Neuroprotection in Ischemic Stroke: AhR We Making Progress?

Running title: Padmanabhan et al.; L-Kyn-AhR pathway in ischemic stroke

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Cerebrovascular accidents, more commonly referred to as “strokes,” remain the second most common cause of mortality and the third most common cause of disability worldwide. It has been estimated that more than three quarters of all strokes in the United States are due to ischemia, with the remainder principally related to intracranial hemorrhage. While our attempts to address modifiable risk factors for stroke have decreased its incidence, stroke-related death and disability are increasing at concerning rates. These observations lie in stark contrast to contemporary metrics for acute myocardial infarction (MI), where both short- and long-term mortality have been decreasing over the past 30 years, concomitant with the increasing use of prompt reperfusion strategies including primary percutaneous coronary intervention (PCI). The cell types affected in the context of stroke and MI (neurons and cardiomyocytes, respectively) are similar in that both are terminally differentiated cells that are exquisitely sensitive to ischemic insults. Following the adage that “time is brain” in the setting of acute ischemic stroke, the benefit of restoring blood flow using thrombolytic therapy decreases in a continuous fashion over time, with studies demonstrating that each 15-minute delay in the time to initiation of therapy is associated with reduced odds of independent ambulation and survival. However, compared with coronary revascularization, the temporal window for safe and effective cerebral reperfusion therapy is exceedingly narrow and complicated by the challenges associated with identifying stroke symptoms and obtaining rapid neuroimaging, issues which can further delay rapid triage and initiation of treatment. As such, there remains a huge unmet need for the development of novel therapeutic strategies for ischemic neuroprotection to improve outcomes in acute stroke. Towards that end, elucidating the precise molecular mechanisms that govern neuronal susceptibility to ischemia is a critical step in identifying pathways that may ultimately be amenable to pharmacologic manipulation for patients with stroke.
In this issue of *Circulation*, Cuartero *et al.* \(^1\) use mouse models of stroke to demonstrate that a signaling pathway involving the tryptophan metabolite L-kynurenine (L-kyn) and the aryl hydrocarbon receptor (AhR) are important mediators of ischemic neuronal damage that can be pharmacologically manipulated *in vivo*. AhR is a member of the Per-Arnt-Sim family of basic helix–loop–helix transcription factors that is activated by a range of structurally divergent ligands, including environmental pollutants (e.g. dioxins) and the endogenous tryptophan catabolite L-kyn \(^11,12\). AhR is normally inactive under basal conditions and retained in the cellular cytoplasm bound to several chaperone proteins. Upon ligand binding, AhR translocates to the nucleus and heterodimerizes with the AhR nuclear translocator (ARNT) to bind DNA and alter gene expression \(^13\). Endogenous AhR signaling has been shown to play important roles in cardiovascular development and physiology as well as in the modulation of inflammatory signals \(^14,15\). Previous work has also demonstrated that ablation of AhR results in enhancement of ischemia-induced angiogenesis \(^16\). As noted above, L-kyn (a byproduct of the tryptophan degrading tryptophan-2,3-dioxygenase (TDO) enzyme), was recently discovered to be an endogenous AhR ligand that plays important roles in cancer pathobiology and immune activation \(^12\).

Based on this rationale, Cuartero *et al.* used *in vitro* and *in vivo* models to test the hypothesis that the L-Kyn-AhR signaling pathway potentiates acute ischemic brain injury. They found that AhR protein abundance, nuclear translocation, and transcriptional activity in cortical neurons are all increased in a mouse model of stroke induced by middle cerebral artery occlusion (MCAO). Using pharmacologic AhR inhibitors and activators, as well as mice with genetic *Ahr*-deficiency, they demonstrate that AhR is an important mediator of acute ischemic damage during MCAO. Mechanistic studies suggest that AhR activation during ischemia may mediate certain
pathologic effects via inhibition of CREB signaling (cAMP response element binding protein). The authors go on to demonstrate that L-kyn accumulates in the brain during acute ischemia where it functions as an endogenous activator of AhR. Exogenously administered L-kyn exacerbates stroke, as assessed by infarct volume, in an AhR-dependent fashion. Perhaps most intriguingly, the authors also demonstrate that inhibition of L-kyn production via pharmacologic blockade of TDO decreased AhR activation and reduced infarct volume after MCAO. Taken together, these studies implicate the L-Kyn-AhR pathway as a novel mediator of brain damage during stroke and identify TDO and AhR as new “druggable” targets in this disease (Figure 1).

