

The role of aquaporin-4 in cerebral water transport and edema

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✓Despite decades of research into the pathogenesis of cerebral edema, nonsurgical therapy for brain swelling has advanced very little after more than half a century. Recent advancements in our understanding of molecular water transport have generated interest in new targets for edema therapy. Aquaporin-4 (AQP4) is the primary cellular water channel in the brain, localized to astrocytic foot processes along the blood–brain barrier and brain–cerebrospinal fluid interface. Multiple studies of transgenic mice with a complete deficiency or altered expression of AQP4 suggest a prominent role for AQP4 in cerebral water transport. In models of cellular (cytotoxic) edema, AQP4 deletion or alteration has been shown to be protective, reducing edema burden and improving overall survival. In contrast, AQP4 deletion in extracellular (vasogenic) edema results in decreased edema clearance and greater progression of disease. The data strongly support the conclusion that AQP4 plays a pivotal role in cerebral water transport and is an essential mediator in the formation and resorption of edema fluid from the brain parenchyma. These findings also suggest that drug therapy targeting AQP4 function and expression may dramatically alter our ability to treat cerebral edema.

KEY WORDS • aquaporin • cerebral edema • cytotoxicity • vasogenic edema • water channel

THE IMPORTANCE OF cerebral edema in the progression of neurological disease has long been recognized in neurosurgical literature and clinical practice.³³ Edema is associated with almost all intracranial lesions, including tumors, spontaneous hemorrhage, infections, and traumatic injuries.⁸ The expanding volume of swelling intracranial contents within a rigid skull leads to a rise in ICP, resulting in secondary injury from decreased cerebral blood flow and herniation. Klatzo¹² first differentiated brain edema into two main subtypes based on their pathophysiology. He described cytotoxic edema, caused by “a noxious factor directly affect[ing] the stranded elements of the parenchyma producing intracellular swelling” (now better known as cellular edema) and vasogenic edema caused by cerebral vessel injury leading to “escape of water and plasma constituents” into the brain parenchyma, also known as extracellular edema. His description of the causative factors underlying the two primary forms of cerebral edema was the foundation of our current understanding of their pathophysiological mechanisms. Klatzo hypothesized that these two mechanisms of swelling usually coexisted; however, the current data now suggest that one form tends to predominate over the other, depending on the underlying lesion and stage of disease progression. Nonetheless, the concept of intracellular/cytotoxic and ex-

tracellular/vasogenic edema remains the framework for much of the research into new therapies for cerebral edema.

Unfortunately, despite our current understanding of the pathogenesis of brain edema after decades of research, little advancement has been made in developing actual clinical therapies for edema. The primary therapies, including CSF diversion, hyperosmotic therapy, and corticosteroids, have been the standards of clinical practice for more than half a century.¹⁵ Although many other areas of medicine and neurosurgery have benefited from advances in genetically and molecularly targeted therapies, application of these principles to the treatment of cerebral edema remains in its infancy. Recently the presence of molecular water channels that facilitate the rapid transport of water across cell membranes has been identified in multiple organ systems, including the brain.^{1,6} This family of membrane water transporters, named AQPs, offer a potential new target for cerebral edema therapy, and as such have generated great interest among researchers of brain water physiology. In this paper we review the most recent functional data on the role of AQPs in cellular and extracellular edema from *in vitro* and *in vivo* laboratory experimentation.

Aquaporins

Aquaporins are a family of transmembrane channel proteins that are highly selective for the transport of water. These proteins function as bidirectional channels, facili-

Abbreviations used in this paper: AQP = aquaporin; BBB = blood–brain-barrier; CSF = cerebrospinal fluid; ICP = intracranial pressure; MCA = middle cerebral artery.

