Increased VEGF expression correlates with severely reduced cerebral perfusion in glutaric acidemia type I (GA-1) mouse model of diet induced encephalopathy

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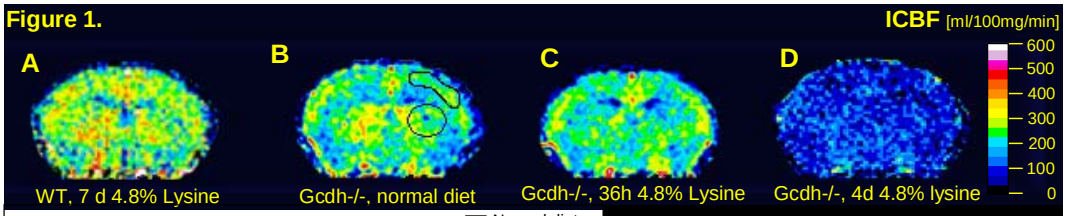
INTRODUCTION: Rapid induction of hypoxia inducible factor 1α (HIF-1α), and its target gene vascular endothelial growth factor (VEGF) has been reported in various animal models of ischemic and hypoxic-ischemic brain injury [1,2]. The proposed neuroprotective role of these genes remains controversial and is a subject of intense investigation [2-4]. Despite intense studies, the consequences of VEGF upregulation on cerebral perfusion are less well characterized. In certain mitochondrial disorders, Krebs cycle dysfunction may lead to rapid induction of hypoxia inducible factor 1α (HIF-1α) [5], and subsequently VEGF. In the current study we investigate the relationship between VEGF expression and cerebral perfusion in a metabolic disorder of lysine and tryptophan metabolism, glutaric acidemia type I (GA-1). GA-1 is characterized by frequent cerebral and retinal hemorrhages, bilateral striatal necrosis, and early death [6]. We hypothesized that frequent hemorrhages and cerebral edema may result as a consequence of increased VEGF expression, further contributing to cerebral edema and reduced cerebral perfusion. We used a previously described GA-1 animal model, where glutaryl-CoA dehydrogenase knockout mice (Gcdh-/-) develop encephalopathy at 4-weeks of age following lysine enriched diet [7].

METHODS: *Gcdh -/-* mice and wild type (WT) littermate controls, both of mixed C57BL6/J X 129SvEv genetic background were used. The high lysine diet was prepared by adding free lysine to a standard diet to achieve 4.7% total lysine. MRI was performed on a 7.0 T Bruker system, with a 2 cm birdcage coil. MR images were obtained before the lysine diet onset (N=11 Gcdh-/- mice, N=4 WT mice), and then on the 2nd, 3rd and 4th day after onset for N=4 *Gcdh -/-* mice, and on the 7th day after onset for N=4 WT mice. Prior to imaging mice were anesthetized with 3% isoflurane, adjusted during the imaging to 1-1.5% in order to maintain a constant breathing rate of 40 bpm. Arterial spin labeling (ASL) was used to acquire a single-slice perfusion-weighted image at the level of the striatum (TR/TE: 2000/12 ms, NAX: 2, 1.06 mm slice thickness, 208^2 μm^2 resolution). A RARE sequence with multiple TR times (100-5000ms), same slice position and resolution, was used to calculate T_1 values. Perfusion values were calculated on a pixel-by-pixel basis using NIH ImageJ and MRI analysis calculator, a plug-in by Karl Schmidt. Following imaging, symptomatic Gcdh-/- mice were sacrificed and tissue was collected for VEGF Western Blot analysis (N=4). Additional WT mice were processed for Western Blot analysis (N=3 normal diet, and N=3 on high lysine diet for 4 days). Additional high resolution MRI was performed post-mortem, in order to visualize vascular changes (3D FLASH sequence, TR/TE: 120/6 ms, NAX: 14, 50^3 μm^3 voxel). Statistical analysis was done using ANOVA, followed by a Holm-Sidak post-hoc test.

