Ultrastructural Observational Studies of the Type 2 Diabetic db/db K Strain Neurovascular Unit in the Midbrain Cortical region

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This ultrastructural observational study of the monogenic leptin receptor deficient [BKS.Cg-Dock7M+/+LeprDB/J] db/db K strain (Db-C) obese, insulin resistant, type 2 diabetes mellitus (T2DM) model was utilized to evaluate and characterize any abnormalities of the cerebral neurovascular unit (NVU) in the mid brain cortical region as compared to controls and Db-C treated with a sodium-glucose co-transporter-2 (SGLT2) inhibitor (empagliflozin). We hypothesized that we would be able to demonstrate abnormal remodeling of endothelial cell(s) (EC) and pericyte(s) (Pc) of the NVU. Additionally, we intended to characterize any remodeling changes in the immediate vicinity of these NVUs. We observed the following remodeling changes of the ECs in the Db-C models: increased cytoplasmic thinning, increased vesicles/vacuoles (50, 100, and 200 nm), increased mitochondria (Mt) fragmentation (electron lucent Mt matrix and loss of cristae), tight junctions/adherens junctions (TJ/AJ) were attenuated and/or lost and basement membrane (BM) thickening. Importantly there was an attenuation and/or loss of Pc soma and end-feet. Also noted was microglia cell (MGC) activation with amoeboid type phenotype indicating a polarization of MGCs from M2 ramified to a M1-like phenotype. Unexpected novel findings included i) loss of EC coverage by NVU astrocyte(s) (Ac) due to detachment – retraction from EC and Pc BMs and dysmyelination of cortical grey matter myelinated neurons, which consisted of splitting and separation of the myelin lamella. All observations were compared to control (CKC) models and interestingly empagliflozin treatment (DbC-E) protected the NVU and surrounding tissues from these abnormal remodeling changes. Future directions for this study will include FIB/SEM milling of these models if possible. The following multipaneled image depicts most of these electron microscopic remodeling changes and the protection provided by treatment with the SGLT2 inhibitor (empagliflozin). This Excellence in Electron Microscopy grant allowed our group to fill in many gaps in our knowledge regarding the cellular and tissue abnormalities associated with a type 2 diabetic model. These novel remodeling changes in the midbrain cortical regions have provided a greater insight as to how T2DM, obesity and insulin resistance may be associated with an increase in the prevalence of Alzheimer's disease and type 2 diabetes mellitus. Each of these chronic age-related diseases are increasing in epidemic proportions as our world population ages and the convergence of these two diseases present an unprecedented increased cause of chronic disability, emotional loss, morbidity, and mortality.