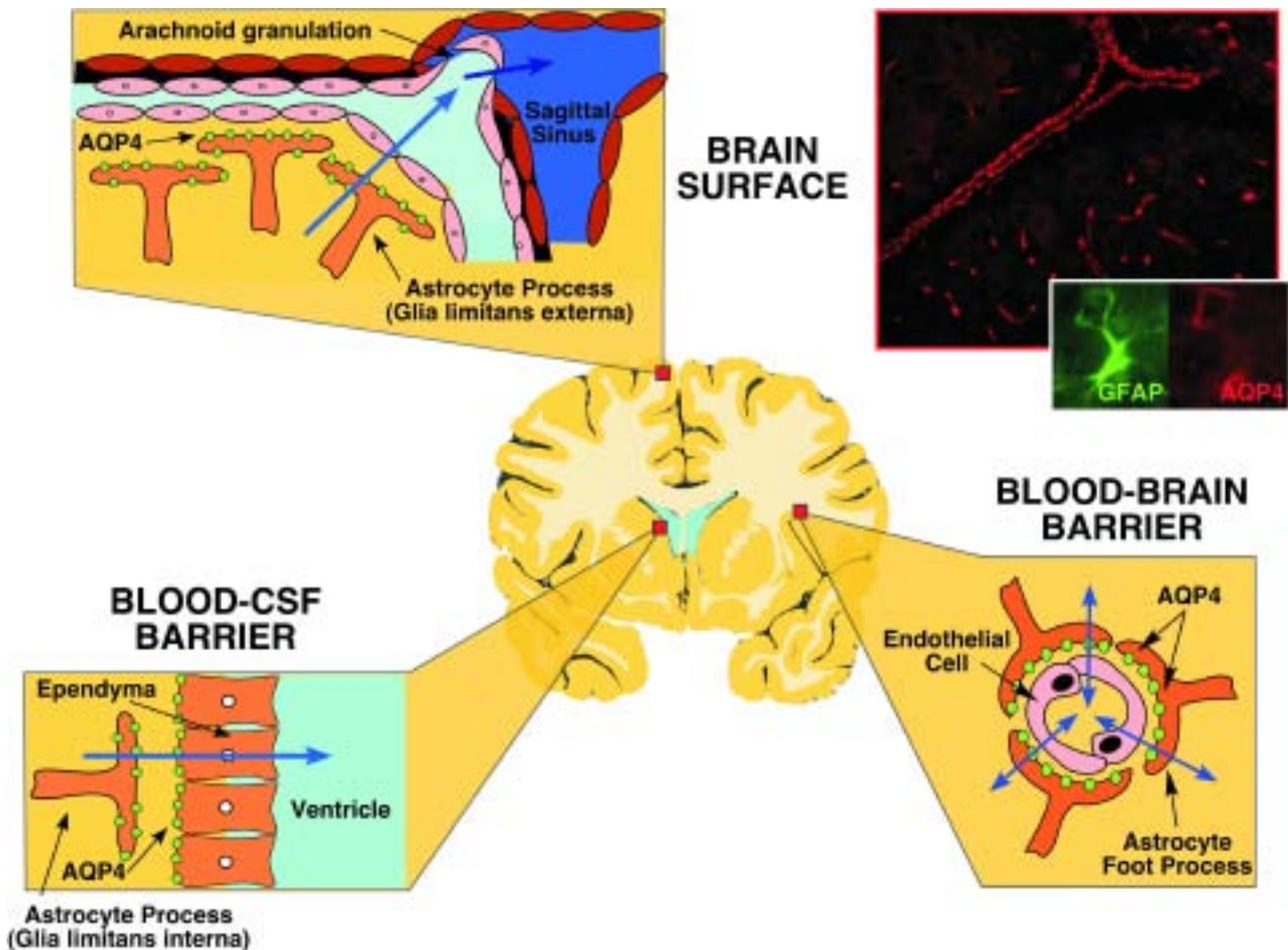


FIG. 1. Schematic illustration demonstrating water flow pathways at the BBB (lower right), brain surface (upper left), and ventricular surface (lower left) through AQP4-containing membranes. The immunofluorescence photomicrograph (upper right) represents a wild-type mouse brain stained with anti-AQP4 antibody demonstrating binding in ventricular ependyma and surrounding microvessels in the subventricular white matter. The inset shows a single astrocyte at high magnification stained with anti-glial fibrillary acidic protein (GFAP) (left) and anti-AQP4 (right) demonstrating localization of AQP4 staining to perivascular foot processes.

