PERSPECTIVE

Current AQP research: therapeutic approaches to ischemic and hemorrhagic stroke

The role of aquaporins (AQPs) in the formation of cerebral edema after stroke: Stroke can be classified into either ischemic or hemorrhagic based on their etiological mechanism. Cerebral edema development often accompanies both ischemic infarct and intracerebral hemorrhage (ICH). Cerebral edema is differentiated according to their underlying mechanism and time course: cytotoxic and vasogenic. Currently, the only approved therapies for treating cerebral edema include decompressive craniectomy and osmotherapy, which both aim to alleviate the downstream effects, rather than addressing the molecular mechanisms underlying edema development (Stokum et al., 2015).

Aquaporins (AQPs) are a class of water channel proteins which have been implicated in the regulation of both physiological and pathological water homeostasis, and represent a promising target for alleviating stroke-induced cerebral edema. Thus far, 13 mammalian AQPs have been identified to be heterogeneously expressed in various tissues. Seven types of AQPs (AQP1, AQP3, AQP4, AQP5, AQP8, AQP9, AQP11) have been found to be expressed in the mammalian central nervous system (CNS), with AQP4 being the dominant AQP channel present in the mammalian brain (Vella et al., 2015).

AQP4 is a bidirectional water-specific channel which is primarily concentrated within the glial limitans and astrocytic endfeet in association with the vasculature at the division between the brain parenchyma and major fluid compartments, such as the blood-brain barrier (BBB). AQP4 plays a role in mediating water influx during the manifestation of edema as well as regulating water efflux during clearance. AQP4 expression is both spatially and temporally regulated based on the type of stroke model, with AQP4 downregulation noted in cytotoxic edema and an upregulation observed at the onset of vasogenic edema, potentially serving to accelerate water clearance (Stokum et al., 2015). AQP4 upregulation plays a significant role in the formation of vasogenic edema following both hemorrhagic and late ischemic stroke. Cytotoxic edema, which appears during the early stages of ischemic stroke, is correlated with a downregulation of AQP4 expression (Ribeiro Mde et al., 2006; Zhao et al., 2016), while AQP1 and AQP9 are both upregulated following ICH (Wang et al., 2015).

ICH is typically associated with the development of vasogenic edema. In cases of ischemic stroke, edema formation proceeds in a cascade, wherein cytotoxic edema manifests during the first few hours following the ischemic insult. Subsequent prolonged periods of ischemia can prompt the breakdown of the BBB and the hemorrhagic conversion of ischemic tissue, resulting in a progression from cytotoxic to vasogenic edema. Cytotoxic edema often occurs in the early stages of ischemic stroke with AQP4 downregulation. Cytotoxic cerebral edema manifests as AQP4 facilitates water passage into the astrocyte compartment, causing cellular swelling and disruption of the basal lamina. The downregulation of AQP4 at this stage may be a response to counteract the influx of water and resultant cerebral swelling. In cases of hemorrhagic stroke and the late stages of ischemic stroke, BBB disruption results in vasogenic edema. Water accumulates in the extracellular space (ECS) after exiting the leaky capillaries. Concurrently, AQP4 channels in the astrocyte endfeet are upregulated, which gradually facilitates the removal of extracellular fluid.

The therapeutic approaches to ischemic and hemorrhagic stroke: Due to their integral role in brain edema formation and resolution, AQPs are promising therapeutic targets to mitigate the damage of ischemic and hemorrhagic stroke. However, no studies have clearly demonstrated how different treatments specifically target ischemic stroke and hemorrhagic stroke. AQP4 is especially attractive because of its prime location for water exchange, between the brain parenchyma and the circulatory system. Clinical utilization of AQP4 modulators is complicated by the bimodal role that AQP4 plays in stroke progression. AQP4 inhibitors may be beneficial during the early stages of stroke, but could be deleterious if administered during the later stages when the clearance of water from the brain into the vasculature is crucial (Tang et al., 2010).

