SIRT1 Activation: A Potential Strategy for Harnessing Endogenous Protection Against Delayed Cerebral Ischemia After Subarachnoid Hemorrhage

Ananth K. Vellimana, MD*
Deepti Diwan, PhD*
Julian Clarke, BS*
Jeffrey M. Gidday, PhD‡
Gregory J. Zipfel, MD*

*Department of Neurological Surgery, Washington University School of Medicine, St. Louis, Missouri; ‡Department of Ophthalmology, Louisiana State University Health Sciences Center, New Orleans, Louisiana

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Correspondence:
Ananth K. Vellimana, MD,
Department of Neurological Surgery,
Washington University School of Medicine,
660 S Euclid Avenue,
St. Louis, MO 63110.
E-mail: vellimana@gmail.com

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SECONDARY BRAIN INJURY AFTER SUBARACHNOID HEMORRHAGE

Aneurysmal subarachnoid hemorrhage (SAH) affects nearly 30 000 Americans every year and survivors experience significant morbidity, with nearly a third of them becoming functionally dependent. The 2 principal contributors to poor outcome after SAH are early brain injury (EBI) and delayed cerebral ischemia (DCI). EBI is initiated by the acute increase in intracranial pressure and consequent transient global cerebral ischemia that occurs after aneurysm rupture, and typically manifests in the first 72 h after ictus.[1] EBI is characterized by neuroinflammation, blood–brain barrier (BBB) breakdown, cerebral edema, and early neuronal cell death. DCI is multifactorial in etiology with large artery vasospasm being the primary contributor and other pathophysiological events including microvessel autoregulatory dysfunction, microvessel thrombosis, and neuroinflammation being significant contributors.[2-5] DCI typically occurs 4 to 14 d after ictus, and is characterized by delayed neurological decline with or without radiographic evidence of cerebral infarction.[5] Due to the stereotypical delay between ictus and DCI, a window of opportunity exists to institute a therapeutic intervention that can ameliorate the effects of DCI, or even prevent the occurrence of DCI. Given the multifactorial etiology of DCI, a therapeutic strategy that influences several of these etiological factors is needed to combat DCI and consequently improve outcome after SAH.

ABBREVIATIONS: BBB, blood–brain barrier; **DCI,** delayed cerebral ischemia; **DMH,** dorsomedial; **EBI,** early brain injury; **Enos,** endothelial nitric oxide synthase; **FOXO1,** Forkhead box O1; **FOXO3,** Forkhead box O3;; **HIF-1α,** hypoxia-inducible factor 1alpha; **LH,** lateral hypothalamic; **MMP-9,** metalloproteinase-9; **Nkx2-1,** Nk2 homeobox; **NPCs,** neural progenitor cells; **NSCs,** neural stem cells; **SAH,** subarachnoid haemorrhage; **SIRT1,** Sirtuin 1

ENDOGENOUS NEUROPROTECTION

Cells and tissues possess an innate ability to resist injury. The innate protective mechanisms can be activated by a process referred to as conditioning, wherein a sub-lethal quantity of a normally injurious stimulus is administered in a controlled manner.[6] The activated endogenous protective mechanisms reduce the severity of subsequent injuries and enhance reparative processes. This protective phenotype is achieved through a cascade of conditioning stimulus-induced cellular changes involving molecular sensors, transducers, and effectors that produces a coordinated response at the genomic, molecular, cellular, tissue, and organ levels.[7] In the central nervous system, conditioning-induced protection is mediated through changes occurring in neurons, glia, and cerebral vasculature.

ROLE OF ENDOGENOUS NEUROPROTECTION IN SAH

For a variety of reasons, DCI after SAH represents an ideal pathology for application of a conditioning-based strategy. First, despite decades of extensive research the incidence of DCI remains unacceptably high and there are no effective therapies to reduce the morbidity resulting from DCI, thereby creating the need for an unconventional approach to combat the problem. Second, the stereotypical delay between the ictus and onset of DCI provides a window of opportunity to intervene with a conditioning based stimulus. Third, the multifaceted protection afforded by conditioning can

be leveraged to target the multiple etiological factors contributing to DCI.