tating the transport of water by simple diffusion along osmotic and hydrostatic gradients.²⁹ Although 12 members of the AQP family have been identified in various tissues, AQP4 is the predominant water channel expressed in the brain.⁶ Aquaporin-4 is primarily an astroglial membrane protein with highly polarized expression localized to astrocytic foot processes surrounding the cerebral capillary endothelium.²⁰ In addition, AQP4 is highly expressed in astrocytes in the glial-limiting border facing the cortical and ventricular surfaces, as well as in the basolateral membrane of the ventricular ependyma.²³ Figure 1 summarizes the expression of AQP4 in the brain and demonstrates immunohistological evidence for the localization of AQP4 in astrocytic foot processes surrounding the cerebral microvasculature.

Given its polarized expression bordering the BBB and the brain-CSF interface, AQP4 has been presumed to play an important functional role in the transport of water in and out of the brain parenchyma by enhancing transmembrane water flux in astrocytes. Solenov and colleagues²⁷ demonstrated that astrocytes lacking AQP4 had a seven-

fold reduced water permeability as compared with equivalent cells with normal AQP4 expression. The functional importance of AQP4-facilitated water transport in diseases of cerebral water imbalance has been primarily tested *in vivo* using mice with altered or absent AQP4 expression. The results of multiple studies from several groups suggest that AQP4 plays an essential role in the development and clearance of intracellular and extracellular cerebral edema.

Cellular Edema and AQP4

Cellular (cytotoxic) edema results primarily from dysregulation of osmotic gradients across cell membranes. This dysregulation is primarily seen early in ischemic and toxic injuries, in which a lack of oxygen or disruption of cellular metabolism decreases membrane ion-pump function, resulting in the accumulation of intracellular osmoles.^{8,10} Because water moves between fluid compartments along hydrostatic and osmotic gradients, the accumulation of intracellular ions results in free water flux