RESULTS: The perfusion-weighted MRI revealed significantly reduced ($p<0.05$) cortical, but not striatal perfusion in Gcdh-/- mice at 4-weeks of age compared to WT littermates, prior to high lysine diet onset (208.9±20.3 *vs*. 244.0±25.1 [ml/100mg/min]). After 7 days of lysine diet exposure, there was a tendency toward increased perfusion in WT mice, **Fig. 1A**, although this was not statistically significant. In WT mice cortical baseline perfusion was 244.0±25.1 *vs*. 265.4±15.7 [ml/100mg/min] after 7 days on high lysine, while striatal baseline perfusion was 235.0±39.4 *vs*. 253.9±25.4 [ml/100mg/min] after 7 days on high lysine for striatum. WT mice remained asymptomatic on high lysine diet. In contrast, Gcdh-/- mice experienced encephalopathy and reduced cortical and striatal perfusion following high lysine diet onset that was significantly different by 4-days after onset ($p<0.005$), **Fig. 1B-D**. In encephalophatic Gcdh-/- mice cortical perfusion was reduced ~60% (216.9±21.9 *vs*. 84.9±1.6 [ml/100mg/min] after 4-days of high lysine diet), while striatal perfusion was reduced ~50% (216.7±19.3 *vs*. 107.6±3.7 [ml/100mg/min]), **Fig. 1D**. Western Blot analysis revealed significantly increased cortical and striatal VEGF expression 4 days after high lysine diet in Gcdh-/- compared to WT mice ($p<0.05$), **Fig. 2**. Additional post-mortem high resolution MRI revealed highly distended vasculature, **Fig. 3,** while hemorrhages and blood brain barrier breakdown have been already reported [7].

DISCUSSION: While a mild dose of VEGF may indeed be neuroprotective [3,4], here we demonstrate overexpression of VEGF to correlate with cerebral hemorrhages, reduced perfusion and poor outcome in the GA-1 mouse model. The exact cause and effect relationship between increased VEGF expression and reduced perfusion needs to be further characterized. Nevertheless, we can speculate mitochondrial dysfunction, due to Gcdh enzyme deficiency, to be the triggering event leading to initiation of compensatory mechanisms, including VEGF induction. Increased VEGF expression, during the sustained metabolic decompensation, is likely enhancing blood-brain barrier dysfunction, causing hemorrhages, and contributing to cerebral edema previously reported in this mouse model [7]. These events could then lead to reduced cerebral perfusion and poor outcome. Presented results are in accord with previous reports, where high doses of VEGF failed to provide neuroprotective effect, but instead increased the risk of hemorrhagic transformation following focal ischemia [4]. In conclusion, our results demonstrate how continuous metabolic derangement can quickly lead to severe global perfusion deficits, and strongly imply perfusion-weighted MRI as an important monitoring technology for individuals affected with GA-1.



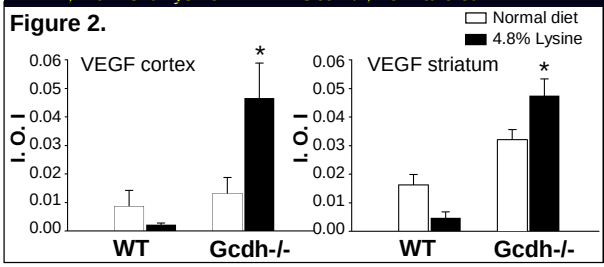


Figure 1. Index of cerebral blood flow (ICBF) at the level of the striatum in (A) WT and Gcdh-/- mice (B-D). Color scale represents different ICBF values. The ROIs used for statistical analysis are outlined in B (ROI was bilateral).

Figure 2. VEGF levels in cortex and striatum expressed as integrated optical density (I. O. I.), and after being normalized for actin expression, *$p<0.05$ (WT on high lysine diet (N=3) vs. Gcdh-/- on high lysine diet, (N=4)).

Figure 3. High resolution 3D FLASH MRI reveals multiple distended blood vessels (white arrows) in Gcdh-/- mice on high lysine diet for 4 days.

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