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Invited review

Aquaporins as potential drug targets[1]

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Abstract

The aquaporins (AQP) are a family of integral membrane proteins that selectively transport water and, in some cases, small neutral solutes such as glycerol and urea. Thirteen mammalian AQP have been molecularly identified and localized to various epithelial, endothelial and other tissues. Phenotype studies of transgenic mouse models of AQP knockout, mutation, and in some cases humans with AQP mutations have demonstrated essential roles for AQP in mammalian physiology and pathophysiology, including urinary concentrating function, exocrine glandular fluid secretion, brain edema formation, regulation of intracranial and intraocular pressure, skin hydration, fat metabolism, tumor angiogenesis and cell migration. These studies suggest that AQP may be potential drug targets for not only new diuretic reagents for various forms of pathological water retention, but also targets for novel therapy of brain edema, inflammatory disease, glaucoma, obesity, and cancer. However, potent AQP modulators for *in vivo* application remain to be discovered.

Introduction

The aquaporins (AQP) are a family of water-transporting proteins with 13 homologous members in mammals. The AQP family members have 25%–60% homology in protein sequence, and structurally have a general homotetrameric assembly in cell membranes, where each monomer contains 6 transmembrane segments that form a water pore[1–3]. Functionally, AQP are divided into 2 subclasses: the subfamily that includes AQP 1, 2, 4, 5, and 8, which selectively transport water; and the subfamily that includes AQP 3, 7, 9, and 10, which transport glycerol and possibly other small solutes as well as water[4]. Members of the mammalian AQP family are expressed diversely in fluid-transporting epithelium, endothelium of various organs, and in other tissues such as skin, white blood cells and fat cells[4,5]. The functional importance of AQP in mammalian physiology and diseases has been studied extensively by analyzing the phenotype of transgenic mouse models of AQP knockout or mutation, and of human mutations such as nephrogenic diabetes insipidus caused by various autosomal dominant or recessive AQP2 mutations[5–7]. These studies have provided new insights into the basic mechanisms of physiology and human diseases, and have indicated that the pharmacological modulation of water and solute transport targeting AQP may provide novel opportunities for therapeutic interventions in a wide range of human disorders. This review will focus on well-defined functions of AQPs in mammalian physiology and the potential of the AQPs as drug targets in developing new therapies for related human diseases.

AQP as novel targets for diuretic drugs

The kidney is the mammalian organ with the most active fluid-transporting epithelia. Seven AQP members are expressed in different segments of the nephron[8-10]. AQP1 is expressed in the apical and basolateral plasma membranes in the proximal tubule, where the majority of glomerular-infiltrated fluids are absorbed. AQP1 is also expressed in the plasma membranes of the thin descending limb of Henle (TDLH) and in the microvascular endothelium of the outer medullary descending vasa recta (OMDVR), which are the structural basis of the countercurrent multiplication mechanism of urinary concentration. AQP2 is a vasopressin-regu-

lated water channel expressed in collecting duct principal cells, where they undergo trafficking between an intracellular vesicular compartment and the cell apical plasma membrane in response to phosphorylation status controlled by the vasopressin V2 receptor signal pathway. AQP3 and AQP4 are coexpressed at the basolateral membrane of collecting duct epithelial cells, with AQP3 prominently expressed in proximal segments of the collecting duct and AQP4 in the inner medullary collecting duct, where they are constitutively expressed and confer high basolateral membrane water permeability. AQP6 is expressed in the intercalated cells of the collecting duct, where its exact localization and function have not been established. AQP7 is expressed in the epithelium of a short distal segment (S3 segment) of proximal tubule. AQP8 is expressed at low levels in the proximal tubules and collecting duct principal cells.