While prior review articles have suggested the suitability of SAH for application of a conditioning strategy,[8,9] our group provided the first experimental evidence demonstrating the protective effect of conditioning against DCI after SAH.[10] We demonstrated that a conditioning stimulus of hypoxia (8% oxygen for 4 h) administered to mice prior to experimental SAH provides robust protection against SAH-induced vasospasm and neurological dysfunction. Furthermore, our study also began unraveling the molecular mechanisms underlying conditioning induced protection and demonstrated that the protective effect of conditioning against SAH-induced DCI is mediated by activation of endothelial nitric oxide synthase (eNOS).[10]

In order to translate a conditioning strategy to clinical use in SAH patients, it is imperative to develop a conditioning stimulus or pharmacological agent that can activate innate protective mechanisms and target the various etiological factors implicated in the development of DCI, can be administered to humans, and is effective when administered after ictus. As an initial step towards this goal, our group subsequently demonstrated that conditioning with isoflurane, an inhalational anesthetic, administered at clinically relevant time points after SAH attenuated multiple components of DCI including cerebral vasospasm, microvessel thrombosis, and microvessel dysfunction, and improved neurological outcome after experimental SAH.[11] In that study, isoflurane conditioning-induced protection was found to be critically dependent on vascular endothelial hypoxia-inducible factor 1alpha (HIF-1α). However, administration of repetitive conditioning stimuli of isoflurane to SAH patients in an intensive care unit is practically difficult, thereby limiting its immediate translational potential. Simultaneously, Gonzalez and colleagues[12] examined remote ischemic limb preconditioning in SAH patients. Remote ischemic conditioning is a strategy wherein administration of a sub-lethal ischemic stimulus at one site confers protection against subsequent ischemia at a distal site. Their work has demonstrated that repetitive lower limb ischemic preconditioning is safe and largely well-tolerated in SAH patients.[12] Preliminary results from their work also suggest a positive effect of repetitive lower limb ischemic preconditioning on functional outcomes after SAH.[13] However, completion of a repetitive limb ischemic preconditioning protocol in the window of opportunity before the onset of DCI can be challenging and could limit its clinical utility, as evidenced by the low proportion (40%) of patients in whom the protocol was initiated in the first 72 h after ictus.[12]

Although the aforementioned preliminary studies have demonstrated a favorable effect of conditioning against DCI after SAH, the translational potential of a conditioning-based therapeutic strategy can be significantly enhanced if a pharmacologically targetable molecular pathway underlying conditioning-induced protection is identified. In this regard, our lab has recently identified Sirtuin 1 (SIRT1) as a possible candidate.

SIRTUINS

Sirtuins are a family of proteins in the category of class III histone deacetylases that play important roles in human epigenetic modification.[14] The mammalian sirtuin family consists of 7 proteins (SIRT1-SIRT7), all of which require the cofactor nicotinamide adenine dinucleotide (NAD+) for their deacetylase activity.[14-16] Sirtuins are expressed throughout the body, including tissues and organs such as heart, brain, kidney, liver, muscle, and adipose.[17,18] Sirtuins are master regulators of a diverse range of cellular processes such as gene expression, metabolism, telomere activity, differentiation, proliferation, DNA repair, senescence, and oxidative stress response.[19] Due to their reliance on NAD+ levels for activity, Sirtuins are considered to be cellular metabolic sensors, and their activity is modified by cellular energy levels.[20] This cellular energy sensing capability of Sirtuins permits them to act as metabolic regulators within a variety of different organ systems.[21]

