PERSPECTIVE

Recent advances and future directions in preclinical research of arginine-vasopressin (AVP) receptor blocker conivaptan in the context of stroke

Stroke is a major cause of mortality and permanent disability. The onset of stroke is followed by life-threatening pathophysiological responses including brain edema, elevation of intracranial pressure, disruption of blood-brain barrier (BBB), brain infarct and permanent tissue damage. Brain edema develops due to accumulation of water in intracellular and extracellular compartments of the brain, which causes an increase in brain volume and elevation of intracranial pressure, (**Figure 1A**). Compression of the brain tissue has an impact on cerebral blood flow which results in secondary brain injury. Often, clinical presentation of stroke is accompanied by hyponatremia caused either by the syndrome of inappropriate release of antidiuretic hormone (SIADH) or cerebral salt wasting (CSW) (Saleem et al., 2014). SIADH is the result of uncontrolled secretion of antidiuretic hormone (ADH) also called arginine-vasopressin (AVP). AVP acts on V1a and V2 receptors triggering vasoconstriction, platelet aggregation, and water retention followed by hypervolemic or normovolemic hyponatremia and low plasma osmolality (Saleem et al., 2014) (**Figure 1A**). CSW is manifested by a dramatic loss of sodium through the kidneys resulting in hypovolemic hyponatremia (Saleem et al., 2014). Both SIADH and CSW can quickly exacerbate post-ischemic brain edema if plasma sodium levels and osmolality are not properly managed and corrected in a patient. Because SIADH can often be confused with CSW the treatments have to be strategized carefully. Although pathophysiological mechanisms of SIADH-caused hyponatremia are identified, mechanisms of CSW-induced hyponatremia are unknown. Current therapeutic applications against brain edema (such as decompression craniotomy or hypertonic saline) do not fully address the complications caused by SIADH or CSW. Therefore, new research approaches are required to identify and target precise causes of stroke-induced brain edema.

Many studies report that AVP receptors (V1a and V2) mediate cascades of events that result in vasoconstriction, platelet aggregation, water retention and hyponatremia (Holmes et al., 2003, Saleem et al., 2014). V1a receptors are found primarily in vascular smooth muscle cells and platelets, and activation of V1a receptors by AVP induces vasoconstriction and platelet aggregation (Holmes et al., 2003). Therefore, blocking of V1a receptors after ischemic brain injury may potentially prevent vasoconstriction and clot formation, which may also improve regional cerebral blood flow (rCBF), and lower the magnitude of brain edema (**Figure 1B**). V2 receptors are localized in the collecting ducts of the kidneys, and activation of V2 receptors by AVP leads to increased water reabsorption and reduction of plasma sodium and osmolality (Holmes et al., 2003). Pharmacological inhibition of V2 receptors has been shown to increase water secretion (aquaresis) in the kidneys, raising sodium concentration in the blood (Li-Ng and Verbalis, 2009; Zeynalov et al., 2015) and thus may help in controlling brain edema.