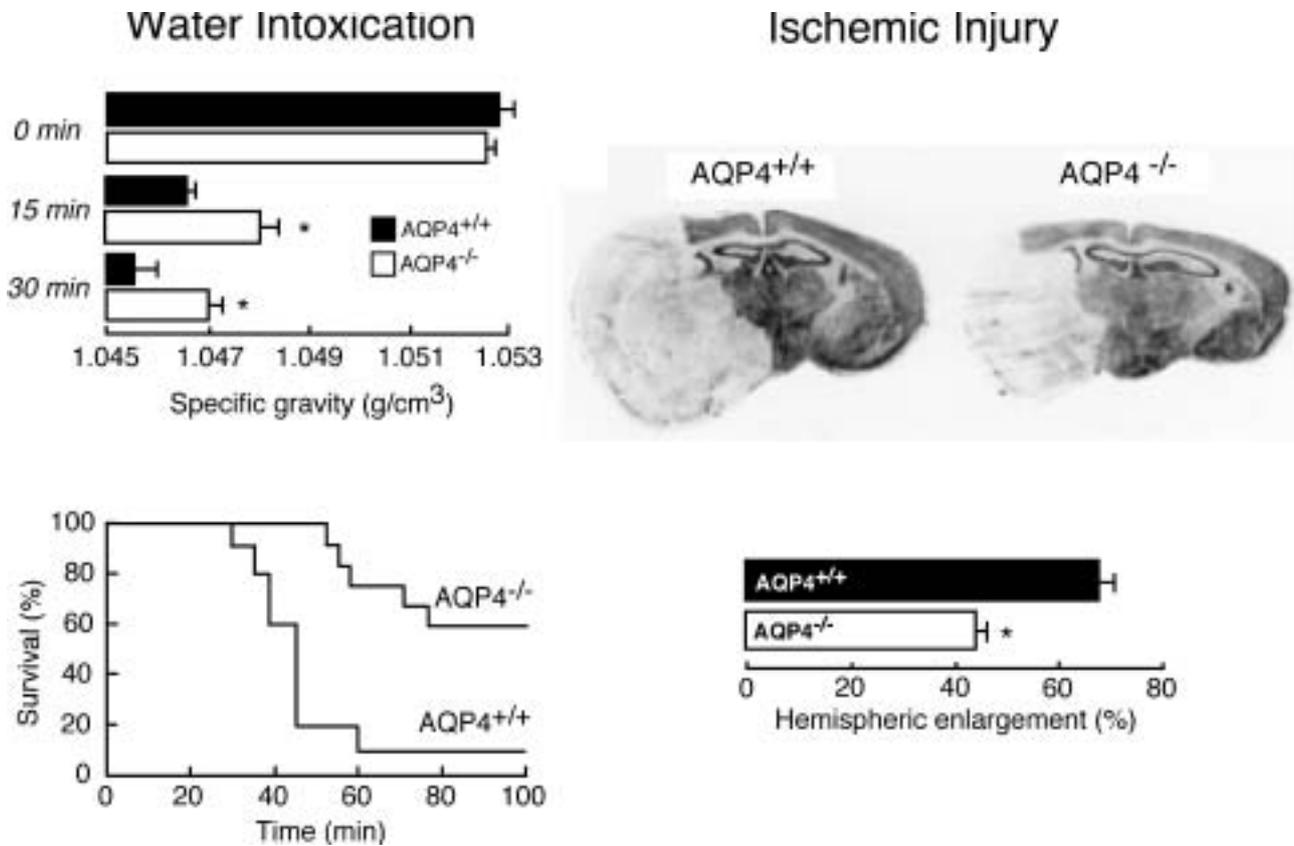


FIG. 2. Decreased cellular edema and improved survival in AQP4-deficient mice (AQP4^{-/-}) compared with normal control mice (AQP4^{+/+}) following water intoxication and cerebral ischemia. The *asterisk* denotes statistically significant differences ($p < 0.01$). *Upper left*: Bar graph showing brain water content measured as tissue specific gravity following water intoxication for 30 minutes in both groups of mice. Differences between the two groups were significant after 15 minutes. *Lower left*: Graph showing mice survival percentage following 100 minutes of water intoxication. *Upper right*: Photographs of representative sections of hemispheric edema following MCA occlusion in both mouse groups. *Lower right*: Bar graph showing quantification of average hemispheric enlargement as a percentage increase following ischemia, determined from image analysis of serial brain sections.

into cells and cell volume expansion. Although hypoxia affects all cell types, including both neurons and astroglia, in vivo cell swelling is seen early in astrocytes.¹⁶ Morphological studies have documented that early astrocytic edema is specifically localized to the perivascular foot processes.^{31,32} Although swelling of the astrocytic foot processes is expected given their proximity to the vasculature, it is particularly devastating because it results in compression of the microvasculature and reduced blood flow to tissue that is already ischemic.

Given the colocalization of early cellular edema with the highest levels of AQP4 expression, it is logically expected that AQP4 has an important role in the development of cellular edema. We tested this hypothesis by examining the extent of cellular edema formation in transgenic mice lacking AQP4 as compared with normal control animals.¹³ Cerebral edema was induced using water intoxication, a well-established model of pure cellular edema. Mice received intraperitoneal injections of free water equivalent to 20% of their body weight, in addition to desmopressin. Neurological function and overall survival of the mice were closely monitored for 90 minutes following induction of edema, and subgroups of mice

were sacrificed at 15-minute intervals to examine total brain water content and degree of astrocytic foot process swelling. Fifteen minutes after induction of cellular edema, AQP4-deficient mice demonstrated significantly less edema (measured as high brain specific gravity) when compared with wild-type control mice (Fig. 2). In addition, wild-type mice had a more rapid decline in neurological function and ultimately worse outcomes, with a nearly fourfold difference in mortality rate after 60 minutes (8% survival in wild-type compared with 76% survival in AQP4-deficient mice). This study demonstrated for the first time that elimination of AQP4 was protective during cellular edema, slowing the rate of edema formation and resulting in not only a measurable difference in peak edema, but also a true mortality benefit.