Among the various Sirtuins, SIRT1 has been most extensively studied. In this review, we will focus on the physiological roles of SIRT1, discuss the evidence surrounding SIRT1 in the context of conditioning, and describe its suitability as a potential target to combat DCI after SAH. SIRT1 deacetylates multiple proteins involved in regulatory pathways, including tumor suppressor p53, Forkhead box O1 and 3 (FOXO1 and FOXO3), peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), liver X receptor, HIF-1 α, and hypoxia-inducible factor 2α.[22-31] As a result, it impacts numerous functions such as stress, inflammation, vessel function, and thrombosis. Importantly, the deacetylation of multiple regulatory proteins by SIRT1 exerts downstream effects on numerous other proteins which have been implicated in the pathophysiology of DCI including eNOS,[32] PARP-1,[33] matrix metalloproteinase-9 (MMP-9),[34] and tissue factor.[35]

SIRT1 IN THE CENTRAL NERVOUS SYSTEM

SIRT1 is located in most regions of the brain, including the cortex, cerebellum, hippocampus, and hypothalamus.[36] Within these areas, SIRT1 expression is mostly within neurons. However, it is also found in neural stem cells and neural progenitor cells (NSCs and NPCs), astrocytes, and microglia.[36-42]

Neurodevelopment, Learning, and Memory

SIRT1 plays a critical role in neurodevelopment and modulates both neurogenesis and gliogenesis. The highest levels of SIRT1 mRNA expression in the brain, spinal cord, and dorsal root ganglia are found as early as embryonic day 4.5, and a high level of expression is still present in the late embryonic stage at day 18.5.[42] SIRT1 influences neuronal structure at many levels, such as axonal growth, neurite growth, and dendritic sprouting, arborization, and complexity.[43-45] In adults, SIRT1 is important for the balance between differentiation and self-renewal of NSCs as evidenced by the elevated hippocampal

neurogenesis via increased proliferation of NSCs/NPCs in SIRT1 knockout mice,[46] and the reduced survival of neurosphere cultures in vitro and hippocampal NPCs in vivo with SIRT1 activation.[47] SIRT1 also exerts a similar effect on oligodendrocytes. SIRT1 inactivation leads to increase in oligodendrocyte progenitor cells and expansion of the oligodendrocyte lineage.[48] In addition to neurodevelopment, SIRT1 has been implicated in synaptic plasticity, learning, and memory. SIRT1-KO mice exhibit a decrease in dendritic branching and branch length, and brain-specific SIRT1-KO mice display reductions in the number of functional synapses and dendritic spine density.[45,49,50] Long-term potentiation in hippocampal CA1 neurons is impaired in SIRT1-KO mice, and these mice show functional deficits in associative memory, immediate memory, and spatial memory.[45,50]

Metabolism

The hypothalamus is a key regulator of metabolism, and SIRT1 mediates many hypothalamic functions. For example, SIRT1 deacetylates Nk2 homeobox 1 (Nkx2-1), a protein that regulates gene transcription, in the dorsomedial (DMH) and lateral hypothalamic (LH) nuclei.[51] Deacetylation of Nkx2-1 leads to the transcription of a promoter, orexin receptor type 2 (Ox2r), and enhances this promoter's activity.[51] This SIRT1/Nkx2-1/Ox2r-mediated pathway leads to neuronal activation that regulates physiological parameters such as physical activity, oxygen consumption, body temperature, and quality of sleep.[51] Moreover, this signaling pathway in the DMH and LH may function as a control center for mammalian aging and longevity, and mice overexpressing SIRT1, specifically in the brain, show a significant increase in life-span.[51] Another example highlighting the role of SIRT1 in systemic metabolic regulation is its regulation of pro-opiomelanocortin neurons of the hypothalamic arcuate nucleus. The lack of SIRT1 in these neurons reduces energy expenditure via alteration of leptin induced remodeling of white adipose tissue.[52,53]