We have recently demonstrated that conivaptan, an AVP receptor blocker, reduces brain edema and BBB breakdown in mice after stroke (Zeynalov et al., 2015). Conivaptan is an antagonist of V1a and V2 receptors which is available for clinical setting to treat patients with hypervolemic and normovolemic hyponatremia (Li-Ng and Verbalis, 2009). The V1a receptor blocking feature of conivaptan may provide vasodilatory effects on cerebral microvessels, which can potentially benefit regional cerebral blood flow (rCBF) near the ischemic region of the brain after stroke. Physiological factors, such as regulation of rCBF and BBB, are greatly influenced by ischemic brain injury. Improved rCBF may facilitate better outflow of the blood and interstitial fluid from the ischemic regional leviating the formation of brain edema, and may also contribute to faster recovery of the brain after ischemic insult. These effects of conivaptan are yet to be demonstrated in clinical or in laboratory settings. A published study showed that selective V1a receptor blockers can improve rCBF after subarachnoid hemorrhage (SAH) in rats (Hockel et al., 2012). In this study, authors use the model of endovascular puncture to induce SAH followed by intravenous delivery of a specific V1a receptor blocker. Monitoring of physiological parameters during the treatment revealed that the V1a receptor blocker can reduce intracranial pressure, rCBF, and mean arterial blood pressure and prevent re-bleeding after SAH. In addition, the V1a receptor blocker also showed anti-edema properties, improved neurological deficits and reduced mortality after SAH. Thus, the prominent advantage of the V1a receptor blocking effect on cerebral vasculature after SAH may be relevant in the context of ischemic stroke. Another study by Rauen and colleagues shows that mice which lack V1a receptor are protected against secondary brain injury after traumatic brain injury (TBI) (Rauen et al., 2013). In this study V1a receptor knock-out mice exhibited smaller brain edema induced by controlled cortical impact (CCI), which was also confirmed by an improved neurological dysfunction. Therefore, targeting of V1a receptors is proven to be a promising strategy in therapeutic intervention against TBI-induced brain injury. Follow-up physiological studies are necessary to explore whether conivaptan is able to improve rCBF after stroke or TBI. It is likely that this unexplored feature of conivaptan can enhance its potential for future clinical trials of stroke and brain edema. A single case report in the literature demonstrated that conivaptan can be promising as an alternative drug to treat brain edema after cerebrovascular accident (Hedna et al., 2014). In this case, a patient suffered a ruptured vertebral artery aneurysm which extended into the basilar circulation. Brain edema was detected in the midbrain and thalamic regions post-hemorrhage. After unsuccessful conventional anti-edema therapy, administration of conivaptan reduced brain edema in the previously swollen regions as well as clinical improvements without significant adverse effects. Thus, the authors demonstrated that conivaptan may be safely administered to reduce brain edema in clinical settings. Although this clinical study revealed the new protective property of conivaptan, more mechanistic preclinical

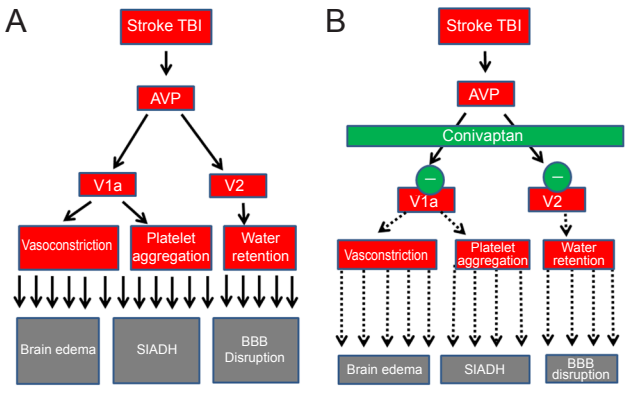

Figure 1 V1a and V2 receptor blocker conivaptan can prevent secondary brain injury after stroke and traumatic brain injury (TBI).
Stroke or TBI results in release of arginine-vasopressin (AVP) followed by V1a and V2 receptor activation. V1a and V2 receptors induce vasoconstriction, platelet aggregation, and water retention in the body. These pathophysiological events can exacerbate brain edema, trigger syndrome of inappropriate release of anti-diuretic hormone (SIADH), and increase blood-brain barrier (BBB) disruption (A). Conivaptan reverses brain edema, SIADH and BBB disruption caused by stroke or TBI (B).

studies are required to explore underlying molecular pathways of this anti-edema effect of the drug.

The molecular mechanisms of V1a receptor blocking effects of conivaptan have not been studied in the brain. However, we have demonstrated that the V2 blocking effect of conivaptan is responsible for excretion of excess water by the kidney and elevation of blood osmolality (Zeynalov et al., 2015), which could potentially help prevent development of brain edema. It has not been established whether V2 receptor exists in the brain. We also reported in our study that orally delivered tolvaptan, a selective V2 receptor blocker, has no beneficial role for post-ischemic brain edema. The oral administration route for tolvaptan, used according to the manufacturer's recommendation, produced the expected aquaretic effect and was sufficient to increase plasma sodium levels. However, tolvaptan failed to increase plasma osmolality overall (Zeynalov et al., 2015). This result suggests that osmolality *per se* may be important in the mechanisms involved in the genesis and/or resolution of cerebral edema. Based on our current results, we speculate that the V1a blocking component of conivaptan is perhaps crucial for protection against brain edema and BBB disruption, but the importance of the V2 blocking component of conivaptan requires further study.

