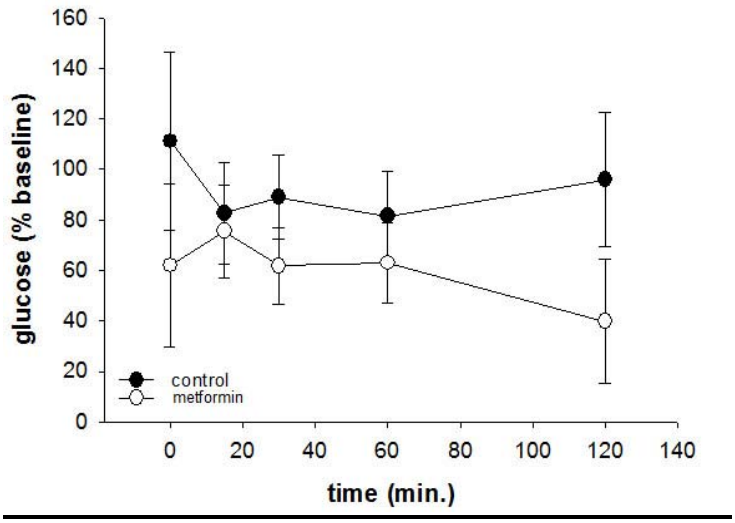

Figure 1: Metformin treatment improves glucose tolerance. Middle-aged d*b/AD* mice were treated with the antidiabetic drug metformin (200 mg/kg/day). Prior to treatment start and again prior to euthanasia, hyperglycemia was assessed using a glucose tolerance test during which fasted mice were challenged with a large bolus of glucose and their blood glucose monitored using a glucometer. Values are expressed as % baseline (prior to start of study) for the same GTT time point. Though the effect is not significant due to small animals numbers, there is a trend towards lower blood glucose with metformin treatment (n = 5 Control; n = 6 Metformin; p=0.29). We are currently treating additional mice and this study is ongoing.

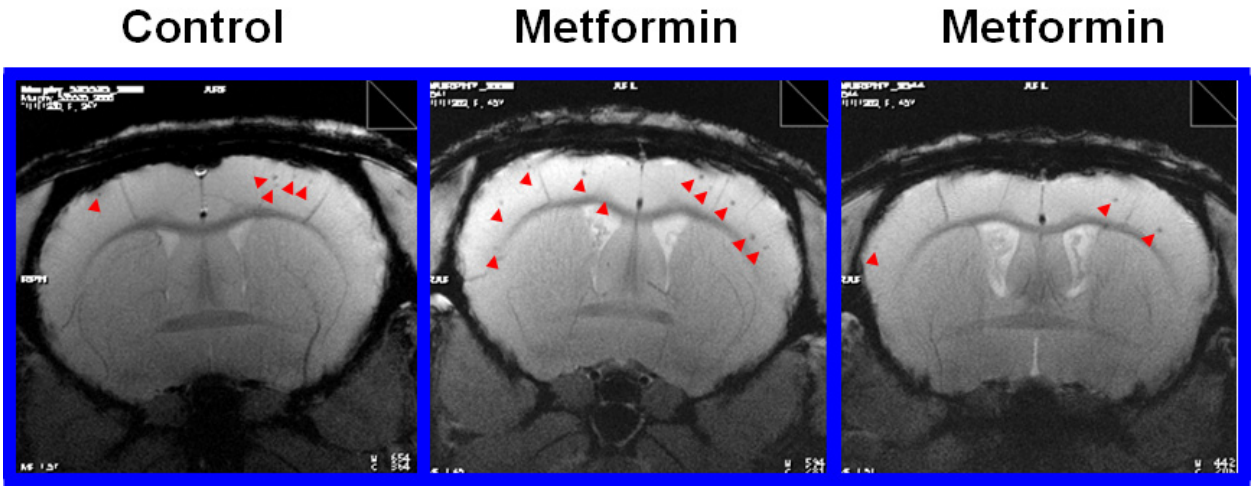

Figure 2: MRI images of metformin-treated mice.T2*MRI scans taken after six months of metformin treatment (mice are approximately 12 months old). Mice in both the control and treatment groups exhibited significant numbers of small strokes (arrowheads). As the study concludes, and we analyze larger numbers of mice, we will determine if any changes are statistically meaningful

3. Publications:

Funding from the Diabetes Complications Consortium contributed to data included in the following publication:

Niedowicz DM, Reeves VL, Platt TL, Kohler K, Beckett TL, Powell DK, Lee TL, Sexton TR, Song E, Brewer LD, Latimer CS, Larson KL, Kraner SD, Ozcan S, Norris CM, Hersh LB, Porter NM, Wilcock DM, Murphy MP (2014) "Obesity and diabetes cause cognitive dysfunction in the absence of accelerated β-amyloid deposition in novel murine model of mixed or vascular dementia" *Acta Neuropathologica Communications* **2**:64

In addition, upon completion of the metformin treatments, we expect to submit at least one additional publciation based on the data obtained during this study.