Our initial results were supported by data from Vajda and coworkers,²⁸ who used transgenic mice lacking the dystrophin gene to examine similar outcome variables. Anchoring of AQP4 to the cell membrane and localization to astrocytic foot processes occurs through binding to α -syntrophin, a member of the dystrophin protein complex. Deletion of α -syntrophin or other components of the dystrophin complex results in mislocalized and unstable AQP4

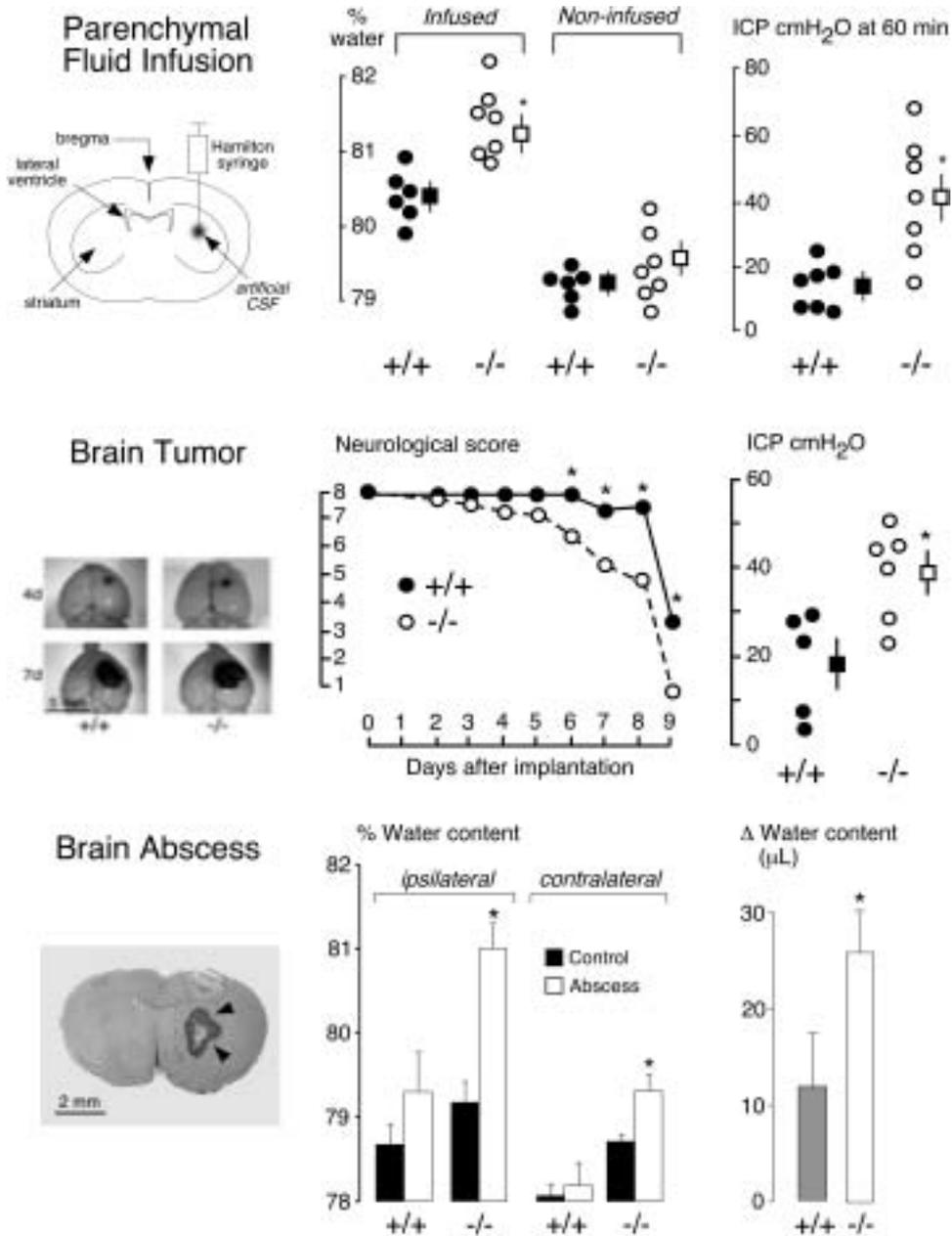


FIG. 3. Increased edema in AQP4-deficient mice in three models of extracellular water accumulation: parenchymal fluid infusion (upper row), brain tumor (center row), and brain abscess (lower row). The asterisk denotes statistically significant differences ($p < 0.01$). Upper row, left: Diagram of experimental setup for intraparenchymal fluid infusion. Upper row, center: Scatterplot showing individual and mean brain water content, measured as percentage of weight, in wild-type (+/+) and AQP4-deficient (-/-) mice before and after 60 minutes of intraparenchymal fluid infusion. Upper row, right: Scatterplot showing individual and mean ICP levels after 60 minutes of intraparenchymal fluid infusion in both mouse groups. Center row, left: Photographs of representative brains 4 and 7 days following melanoma implantation. Center row, center: Scatterplot showing the comparative neurological scores in wild-type and AQP4-deficient mice over 9 days following tumor implantation, demonstrating significant differences in neurological score after 6 days. Center row, right: Scatterplot showing individual and mean ICP levels after 7 days of tumor implantation. Lower row, left: Photograph of representative brain