

A potentially new target for stroke recovery... 'Nox' at the door

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It is well established that hypertension creates a vascular environment ripe for both hemorrhagic and ischemic stroke. Recovery from ischemic stroke is facilitated clinically by interventions that remove vascular occlusions via tissue plasminogen activator-mediated thrombolysis¹. While patients have largely benefited from this therapy, enjoying leading remarkable recovery rates, a not insignificant proportion of individuals experience vascular complications which include leakage of the blood brain barrier and hemorrhage², coupled with reactive oxygen species (ROS) overproduction which can worsen outcomes¹.

The source(s) of such ROS generation have until now remained obscure and therefore beyond the reach of potentially beneficial targeted therapeutic intervention. However, a recent study by Casas and co-workers sheds new light upon the enzymatic source of such deleterious ROS produced during recovery from ischemic stroke³. In this novel work, the authors focus upon a relatively unique member of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) family – the Nox5 isoform, and show that its expression in the vascular endothelium is sufficient to drive oxidative stress thus rendering the brain susceptible to impaired recovery from post-occlusion-mediated ischemic stroke in a mouse model.

While many sources of ROS contribute to oxidative stress (xanthine oxidases, lipoxygenases and mitochondrial respiratory chain oxidation), Nox enzymes produce physiologically significant $O_2^{\cdot-}$ and H_2O_2 throughout the body (reviewed in⁴). The Nox family consists of seven members (Nox1-5, Duox1-2). Nox5 is potentially a good target for therapies because it differs from other Nox isoforms in terms of its activation and tissue distribution. Most Nox isoforms are dependent on protein co-factors for their activity (e.g., p22phox), but Nox5 is not⁵. Furthermore, while Nox5 is structurally similar to other Noxes in that it contains conserved C-terminal NADPH and FAD binding domains and 6 transmembrane-spanning regions, its amino terminus harbours multiple EF-hands that regulate its activity in response to intracellular Ca^{2+} ⁶. Phosphorylation of amino acid residues alters EF-hand domain conformation to enhance Nox5 sensitivity to physiological Ca^{2+} levels⁷.

Research into the precise physiological and pathophysiological roles of Nox5 has been limited by the fact that it is absent from the mouse/rat genomes. While Nox5 is highly expressed during fetal development, its levels are low in healthy adult tissues with the exception of spleen and testis^{8,9}. It is upregulated in disease (e.g., in intramyocardial blood vessels and myocytes¹⁰ following infarction and in abdominal aortic aneurysms¹¹). Nox5 was detected in glomeruli of individuals with diabetic kidney disease but not in non-diabetic individuals and it was recently reported that Nox5 is found in renal proximal tubules of individuals with hypertension¹². Transgenic mice with vascular smooth muscle / mesangial cell Nox5 expression are susceptible to diabetic kidney injury, although BP was unaffected¹³. Work in other animal models suggest that NOX5 contributes to the pathophysiology of stroke. Mice with human Nox5 expression in the endothelium exhibit elevated blood pressure along¹⁵ with enhance stroke risk¹⁴. Furthermore, Genome wide association studies have suggested a role for NOX5 in hypertension.

Specifically, *NOX5* was identified as a putative blood pressure-associated gene being positively linked to elevated blood pressure¹⁵. Lastly, SNPs (e.g., T253M) in *Nox5* were identified that alter phosphorylation-dependent Nox5 activity¹³. Interestingly, T253M is a low-frequency SNP (0.37%), suggesting that it is not well tolerated, and it was limited to African Americans – who are at high risk for CKD. These studies imply that interventions that abrogate NOX5 activity could be both vasculoprotective and neuroprotective.

Along these line, the studies of Casas and colleagues in the April issue of *The Journal of Clinical Investigation* provide new evidence that Nox5 is sufficient to impair recovery from occlusion-mediated ischemic stroke. They employ both *in vitro* and *in vivo* approaches to support this novel hypothesis.

For *in vivo* studies, since Nox5 is absent from the rodent genome, this group engineered an elegant humanized mouse model wherein Nox5 is knocked into the *Hprt* locus and placed under the endothelium-specific *Tie2* promoter. The result is a mouse line that specifically expresses Nox5 in the vascular endothelium.

These mice were subsequently subjected to a model of stroke by a transient occlusion of the middle cerebral artery. Upon reperfusion, the blood brain barrier typically becomes abnormally permeable (leaky). This impairment is known to be both Ca²⁺ and ROS-dependent which are thought to disrupt tight junction maintenance in the blood brain barrier.

As Nox5 is activated by increased intracellular calcium levels, it was appropriate that Nox5-knockin mice exhibited significantly worsened blood brain barrier leakage as compared to wild type controls. Such blood brain barrier injury was accompanied by excessive poststroke ROS formation, infarct size, and worsened neuromotor performance. For *in vitro* studies, cultures of human brain microvascular endothelial cells were subjected to hypoxic conditions followed by reoxygenation and cell permeability assessed. A Nox5-specific inhibitor (ML090) was protective in this setting thereby implicating this Nox family member at a cellular level.

While these informative studies suggest a role for Nox5 in limiting recovery of the permeability of the blood brain barrier under conditions of post-ischemic stroke, a number of new questions and avenues for investigation have emerged. Importantly, whether such Nox5-dependent injury occurs in human patients remains to be determined. While the ROS-dependent blood brain injury and infarct sizes were substantial, the question of whether the expression level of Nox5 was reflective of physiological levels remains. Furthermore, the determinants of Nox5 regulation in the brain endothelium—namely at the level of its expression and activity await identification. As for new avenues, this important study opens the door and provides further impetus for the development of novel therapeutic compounds which specifically target the Nox5 isoform. Up until now, most pan-specific antioxidants and non-selective Nox inhibitors have been largely ineffective in reducing disease progression in a number of contexts. Could this be a “Nox at the door” ushering in a new therapy using yet to be developed, highly selective Nox5 inhibitors to improve neuroprotection and recovery from conditions such as stroke?

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