SIRT1 IN CEREBRAL ISCHEMIA

Multiple studies have demonstrated a protective role for SIRT1 in various models of neuronal injury and neurodegeneration including cerebral ischemia.[38,54-57] Initial experiments utilizing an oxygen and glucose deprivation in vitro model of cerebral ischemia demonstrated that pharmacological preconditioning with Resveratrol, a nonspecific activator of SIRT1, protected hippocampal neurons against ischemia and this protection was lost when cultured neurons were pretreated with the SIRT1 inhibitor Sirtinol.[58-61] Subsequent in vivo studies found that resveratrol-conditioning induced protection against cerebral ischemia is effective at early (24 h) and delayed (>7 d) time points after injury. This protection against cerebral ischemia has subsequently been observed in multiple experimental models including ischemic stroke, recurrent stroke, neonatal hypoxia-ischemia, and asphyxia cardiac arrest.[58,60,62,63] More recent studies utilizing mice with genetically manipulated SIRT1 have more definitively established the protective role of SIRT1 activation against cerebral ischemia. *Sirt1*$^{-/-}$ mice were found to have larger infarct volumes and worse neurological outcome after experimental ischemic stroke.[64] In contrast, mice overexpressing SIRT1 had preserved cerebral blood flow following bilateral common carotid artery stenosis and bilateral common carotid artery occlusion.[64-66]

POTENTIAL ROLE FOR SIRT1 IN CONDITIONING-INDUCED PROTECTION AGAINST DCI

Recent evidence indicates that hypoxic conditioning increases SIRT1 expression.[67] SIRT1 is an established regulator of various molecular pathways that have been implicated in the pathophysiology of EBI and DCI. For example, an increase in SIRT1 expression has been shown to increase the catalytic activity of eNOS resulting in increased nitric oxide (NO) synthesis,[32,68] both of which have been strongly implicated in the pathophysiology of DCI[69] and were shown in our prior study to be essential for hypoxic conditioning induced protection against DCI.[10,68] This suggests that SIRT1 activation could mimic hypoxic conditioning-induced protection against neurovascular dysfunction after SAH.

In addition to the downstream effects on eNOS and NO, SIRT1 also regulates other cellular pathways implicated in the pathophysiology of secondary brain injury after SAH. SIRT1 downregulates matrix MMP-9,[33] a member of the zinc-dependent endoprotease family that has been demonstrated to play a critical role in the pathogenesis of EBI by contributing to BBB breakdown, cerebral edema, and neuronal cell death,[70-73] and in the pathogenesis of DCI by contributing to large artery vasospasm, and microvessel thrombosis.[74] Recent studies also suggest that an increase in SIRT1 expression may directly protect against vascular thrombus formation as SIRT1 inhibition enhances tissue factor, a key trigger of the coagulation cascade.[35] This suggests a potential for SIRT1 activation to attenuate microvessel thrombosis after SAH. Moreover, SIRT1 activation also promotes cell survival under stress by a variety of mechanisms including inactivation of PARP-1, a key component of cell signaling pathway that promotes neuronal cell death after cerebral ischemia,[75] deacetylation of p53 and consequent inhibition of p53 mediated cell death, augmentation of FOXO3-induced cell cycle arrest and resistance to oxidative stress, among others. In addition to its direct effects on multiple intracellular signaling pathways, SIRT1 activation has also been implicated in stimulating ischemic tolerance by stabilizing HIF1α, which we have previously demonstrated to be a mediator of isoflurane conditioning induced protection against DCI.[76-78]

CONCLUSION

Activation of endogenous protective mechanisms is a multifaceted strategy that could attenuate secondary brain injury after SAH by targeting multiple components of EBI and DCI. Various conditioning stimuli with limited direct translational ability have demonstrated preclinical efficacy in activating endogenous protective mechanisms to combat SAH-induced neurovascular dysfunction. In order to enhance the translational potential of a conditioning strategy, it is imperative to identify a pharmacologically targetable cellular mediator of conditioning induced protection. SIRT1 is a cellular energy sensor with known activation after hypoxic conditioning that functions as a master regulator and is capable of favorably influencing various molecular pathways, many of which have been implicated in the pathophysiology of EBI and DCI after SAH. Preclinical studies examining the efficacy of SIRT1 activation in attenuating secondary brain injury after SAH are therefore desperately needed.

Disclosures

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