section 3 days after implantation of staphylococcus aureus-containing microbeads, demonstrating brain abscess formation (arrows). Lower row, center: Bar graph showing an increase in brain water content at 3 days, measured as percentage by weight, in the abscess-containing hemisphere and contralateral hemisphere of mice with experimentally induced brain abscess compared with sterile-bead injected controls. Lower row, right: Bar graph showing increases in brain water content of the mice with abscess-containing hemispheres at 3 days, expressed as increased volume in microliters.

protein expression.¹⁹ Dystrophin-deficient mice have normal levels of AQP4 mRNA expression; however, the AQP4 protein is mislocalized, rendering them functionally similar to AQP4-deficient mice.²⁸ As in the AQP4-deficient mice, dystrophin-deficient mice demonstrated a delayed onset to peak swelling, conferring a temporary survival advantage. Vajda et al.²⁸ measured apparent diffusion coefficients using diffusion-weighted magnetic resonance imaging as a surrogate marker of the degree of cerebral edema following water intoxication in dystrophin-deficient and normal control mice. The drop in apparent diffusion coefficients, which corresponded directly with animal survival, began at 35 minutes postinduction in wild-type mice as compared with 52 minutes in dystrophin-deficient mice. Although all mice in both groups eventually died as a result of severe cerebral edema, dystrophin-deficient mice with altered AQP4 localization survived 50% longer, suggesting that concentrated AQP4 expression in astrocytic foot processes facilitates rapid water transport into cells and that alteration of expression is protective.

