Insulin Reverses Attenuation of Cerebral Blood Flow (CBF) Caused by Hyperglycemia in a Mouse Model of Diabetes: Potential Impact on Acute Ischemic Stroke

S. K. Amin[1], F. Serrano[1], T. Terashima[2], L. Chan[2], L. Hu[1], and R. G. Pautler[1]

[1]Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, United States, [2]Medicine, Baylor College of Medicine, Houston, TX, United States

Introduction:

Diabetes is associated with microvascular disease and is believed to increase cerebrovascular complications, particularly acute ischemic strokes. Admission hyperglycemia is associated with complications in one-third of acute ischemic strokes, worsening the outcome. [1] Both human and rat studies have shown that both acute and chronic hyperglycemia cause a reduction in cerebral blood flow (CBF), however, little is known about the mechanism of this change. A recent review article discussed the biochemical and molecular pathways in the vasculature affected by hyperglycemia and concluded that the endpoint was a pro-vasoconstrictive, pro-thrombotic and pro-inflammatory state, increasing the vulnerability to reperfusion injury.[1] In an attempt to evidence the pathways elucidated by this article (Fig. 1) at a systemic level, our goal was to use an *in vivo* mouse model of diabetes not only to evidence reduced CBF, but also to determine if this vascular effect is reversible by modifying glucose levels with insulin.

The experimental model for diabetes is produced by the administration of streptozotocin (STZ), which causes a selective destruction of the beta cells of the pancreas. This results in a decreased production of insulin (hypoinsulinemia) and high levels of serum glucose (hyperglycemia). Our lab has demonstrated *in vivo* that STZ-treatment causes impairment in axonal transport in both central neurons (data not shown); however, little to no work has been done to assess CBF in diabetic mice and none exploring the reversibility of hyperglycemic-induced vascular changes.

We utilized Flow-sensitive Alternating Inversion Recovery (FAIR) arterial spin labeling (ASL) to determine CBF. The difference of the inverse of T_1 global and selective inversion datasets yields a cerebral blood flow (CBF) measurement. This modality is advantageous to previously used techniques to assess CBF because it is a noninvasive modality that provides quantitative data unlike Doppler.

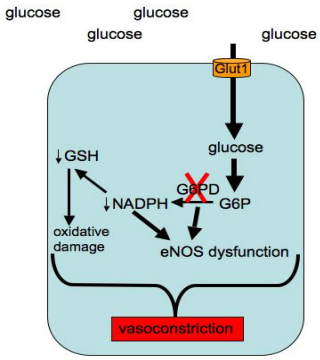

Figure1: Endothelial biochemical pathways in hyperglycemia.[1]

Methods:

Induction of diabetes: C57BL/6 (23-25g) mice were injected intraperitoneally (IP) with 170mg/kg streptozotocin (STZ) made in sodium citrate buffer pH4.5. Body weight and glucose levels were checked before and after STZ-injections. Mice are considered diabetic if glucose levels were higher than 200mg/dl. In this study we examined: mice with no STZ injection (n= 4); mice injected with STZ (n = 3); mice injected with STZ and also an insulin pellet (n = 5). The average glucose values for each group are listed in Table I.

Perfusion Arterial Spin Labeling (ASL): The same groups of mice listed above were utilized to assess CBF with ASL. Two weeks after STZ injection and four weeks after STZ + insulin injection, mice were sedated with 2% isoflurane in 100% oxygen. Following anesthesia, the mice were placed in a horizontal bore 9.4T Bruker Avance imaging system with the head positioned in the center of the probe and maintained in 1-2% isoflurane for the remainder of the imaging session. The body temperature of the mice was monitored and maintained at 37°C using an air heater. The imaging parameters were as follows: TR = 7145.973 ms, TE = 24.26 ms, Number of averages = 1. The inversion recovery time (TIR) = 100ms, the number of TIR = 8, the TIR increment = 1000 ms, the inversion slab thickness = 6 mm, and the slice package margin = 2.50 mm. The effective bandwidth = 200kHz. The field of view (FOV) = 15 x 15 mm, matrix = 64 x 64 and slice thickness = 1mm. We utilized Bruker Biospin's Paravision 4.0 software to calculate the rCBF. Briefly, the imaging slice was positioned transversely through the center of the cortex for both the slice selective and non-selective slice T1 series. Regions of interests (ROI) were defined for the calculation of the T1 images for both the T1 series: right cortex, left cortex. The data points from each ROI were fitted to a T1 inversion regression curve. The values are then to calculate CBF according to: Relative CBF (rCBF) = $\lambda (1/T_{1selective} - 1/T_{1nonselective})$ where λ is the blood-brain partition coefficient, which was set to 90ml blood/100g of tissue. The factor 60000 was used to convert ms to min and, consequently, the unit for rCBF is ml/(100g*min). The rCBFs were compared among groups and analyzed utilizing the software, Prism. P values were calculated utilizing a t-test.

Results:

We observed that the STZ-injected mice had significantly reduced CBF (p value <0.036) compared to the control group (Fig. 2). Furthermore, when insulin was injected in STZ mice (STZ + Ins), a reversal in CBF was observed that was not only significantly higher than that of the STZ group, but also comparable to that of the control group.

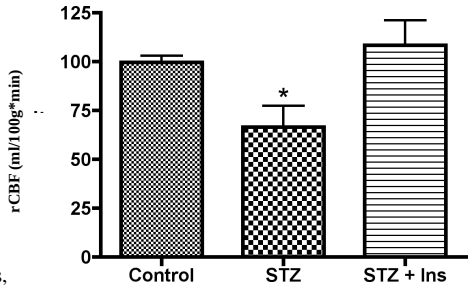

Figure 2: Insulin reverses attenuation of CBF caused by hyperglycemia in STZ mice. Inverse regression curves using ASL shows the following rCBF: Control (99.64, n=4), STZ (66.62, n=3), STZ + Ins (113.54, n=5). * = p < 0.036

Conclusion and Future Directions:

In conclusion, we observed a reduction in CBF in a diabetic mouse model as well as witnessed an "insulin rescue" in CBF in STZ mice. The fact that the only difference between the STZ group and STZ + Ins group was the administration of insulin, and thus the level of blood glucose, the decrease in CBF seen in the STZ mice is secondary to the glucose level rather than to STZ toxicity. Therefore, our results evidence the biochemical pathways illustrated in Figure 1 at a systemic level. However, it is still also possible that the effect could be due to insulin itself and warrants further investigation.

Because hyperglycemia is thought to induce a complexity of biochemical changes within endothelial cells, the next step would to determine if a rescue phenomenon in CBF can be observed by directly increasing the intracellular antioxidant potential of cerebral vasculature, thus reducing eNOS dysfunction. This may elucidate the vascular injuries that occur acutely in hyperglycemic stroke and lead to neuronal injury. Therefore, the information garnered will facilitate in developing intervention for hyperglycemic stroke patients.

Table I	Control (N = 5)	STZ (N = 5)	STZ + Insulin (N = 5)
Glucose (mg/dl)	175 ± 13	473 ± 61	255 ± 22

References: 1. Martini SR, Kent TA. Journal of Cerebral Blood Flow & Metabolism 2007; 27:435-451.