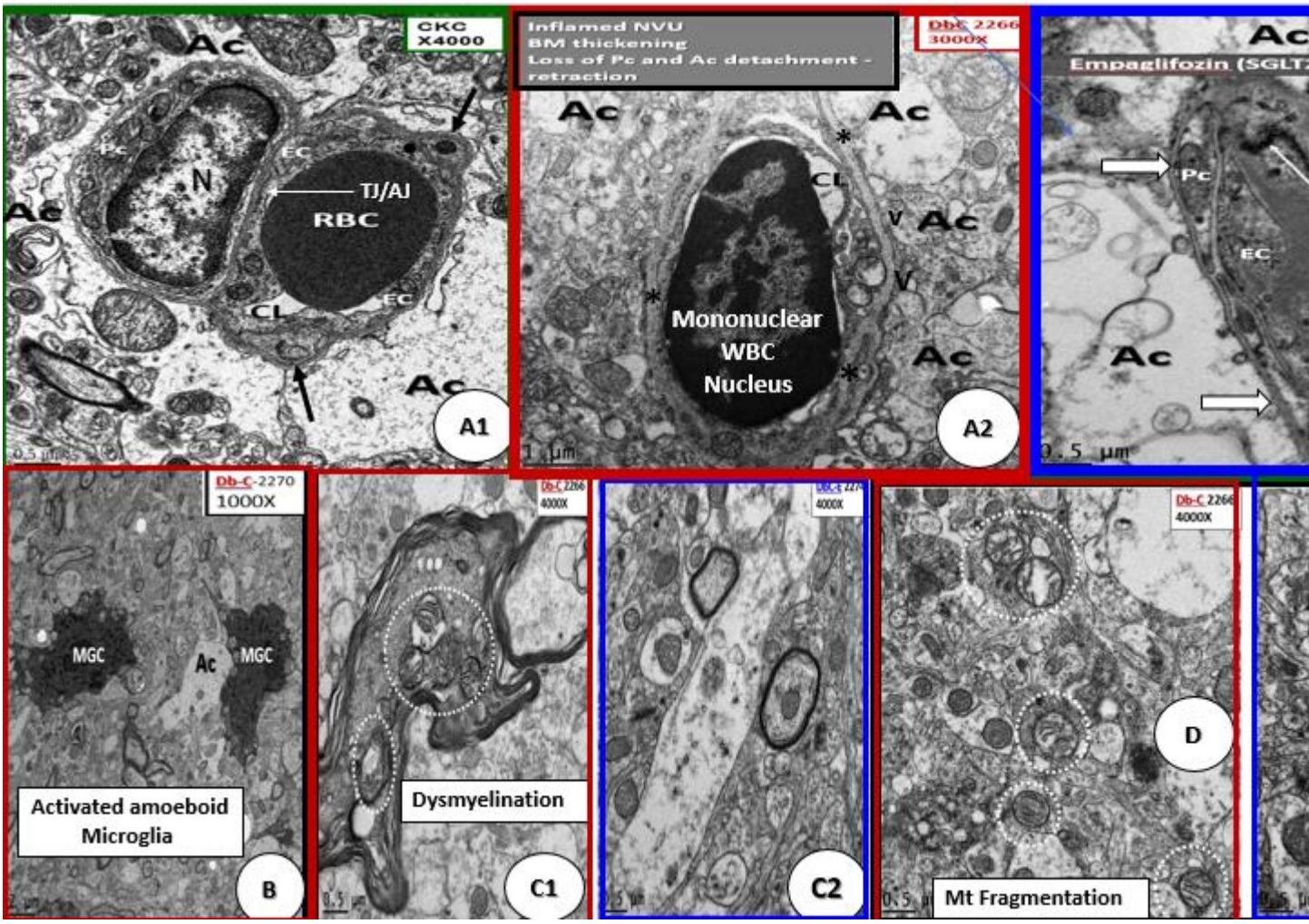

Figure Legend

Panels A1-A3 depict representative images of the neurovascular unit (NVU) in the grey matter midbrain cortex. Note that in the control (NVU) model (CKC) (A1) the NVU has a normal pericyte (Pc) soma with nucleus (N) and cytoplasmic Pc foot processes – end feet (black arrows) adjacent to the endothelial cell (EC) and enclosed by an inner and outer basement membrane (BM). Also, note the electron dense tight junction/adherens junction complex of the NVU (white arrow). Additionally, note the prominent electron lucent cytoplasmic corona of astrocyte end feet (Ac) surrounding the EC and Pc. Middle panel depicts a representative NVU in the diabetic db/db K strain model (Db-C) (A2) and note the absence of Pc soma and Pc foot processes. Importantly, note the thickening of EC and Pc BM (asterisks) as compared to control in panel A1. Also, note the vesiculation (v) and vacuolization (V) in the EC cytoplasm. Importantly, note the absence of TJ/AJ in the EC. Panel A3 is a representative NVU in the treatment model (SGLT2 inhibitor – empagliflozin): Note the presence of Pc foot processes (open arrows) and the normal thickness of the inner and outer BMs (like the control model

(panel A1). Also, note the prominent electron dense TJ/AJ (closed arrows). Importantly, note the presence of the electron lucent corona of Ac without detachment or retraction. This treatment model strongly suggests that empagliflozin protects the NVU from deleterious remodeling changes.

Panel B demonstrates the amoeboid M1-likw phenotype of the activated microglia cells (MGC) in the db/db K strain models (Db-C). These amoeboid MGCs were not present in either the control models or the db/db K strain models treated with empagliflozin.

Panels C1-C2 demonstrate myelinated neurons in the grey matter midbrain cortex. Panel C1 depicts dysmyelination with myelin lamellar splitting and separation in the Db-C models. Also, note the Mt fragmentation within the neuronal axon (encircled dashed lines). These abnormal myelin remodeling changes and Mt fragmentations were not observed in either the control models (not shown) or the db/db K strain models treated with empagliflozin (DbC-E) as depicted in panel (C2).

Panels D-D1 illustrate mitochondria (Mt) in the neuropile adjacent to the NVU. Note the abnormal Mt in the db/db K strain (Db-C), which are representative of Mt fragmentation (encircled with white dashes) (D). These fragmented Mt are characterized by an electron lucent Mt matrix with attenuation and/or loss of Mt cristae. Fragmented Mt were also found in the cytoplasm of EC, Pc and AC in the db/db K stain models (not shown) but were not observed in the empagliflozin treated models (DbC-E) or control models. Panel D1 is a representative image from the model treated with empagliflozin (DbC-E) and note the electron dense Mt matrix with intact cristae.

Magnifications vary (see upper right box) and scale bar (lower left) for each image.