Although two separate studies have documented the protective effect of AQP4 deletion/mislocalization in cellular edema caused by water intoxication, this mechanism of edema formation rarely occurs clinically. To address the role of AQP4 in more clinically relevant causes of cellular edema, we examined the benefit of AQP4 deletion in stroke following permanent MCA occlusion.¹³ As during water intoxication, wild-type mice had significantly worse neurological outcome scores and slightly increased mortality rates after 24 hours when compared with AQP4-deficient mice. These data correlated with a 50% greater increase in hemispheric volume secondary to edema in wild-type mice (Fig. 2), indicating that AQP4 deletion is protective in ischemia-related cellular edema. Our results were independently verified by Amiry-Moghaddam and colleagues² in a study of transgenic mice lacking α -syn-trophin. These mice have the same AQP4 mislocalization defect as dystrophin-deficient mice.³ Amiry-Moghaddam and colleagues induced cerebral ischemia in α -syn-trophin-deficient mice and wild-type controls using transient MCA occlusion for 90 minutes. Similar to our results with AQP4-deficient mice, the volume of edema and infarction was 50% greater in wild-type mice as compared with α -syn-trophin-deficient mice with mislocalized AQP4. Together, these studies strongly imply a significant role for AQP4 in the transport of water and development of cellular edema following cerebral ischemia.

Extracellular Edema and AQP4

Extracellular (vasogenic) edema is primarily caused by a disruption of the BBB and a subsequent leakage of intravascular fluid into the extracellular space of the brain.⁸ Water movement across the cerebral capillary endothelium is determined by the balance of the hydrostatic and osmotic forces across the capillary wall. Under normal conditions, the tight BBB restricts the movement of osmoles across the vascular endothelium, and water absorption into the parenchyma is primarily driven by hydrostatic forces (cerebral perfusion pressure) and ionic cotransportation. This movement is opposed by osmotic forces, resulting in minimal net fluid absorption into the parenchyma

and maintenance of brain water homeostasis. When the tight junctions of the BBB are disrupted and the intravascular contents are allowed to extravasate into the parenchymal extracellular space, normal homeostasis is disrupted.¹¹ In this situation, bulk isotonic fluid flows into the parenchyma along hydrostatic gradients with no opposing osmotic forces. This flow results in net fluid accumulation in the brain extracellular space. Because intracellular osmotic content is not affected by this process, cell volumes remain constant while the extracellular space expands. This form of edema is primarily associated with intracranial lesions that upregulate cytokine production and disrupt the BBB, including tumors, hemorrhaging, and intracranial infections.

Because the formation of vasogenic edema involves fluid movement from the vasculature directly into the extracellular space, cellular water regulators such as AQP4 do not affect the rate of edema formation. Less is known about the mechanisms of fluid clearance from the extracellular space, however, which similarly affect the net volume of edema accumulation. Tracer studies in animals with normal ICP have demonstrated that radioactive and immunofluorescently labeled proteins injected into the extracellular space move into the cerebral ventricles by bulk flow and are eventually cleared with CSF, which is presumed to be the normal intracerebral fluid pathway.^{14,22,24} Yet Wrba and coworkers³⁴ showed that, as ICP increases, the CSF clearance of these labeled tracers decreases, suggesting an alternative route of fluid clearance. Tracer injection into the ventricles of hydrocephalic animals with obstructed CSF pathways resulted in tracer accumulation around epithelial-like tight junctions in the parenchymal capillaries, suggesting a reversal of fluid clearance into the cerebral vasculature under these conditions.¹⁸ Therefore, several authors have hypothesized that there is a parenchymal route of extracellular fluid resorption, which is especially pronounced under conditions of high ICP and abnormal CSF flow dynamics.^{14,17,26} Although intuitively this fluid resorption pathway would not be expected to rely on a cellular water channel such as AQP4, the high density of AQP4 protein in astrocytic foot processes surrounding the cerebral capillaries raises the possibility of a role for AQP4 in extracellular fluid clearance.