Among the 7 renal AQP, AQP1–3 seem to play essential roles in urinary concentrating mechanisms, as indicated by the severe nephrogenic diabetes insipidus (NDI) phenotype seen in AQP1 and AQP3 knockout mice, and in AQP2 mutant mice. Under normal feeding conditions with free access to food and water, AQP1 and AQP3 null mice are remarkably polyuric and polydipsic[11,12]. An additive effect of polyuria was seen in AQP1/AQP3 double knockout mice, indicating a more severe loss of renal concentrating function due to a combination of defects in different nephron segments[13]. Urine osmolality was markedly decreased in the AQP1 and AQP3 null mice. However, in response to vasopressin treatment or 36 h water deprivation, urine osmolality in AQP1 null mice did not increase[11], indicating severe loss of urinary concentrating ability caused by a combination of impaired proximal tubule fluid absorption and defective countercurrent multiplication. Indeed, microperfusion and micropuncture studies have shown dramatically decreased transcellular water permeability of the proximal tubule, TDLH and OMDVR, indicating that AQP1 is the principal water channel in the proximal tubule, TDLH and OMDVR, and plays a key role in the osmotic equilibration of fluids along these nephron segments, and thus in proximal tubule water absorption and in the renal countercurrent concentrating mechanism[14-17]. Human subjects with loss-of-function AQP1 mutations had a moderate urinary concentrating defect with no obvious polyuria[18]. AQP3 null mice can partially concentrate their urine upon vasopressin treatment or water deprivation. However, AQP2 expression is dramatically decreased in AQP3 null mice and thus the severe polyuria in AQP3 null mice seems to be the consequence of AQP2 downregulation[12,19]. The mechanism associated with this phenomenon remains unknown. In AQP3 null human subjects negative for the GIL blood group antigen, no obvious polyuria was identified, although detailed clinical analysis was not carried out[20]. Therefore, the role of AQP3 in the urinary concentrating mechanism may be different in different species. AQP4 null mice do not have polyuria, despite having a small defect in maximal urine osmolality after 36 h water deprivation[21]. AQP7 knockout mice did not have a urinary concentrating defect; however, AQP1/AQP7 double-knockout mice had a significant reduction in urinary concentrating ability compared with AQP1 knockout mice, suggesting a role of AQP7 in water reabsorption in the proximal straight tubules[22]. AQP8 was localized by immunocytochemistry to the intracellular vesicular fractions of rat kidney proximal tubules[23], therefore it is not surprising that urinary concentrating ability was unimpaired in AQP8 knockout mice[24]. The phenotypes of AQP6 knockout mice have not been reported; however, AQP6 is not expected to play a key role in collecting duct water transport or urinary concentration because its localization is restricted to intercalated cells, where it could be involved in maintaining the acid-base balance[25].

The role of AQP2 in collecting duct water absorption is well established from studies in human subjects with autosomal NDI. Loss-of-function mutations in AQP2 causing both autosomal recessive and dominant NDI have been identified and characterized[6]. The AQP2 mutation T126M causes autosomal recessive NDI by a mechanism involving retention of the mutant protein at the endoplasmic reticulum (ER) of mammalian cells, which was confirmed in an AQP2-T126M knock-in mouse model, in which mice developed severe polyuria and died less than 1 week after birth[26]. Similarly, the autosomal dominant mutations in human AQP2 result in a trafficking defect that prevents translocation of AQP2 from non-ER compartments to the apical membrane of collecting duct principal cells, which also leads to diuresis in patients[27,28].

The impairment of renal concentrating ability in loss-of-function mutations of AQP1 and AQP2 in both transgenic mice and humans indicates that inhibitors of the two AQP may prove novel effective diuretic reagents that are superior to the existing diuretic drugs, which often cause electrolyte imbalances. In addition, in clinical conditions associated with retention of water, such as congestive heart failure, liver cirrhosis and pregnancy, significant increases in apical AQP2 expression have been observed[8,29]. AQP2 may also be involved in the pathogenesis of spontaneous hypertensive rats (SHR), because protein expression and apical targeting of AQP2 were increased in the inner medullary collecting duct in SHR[30]. Treatment of SHR with vasopressin V2 receptor antagonist resulted in significantly lower blood pressure, higher urine volume, lower urine osmolality and

higher urinary AQP2 excretion than in untreated animals[31]. Therefore, AQP2 inhibitors could become effective drugs in reducing water retention in these common chronic disorders.

AQP as therapeutic targets for exocrine disorders

Several AQP are expressed in exocrine glands of the gastrointestinal system, airways, eyes, and skin and in the choroid plexus in the brain. The role of AQP in fluid secretion by exocrine glands including salivary, submucosal, sweat, and lacrimal glands, by the liver and pancreas, and by the choroid plexus and the ciliary bodies has been characterized using appropriate knockout mouse models[7,32-36]. AQP5-dependent fluid secretion by salivary glands and airway submucosal glands, and AQP1-dependent fluid secretion by the choroid plexus have been established[7,34].

