Characterization of Cerebrovascular Parameters using MRI in eNOS and sGCα1 Knockout Mice

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INTRODUCTION: Endothelial nitric oxide synthase (eNOS) is a key signal-transduction enzyme responsible for endothelium-dependent vasodilation, controls cerebral blood flow, and the absence of eNOS can induce larger cerebral infarction upon ischemia [1]. NO signaling involves the binding of NO to soluble guanylyl cyclase (sGC), resulting in the downstream signaling processes[2]. Therefore, we posit that cerebrovascular function can be modulated in mice deficient for eNOS or for sGCα1 subunit. In this study, we aimed to quantify MRI-derived vascular parameters (cerebral blood volume (CBV), transvascular water exchange index (WEI), and vessel size index (VSI)) in eNOS knockout (KO) and sGCα1 KO mice using two different MRI intravascular contrast agents (Gd-PGC and superparamagnetic iron oxide nanoparticle (SPION)) and compared these parameters with those in wild type (WT) mice. Finally, we also investigated whether perivascular aquaporine (AQP) proteins, which play a central role in formation and resolution of edema in the pathophysiology of many diseases, are differently expressed in eNOS KO mice.

MATERIALS & METHODS: A 3D flip angle dependence of the MRI signal intensity on transvascular water exchange rate was used to quantify CBV and WEI before and after intravenous administration of Gd-PGC using wild type (C57BL/6, n=10), eNOS KO (n=5) and sGCα1 KO mice (n=4). 2D T2 and T2* maps were also acquired before and after the SPION administration for measurements of cerebral VSI (see Fig. 1). WEI was calculated using low flip angles as compared to relatively high flip angles, after creation of subtraction images (i.e., SI_{post_Gd}–SI_{pre_Gd}) for each flip angle. VSI was calculated using the following relationship: $VSI = \Delta R_2^* / \Delta R_2$. For the Western blot, three WT and three eNOS KO mice brains prepared to compare the expression of AQP proteins.

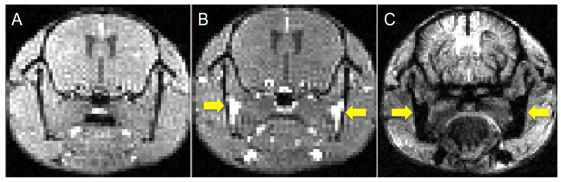

Fig. 1. Representative 3D images obtained before (A) and after (B) injection of Gd-PGC, and 2D T2* imaged obtained after injection of SPION (C). Note the significant change of signal intensity attained by two different intravascular contrast agents.

RESULTS & DISCUSSION: Both the eNOS and sGCα1 KO mice have lower VSI values than those measured in the WT mice (Fig. 2A). However, the CBV of both KO groups is not significantly different from WT CBV (Fig. 2B). Furthermore, the baseline WEI is significantly higher in KO than WT mice, indicating an increased water permeability across the BBB (Fig. 2C). However, both AQP1 and AQP4 were less expressed in the eNOS KO mice than those in the wild type mice (Fig. 2D). Surprisingly, the presence of basal AQP proteins in the plasma membrane do not appear to be correlated with the increased water exchange rate. Since we hypothesized that these proteins are directly associated with the water movement across the BBB interface, such unexpected lack of correlation between AQP protein expression and WEI precludes the channel-mediated water exchange. In this regard, the elevated WEI values in eNOS KO mice imply the inherently weakened BBB function, which increases the rate of the water movement across the cerebrovascular wall. On the other hand, such increases in WEI may serve as a predictive index for abnormally heightened stroke formation, previously observed in the eNOS KO mice group. Our findings suggest that the loss of eNOS concurrently contributes to the decrease of the vessel diameter and the increase of water permeability. Further investigations are required to elucidate the pathophysiological relationship between NO synthase and the BBB integrity, and endothelial NO-sGC involvement with ischemic damage.

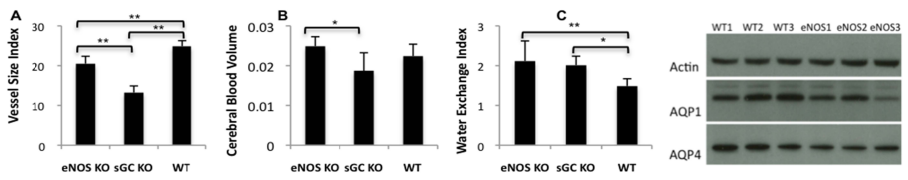

Fig. 2. Comparison of VSI, CBV, and WEI of both KO and WT mice, and the difference in AQP expressions between eNOS and WT mice (**: p<0.01, *: p<0.05)

REFERENCE [1] Huang et al. Nature. 1995;377:239-242; [2] Atochin et at. Stroke. 2010;41(8):1815-1819