It has been shown that the aquaporin-4 (AQP-4) in the brain plays an important role in reduction of brain edema and preservation of BBB after stroke (Zeynalov et al., 2008). AVP has a significant influence on the role of AQPs in water movement across membranes both in the brain (Liu et al., 2010) and the kidneys (Deen et al., 1994; Fenton and Moeller, 2008). The experimental studies of selective V1a receptor blockers in stroke, intracerebral hemorrhage (ICH), or TBI indicate that there is an association between AQP-4 expression and protection induced by these drugs (Liu et al., 2010; Manaenko et al., 2011; Kleindienst et al., 2013). In the study by Liu and colleagues, the authors use the filament stroke model (MCAO) in mice and the intracerebroventricular delivery route for V1a or V2 receptor blockers. The study reports that the V1a receptor blocker treatment reduced stroke-induced infarct and brain edema, and these beneficial events were linked to an upregulation of AQP-4 expression in the brain. The intracerebroventricular treatment with V2 receptor blocker after stroke in mice failed to produce protective effects on infarct volume and brain edema. Manaenko and colleagues investigated the V1a receptor blocking effect on neurological outcomes after ICH in mice (Manaenko et al., 2011). ICH was induced by collagenase injection into the brain and followed by treatment with the experimental V1a receptor blocker SR49059. Neurobehavioral testing, evaluation of brain edema and BBB disruption suggested that the V1a receptor blocker is protective against collagenase-induced ICH. This protection coincided with AQP-4 upregulation in the brain. Kleindienst and colleagues produced TBI in rats by the CCI model (Kleindienst et al., 2013). They used the same experimental V1a receptor blocker (SR49059) after the injury and observed that the drug was protective against TBI-induced brain edema in rats. This protection also correlated with AQP-4 upregulation in the brain.

Regardless of the brain injury model, all studies report strong protective properties of V1a receptor blockers against brain edema. However, the role of V2 receptors in cerebrovascular physiology remains unclear. It has been demonstrated that conivaptan, being a blocker ofboth V1a and V2 receptors, influences AQP-2 in the kidneys (Li-Ng and Verbalis, 2009). Interaction between the renal tubular V2 receptors and AQP-2 has been reported to be necessary for the function of V2 receptors (Deen et al., 1994). It is yet to be demonstrated if conivaptan's protective effects against brain edema and BBB disruption are linked to AQP-4 in the brain. If interactions between AVP receptors and AQP-4 are confirmed in the brain, this finding will greatly advance neuroscience and open new avenues for medical research in the field of brain edema research.

Our recently published study (Zeynalov et al., 2015) offers the mixed V1a and V2 receptor blocker conivaptan as an alternative or, possibly supplemental, drug to the currently used therapeutic approaches to treat stroke-evoked brain edema. Results of our research can yield important knowledge about the role of the AVP system in the pathophysiology of stroke-induced brain edema. This study will also assist translation of experimental results into clinical practice, help improve therapeutic approaches, and address existing challenges in clinical management of stroke.

In summary, the AVP receptor blocker conivaptan may have a potential for alleviating post-ischemic brain edema and minimizing BBB disruption in patients after stroke. Although effects of conivaptan on vascular physiology are yet to be demonstrated, strong evidence suggests that blocking both V1a and V2 receptors improves overall recovery after ischemic brain injury regardless of SIADH presence. Extensive preclinical studies of conivaptan in the context of stroke and brain edema will provide a complete spectrum of newly discovered features of the drug and its role in pathophysiology of stroke.

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