Endothelial nitric-oxide synthase overexpression rescues cerebral blood flow and cerebrovascular reactivity in diabetic brain

Saurav B Chandra[1], Sumathy Mohan[2], Preethi Janardhanan[2], Kaiwalya S Deo[1], Eric R Muir[1], and Timothy Q Duong[1]
[1]*Research Imaging Institute, UT Health Science Center, San Antonio, TX, United States,* [2]*Pathology, UT Health Sc. Center, San Antonio, TX, United States*

TARGET AUDIENCE: Researchers interested in diabetes, cerebral blood flow regulation and endovascular dysfunction.

PURPOSE: Reduced bioavailability of nitric oxide plays a crucial role in endothelial dysfunction in diabetes.[1] Endothelial nitric-oxide synthase (eNOS) overexpression has been shown to protect against endotoxin shock, skeletal muscle ischemic/reperfusion injury, and congestive heart failure after severe myocardial infarction in mice.[2,3] The goal of this study was to investigate the effect of eNOS overexpression on cerebral blood flow (CBF) and cerebrovascular reactivity (CR) in diabetic mice, eNOS-overexpressed (TgeNOS) mice, Akita diabetic (Ins2 ^{Akita}) mice, and a genetic cross of TgeNOS⊗Ins2 ^{Akita}. We hypothesized that eNOS overexpression rescues CBF and CR dysfunction in the diabetic brain.

METHODS: Male wild-type (WT) C57BL/6J mice (n=6), TgeNOS (n=7), Ins2^{Akita} (n=6) and a cross (n=5) were studied. Mice were 5-7 months old (18-23g). The Ins2^{Akita} and the TgeNOS⊗Ins2^{Akita} cross mice had blood glucose level of >450mg/dl. Tail biopsies from these mice were genotyped by standard PCR procedure followed by restriction digestion.[2] The mice were anesthetized with 1-1.2% isoflurane. Temperature was maintained at 37°C and respiratory rate at 80-110 bpm. MRI scans were performed in a 7T magnet (Bruker Biospec) with a 150 Gauss/cm gradient. A small circular surface coil designed for brain imaging (ID=1.1 cm) and a circular labeling heart coil (ID=0.8 cm) was used for ASL.[4] CBF was acquired using the continuous ASL technique and single-shot EPI with FOV=1.2x1.2cm, matrix=64x64, THK=1mm, 9 slices, labeling duration=2.2s, TR=3s, and TE=8ms. CR was measured as CBF % changes due to 5% CO_2 (in air) inhalation.

RESULTS: Genotyping of Ins2Akita and TgeNOS-Ins2Akita cross mice are shown in **Figure 1**. Lane 1 (WT mice) shows only a single band of the WT gene. Lane 2 (Akita mice) shows the WT gene and the mutant Akita gene (heterozygote) but no eNOS gene. Lane 3 (TgeNOS⊗Ins2 ^{Akita} mice) shows the WT gene, the mutant Akita gene, and eNOS gene. Typical CBF images are shown in **Figure 2**. Whole-brain basal CBF of TgeNOS was statistically higher than that of WT (P<0.05) (**Figure 3**). Akita and TgeNOS⊗Akita CBF were not statistically different from WT CBF (P>0.05), or from each other (P>0.05). CR of TgeNOS was reduced slightly but not significantly compared to WT (**Figure 4**). CR of Akita was significantly smaller than CR of WT (P<0.05) and TgeNOS⊗Akita (P<0.05).

DISCUSSION: eNOS overexpression elevated basal CBF compared to WT, consistent with nitric oxide being a potent vasodilator. Basal CBF was not statistically different between diabetic mice and WT, likely diabetes was mild at this early stage and that the brain was able to adapt to maintain normal basal CBF. The TgeNOS⊗Akita CBF was also not different from WT, again suggesting basal CBF was maintained within normal ranges by adaptation. CR of TgeNOS was slightly but not significantly reduced. This is not surprising because eNOS overexpression likely results in dilated vascular tone and thus makes it less responsive to dilatory effects of 5% CO_2. Akita CR was markedly and significantly reduced, indicative of vascular dysfunction in its ability to vasodilate in diabetes under challenged condition. TgeNOS⊗Ins2Akita cross mice showed improved CR, suggesting that endovascular dysfunction in diabetes can be rescued by overexpressing eNOS, which enhances nitric oxide production.

CONCLUSIONS We found that eNOS overexpression does not perturb basal CBF but significantly perturbs CR at relatively early stage of diabetes. With respect to our central hypothesis, we found eNOS overexpression rescues CR dysfunction in diabetes. This work suggests a potential target for rescuing endovascular function in the diabetic brain. Future studies will investigate stimulus-evoked changes and multiple stages of diabetes.

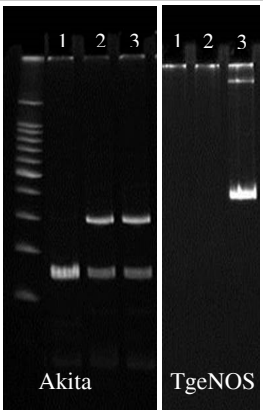

Fig 1: Genotyping of Ins2^{Akita} and TgeNOS-⊗ Ins2^{Akita} cross mice. **1: WT 2: Akita 3: Cross**

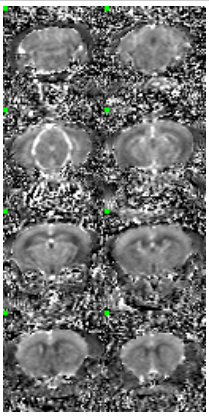

Fig 2: Multi-slice CBF images. Whole-brain ROIs were used for subsequent analysis.

REFERENCES: [1] Cosentino et al. *Cardio Pharm* 1998; 32:S54. [2] Mohan et al. *Lab Invest* 2008; 88:515. [3] Toda et al. *Pharm Rev* 2009;61:62. [4] Muir et al. *MRM* 2008;60:744. Support by DK096119.

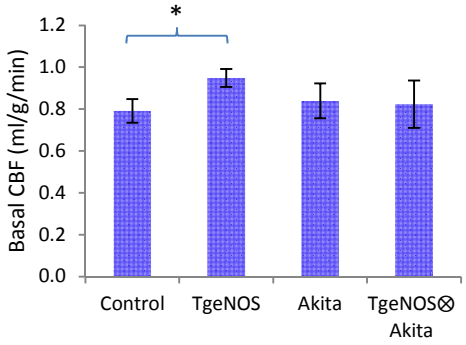

Fig 3: Basal CBF. SEM, * p<0.05

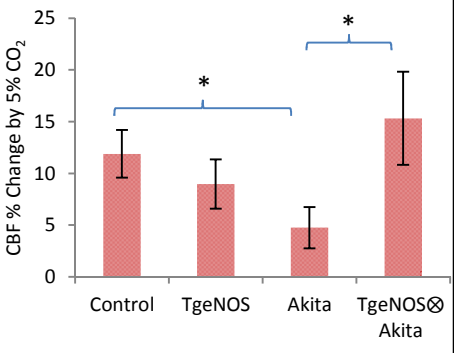

Fig 4: CBF % changes due to 5% CO_2. SEM, * p<0.05.