In salivary glands, AQP5 is expressed in the luminal membrane of acinar cells, AQP1 in the microvascular endothelial cells, and AQP8 in the basolateral membrane. Saliva secretion by the salivary gland upon stimulation is a rapid process of fluid movement from blood to the lumen of acini, which is driven by the interstitial-to-luminal transport of sodium and chloride across the acinar epithelium followed by osmotic water flow. We found that upon pilocarpine stimulation AQP5 null mice produced viscous saliva with more than 60% reduction in volume compared with wild-type mice[37]. The saliva from AQP5 null mice was hypertonic and hypernatremic, whereas amylase and total protein secretion, representing functions of salivary mucous cells, were not affected by AQP5 deletion. A subsequent study confirmed the defect in saliva secretion in AQP5 null mice and reported reduced water permeability in acinar cells isolated from the null mice[38]. In human studies, defective cellular trafficking of AQP5 in the lacrimal gland and abnormal distribution of AQP5 in salivary glands have been reported in patients with Sjogren's syndrome[39,40].

AQP5-dependent fluid secretion has also been seen in airway submucosal glands, where a mixture of water, ions, and macromolecules is secreted onto the airway surface. Glandular secretions are important in establishing airway surface liquid composition and volume, and in antimicrobial defense mechanisms. Submucosal glands contain serous tubules, where active salt secretion into the gland lumen creates an osmotic gradient, driving water transport across a water-permeable epithelium, as well as mucous cells and tubules, where viscous glycoproteins are secreted. AQP5 is expressed at the luminal membrane domain of serous epithelium. The volume of pilocarpine-stimulated fluid secretion was reduced by >50% in AQP5 null mice compared with wild-type mice[33]. Analysis of secreted fluid showed a more than 2-fold increase in total protein in AQP5 null mice. Increased viscosity of airway surface liquid can impede ciliary clearance of bacteria, which could promote airway infections via a mechanism similar to that occurring in individuals with cystic fibrosis[41]. As in salivary glands, the luminal membrane of submucosal gland epithelial cells is the rate-limiting barrier to water movement, where AQP5-mediated high water permeability is essential for normal fluid secretion.

Therefore, stimulation of glandular AQP5 expression or function may provide a novel approach for treating hyperviscous and diminished gland secretions in patients with cystic fibrosis and Sjogren's syndrome. Conversely, inhibition of glandular AQP5 expression or function may provide new treatment for airway fluid hypersecretions in infectious or allergic bronchitis and rhinitis.

AQP as novel therapeutic targets for brain edema

Pathological accumulation of water in the brain is closely associated with morbidity and mortality observed in patients suffering stroke, traumatic brain injury, brain tumors and hydrocephalus. Several lines of evidence have shown that AQP are important in water homeostasis in the central nervous system[42–44]. AQP4 is predominantly expressed in astrocytes in the brain and spinal cord at putative sites involving fluid transport at the blood-brain and brain-cerebrospinal fluid (CSF) interfaces. The expression of AQP4 is polarized to the membrane of astrocytic foot processes adjacent to the vascular endothelium that forms the blood-brain barrier, and of astrocyte dense processes that form the glia limitans, which lines the CSF-bathed pial and ependymal surfaces in the subarachnoid space and the ventricles, suggesting a role of AQP4 in vascular-glial and glial-CSF water exchange, respectively.

Studies using AQP4 knockout mice demonstrated increased protection from cytotoxic brain edema induced by water intoxication and cerebral ischemic injury[45]. In water intoxication-induced brain edema, the AQP4 null mice had much less swelling of astrocytic foot processes than the wild-type mice, as assessed by transmission electron microscopy. Brain swelling and hemispheric enlargement following permanent middle cerebral artery occlusion was also significantly lower in the AQP4 null mice than in the wild-type mice. In both cases, AQP4 null mice had a lower mortality rate and significantly less neurological deficit compared with wild-type mice. In a bacterial meningitis model of brain edema, AQP4 protein was strongly upregulated in meningitis, resulting in an approximately 5-fold higher water

permeability across the blood-brain barrier compared with non-infected wild-type mice[46]. AQP4-deficient mice had more than 2-fold lower intracranial pressure (ICP) and brain water accumulation, and improved survival. Meningitis produced marked astrocyte foot process swelling in wild-type but not AQP4 null mice, manifesting a primarily cytotoxic brain edema mechanism.

Vasogenic brain edema is caused by increased permeability of the blood-brain barrier, which often occurs during cerebral infections, vascular disorders and tumor growth[42]. In contrast to the findings in cytotoxic brain edema, recent studies have shown that AQP4-mediated vascular-glial water transport plays a key role in fluid clearance in vasogenic brain edema[47]. AQP4-deficient mice had significantly higher ICP, greater brain water content and a worse clinical outcome than wild-type mice in vasogenic brain edema induced by continuous intracerebral fluid infusion, freeze-injury, intraparenchymal bacterial abscess and brain tumor growth [46–48]. The impaired clearance of vasogenic brain edema fluid suggests that AQP4 provides an efficient water transport route that allows extracellular edema fluid to move across the astrocyte cell membranes of the blood-brain barrier into the blood and of glia limitans into the CSF.