The work of Cuartero and colleagues raises a number of interesting questions that have important translational implications for cerebrovascular disease. While the authors demonstrate that L-Kyn functions as an endogenous activator of AhR, they also find increased protein abundance of AhR during ischemia. Are these additional mechanisms governing AhR accumulation potential targets for ischemic neuroprotection? Secondly, this study uses systemic delivery of L-Kyn or TDO inhibitors in the MCAO model of stroke. It will be important to understand which cell types are responsible for producing L-kyn during cerebral ischemia, as a significant quantity of this metabolite is produced by the liver. Mouse models harboring a conditional allele of TDO may prove useful in this regard. Given the link between tryptophan metabolism, AhR and immune cell activation, the current observations in the brain raise the possibility that similar pathways may be involved in other cardiovascular conditions (e.g. atherogenesis, myocardial infarction, heart failure) or neurodegenerative disorders (e.g. Alzheimer’s disease). Thirdly, what pathways downstream of AhR are responsible for mediating ischemia-induced neurotoxicity? While repression of CREB signaling is certainly plausible, AhR may well be working through multiple mechanisms. Unbiased approaches to identify AhR-
mediated effects in neurons coupled with studies of neuron-specific AhR deletion will be particularly informative. This work also begs the epidemiological question of whether environmental dioxin exposure contributes to stroke risk. Studies of individuals exposed to agent orange, a herbicide used extensively during the Vietnam War and known to be contaminated with the dioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), may be informative in this context. Finally, apart from L-kyn, are there other yet uncharacterized endogenous or exogenous AhR ligands that confer stroke risk? Conversely, are there any endogenous inhibitors of the AhR pathway? Future work that builds on the index observations of Cuartero and colleagues may elucidate additional therapeutic strategies for the prevention and treatment of stroke.

Providers caring for patients suffering from acute stroke are rarely afforded the opportunity to intervene immediately after the onset of ischemia. While this study provides proof of principle that early inhibition of the L-Kyn-AhR axis confers neuroprotection in the setting of ischemic stroke, the most pressing question that arises is whether pathway blockade at a later time point remains efficacious. Furthermore, how does manipulation of this pathway interact with reperfusion therapies and could this strategy potentially expand the relatively limited window during which cerebral revascularization is effective? Ultimately, the questions as to whether such observations in mouse models such as MCAO eventually translate in to larger mammals or humans remain a major challenge for the field. Despite these long-term hurdles, this work represents an exciting new inroad for the development of novel neuroprotective strategies and suggests that we indeed are making progress.

**Conflict of Interest Disclosures:** None.
References:


**Figure Legend:**

**Figure 1.** The L-Kyn-AhR signaling pathway mediates neuronal susceptibility to ischemia.

Using the MCAO mouse model of stroke, the authors demonstrate that genetic Ahr deficiency, pharmacologic AhR inhibition or inhibition of L-Kyn production (via pharmacologic inhibition of TDO) all confer ischemic neuroprotection (left). On the right, a mechanistic framework for the current findings is provided. Abbreviations: AhR, Aryl hydrocarbon receptor; TDO, tryptophan-2,3-dioxygenase; CREB, cAMP response element binding protein.
Ischemia

L-kynurenine

AhR

Larger Stroke

Other Endogenous/Exogenous AhR Ligands?

Other Pathways?

CREB Signaling

TDO

AhR inhibitor

TDO inhibitor

AhR+/

AhR-/

L-kynurenine

drugs?

drugs?

Other Endogenous Exogenous AhR Ligands?
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