Our group first tested the role of AQP4 in extracellular edema using intraparenchymal water injection in AQP4-deficient mice. We measured ICP and brain water content in wild-type and AQP4-deficient mice receiving a continuous infusion of artificial CSF into the brain extracellular space for 60 minutes.²¹ Despite equivalent rates of extracellular fluid infusion, AQP4-deficient mice had significantly greater increases in brain water content and twofold greater increases in ICP (Fig. 3), suggesting delayed fluid clearance in these mice compared with wild-type controls. We also tested the role of AQP4 in vasogenic edema induced by BBB breakdown using cortical freeze injury and melanoma tumor implantation, two experimentally proven methods of BBB disruption and edema formation.²¹ In both experiments, equivalent lesions were created in wild-type and AQP4-deficient mice and allowed to mature for hours to days. Postinjury brain water content, ICP, and neurological impairment were measured over the course of tumor- or freeze-injury maturation. As was found with intraparenchymal fluid injection, the AQP4-deficient

mice developed significantly greater increases in brain water content and ICP, corresponding to greater degrees of neurological impairment. Additionally, we studied vasogenic edema formation and clearance in cerebral infection by creating staphylococcal brain abscesses.⁷ When compared with wild-type control animals with brain abscesses, the AQP4-deficient mice had more than twice the increase in brain water content, associated with significantly higher ICP. Despite these differences, the measured levels of brain cytokines and BBB permeability were equivalent, suggesting that the forces driving the development of vasogenic edema were the same in both groups and that differences in outcome must be due to differences in fluid clearance from the extracellular space.

Therapeutic Implications

The data from cellular and in vivo animal studies presented in this review strongly demonstrate a functional role for AQP4 in water transport across astrocyte membranes. Aquaporin-4 has been shown to affect edema fluid accumulation and resorption in multiple studies and provides a novel molecular target for edema-targeted therapy. These conclusions have been derived primarily from functional experiments in transgenic rodents because, to date, no effective drugs to alter AQP4 expression or function have been identified, making large animal and human experimentation impossible at this time. Yet the possibility of altering AQP4 expression levels for therapeutic benefit in the future is quite realistic, given that AQP4 expression is already naturally altered in many disease states. Data from immunostaining and protein quantification in human and animal tissues demonstrate upregulation of AQP4 expression in primary brain tumors and stroke, and after traumatic brain injury.^{4,25,30} Although the signaling pathways involved in disease-related expression upregulation remain unknown, upregulation in response to hormonal and osmotic stimulants has been demonstrated in cultured astrocytes.^{5,9} It is only a matter of time before small molecular compounds that can inhibit AQP4 function and upregulate AQP4 expression become available, as several investigative groups have already begun testing for such compounds.

Drugs that target AQP4 function would provide a major therapeutic advancement in the treatment of all forms of cerebral edema, working synergistically with current therapies. The current approach in nonsurgical treatment of edema consists primarily of systemic hypertonic fluid and corticosteroid administration, therapies considered the standard of care for decades.¹⁵ Steroids are primarily beneficial in extracellular (vasogenic) edema by suppressing inflammatory mediator release, thereby limiting BBB permeability and preventing the extracellular accumulation of edema fluid. This approach to edema is primarily preventative, and is more effective in early edema development. In contrast, hypertonic fluids such as mannitol are effective in intracellular and extracellular edema, drawing edema fluid from the parenchyma into the vasculature, thereby enhancing the clearance of edema. Although temporarily effective in the treatment of malignant ICP, hypertonic therapy does not prevent edema formation, and parenchymal fluid will reaccumulate after hypertonic treatment if the underlying condition is not addressed. By

selectively enhancing or suppressing AQP4 activity in particular disease states, both the formation and clearance of edema from any cause can be targeted, providing effective therapy at any stage in the progression of disease. Blocking AQP4 function, as seen in the studies of water intoxication and stroke, decreases the rate of edema formation and enhances survival. Coupled with hypertonic therapy, AQP4 inhibitors would be expected to limit the reaccumulation of edema after treatment with hypertonic fluids, making combined treatment longer lasting and more effective. Similarly, the use of AQP4 expression up-regulators would enhance edema fluid resorption in extracellular edema and could be combined with corticosteroid administration to more rapidly resolve edema associated with tumors and infection/inflammation. We eagerly await the development of AQP4 modulating agents that we expect will be the future of molecularly targeted cerebral edema therapy.

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