Several studies have shown that endogenous hormone levels can modulate AQP4 expression. Animal models of stroke and brain edema have indicated arginine vasopressin V1 (AVPV1), erythropoietin, estrogen, progesterone, melatonin, and thyroid hormone as possible targets.Thyroid hormone (T3 [3,3′,5-triiodo-L-thyronine] and T2 [3,5-diiodo-L-thyronine]) has been observed to confer neuroprotective effects *via* AQP4 modulation (Ambrosius et al., 2011). Low T3 is a predictor of worse outcomes following stroke, suggesting that exogenous supplementation of thyroid hormone may be a promising neuroprotective therapy for the treatment of ischemic stroke. Melatonin has also been observed to confer neuroprotective effects through the inhibition of AQP4 in models of early cerebral ischemia (Borlongan et al., 2000). Melatonin, with small size and high lipophilicity allow for crossing of the BBB, is highly beneficial in the development of neurotherapeutics. And melatonin may alleviate cytotoxic cerebral edema by acting as an activator of PKC, and thus by promoting AQP4 inhibition indirectly (Bhattacharya et al., 2014). Bumetanide has been used in the experimental treatment of various neurological disorders. Despite promising preclinical data, there are encountered problems, including poor BBB penetration and adverse systemic side effects: diuresis, hypokalemic alkalosis, and hearing loss (Tollner et al., 2015). Piroxicam, a non-steroidal anti-inflammatory drug (NSAID), has been reported to inhibit AQP4 expression and resultant edema formation in models of cerebral ischemia/reperfusion injury.

Recent studies have shown that the effects of precondition-

ing are often mediated by AQP4 expression (Gidday, 2015). Chemical preconditioning has been explored in recent years with studies using thrombin and 3-nitroproprionic acid. Low-doses of these substances delivered over weeks prior to middle cerebral artery occlusion greatly attenuated the subsequent ischemic event and reduced AQP4 levels (Hoshi et al., 2011). Hyperbaric oxygen preconditioning has also been proposed as a means of alleviating cerebral edema *via* AQP4 inhibition (Fang et al., 2015).

Remote ischemic post-conditioning, an alternative noninvasive neuroprotective approach, has been purported to suppress AQP4 expression, and thus limit edema formation in models of focal cerebral ischemia (Qi et al., 2016). Employing hindlimb clamping to occlude blood flow post-middle cerebral artery occlusion resulted in fewer AQP4-positive cells and reduced AQP4 mRNA levels at 24 hours compared to controls. Additionally, remote ischemic post-conditioning suppressed activation of the transcription factor nuclear factor-κB (NF-κB), suggesting that the therapy may modulate AQP4 expression by downregulating the NF-κB pathway (Qi et al., 2016).

Another approach to mitigating the damage of ischemic stroke is to induce a hibernation-like state of low metabolic activity. Recent studies revealed that ethanol decreases cerebral edema and improves BBB integrity following ischemic stroke (Zeng et al., 2012; Peng et al., 2013). In addition, therapeutic hypothermia (30–34°C) resulted in increases AQP4 mRNA and protein levels after ICH (Gao et al., 2015; Kim and Yenari, 2015).

Future directions: The therapies for treating cerebral edema currently aim to alleviate the effects of AQP. Although many of the agents presented here have seen success in experimental models, further research is necessary to assess their efficacy and to identify any unwanted side effects which may manifest because of therapy administration. Additionally, ischemic and hemorrhagic strokes should be considered in the development of therapies targeting AQP, which may enhance this effort.

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***orcid:** 0000-0001-8169-8153 (Jessie NW. Ding)*
***doi:** 10.4103/1673-5374.197128*
***How to cite this article:** Ma L, Guan L, Ding JN, Geng X (2016) Current AQP research: therapeutic approaches to ischemic and hemorrhagic stroke. Neural Regen Res 11(12):1918-1919.*

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