These data suggest that inhibitors and activators of AQP4 function and expression could provide potentially efficient therapies for cytotoxic and vasogenic brain edema, respectively.

In the choroid plexus of the brain ventricle, AQP1 is expressed in the apical membrane of the choroid plexus epithelium, where it is involved in the production of CSF. AQP1 deletion did not affect the structure or size of the choroid plexus. However, osmotically driven water transport in isolated choroid plexus is reduced by 5-fold in AQP1 knockout mice, indicating that AQP1 provides the major water pathway in choroid plexus. In AQP1 null mice, CSF production is reduced by 25% and ICP is reduced by more than 2-fold compared with wild-type mice[34]. The role of AQP1 in CSF secretion and ICP regulation indicated that AQP1 inhibitors might provide a novel treatment for various types of hydro-cephalus.

AQP as novel therapeutic targets for glaucoma

In the eye, the rates of continuous formation of aqueous humor in the non-pigmented epithelia of the ciliary body and its drainage through the trabecular meshwork and canals of Schlemm are the major determinants of intraocular pressure (IOP). The fluid dynamics of the aqueous humor are frequently associated with glaucoma, a potentially blinding disease. A major strategy in the medical treatment of glaucoma is to reduce aqueous humor production and thereby IOP[49]. Aqueous humor is secreted across the ciliary epithelium by active transport of solutes from the stroma to the posterior chamber of the eye, with water passively following. AQP1 is expressed in non-pigmented ciliary epithelia and trabecular meshwork, and AQP4 in ciliary epithelium. Studies using transgenic mice demonstrated a significantly lower IOP in AQP1 knockout or AQP1/AQP4 double knockout mice compared with wild-type mice or AQP4 knockout mice[50]. The aqueous fluid production rate was reduced significantly in AQP1-deficient mice, whereas aqueous fluid outflow was not affected. Therefore, the decreased IOP in AQP1 null mice is due to reduced aqueous fluid secretion in the ciliary epithelium.

These findings suggest that pharmacological inhibitors of AQP1 could efficiently reduce the inflow of aqueous humor, thus providing a new approach in the treatment of glaucoma.

AQP as novel therapeutic targets for obesity

In addition to the obvious involvement of AQP in various types of fluid transportation, increasing evidence is uncovering the physiological importance of AQP associated with their solute transport properties. The aquaglyceroporins AQP3, AQP7 and AQP9 are expressed in skin, adipose tissue and liver, respectively[51,52]. Glycerol produced by lipolysis in adipose tissue is transferred from adipocytes to the plasma through AQP7. Plasma glycerol is taken up by liver via AQP9, where it is used as a substrate for gluconeogenesis during prolonged fasting. Coordinated upregulation of AQP7 and AQP9 mRNA expression has been shown in rodents during fasting, diabetic insulin deficiency and insulin-resistant hyperinsulinemia, which indicates the possible involvement of aquaglyceroporins in physiological and pathophysiological glucose metabolism[52]. In humans, loss-of-function genetic defects of AQP7 are associated with an inability to elevate plasma glycerol during exercise[53].

Phenotype studies on AQP7 knockout mice[54–56] revealed an adult-onset obesity featuring markedly greater fat mass compared with wild-type mice. The AQP7 null mice developed obesity and insulin resistance even at a young age after consumption of a high-fat/high-sucrose diet. Histologically, adipocyte size is markedly larger in AQP7 null mice than in wild-type mice, suggesting that the obesity in the AQP7 null mice results from adipocyte hypertrophy. Adipocyte glycerol and triglyceride concentrations are also significantly elevated in the AQP7 null mice. AQP7 mice

exhibit low plasma glycerol levels and impaired glycerol release in response to β3-adrenergic agonist. Finally, AQP7 disruption has been found to elevate adipose glycerol kinase activity and accelerate triglyceride synthesis in adipocytes.

These findings suggest that AQP7 is required for efflux of glycerol from adipocytes, and lack of AQP7 influences not only adipocyte biology but also whole-body glucose homeostasis and insulin sensitivity. Adipocyte glycerol permeability is a novel regulator of adipocyte size and whole body fat mass. Thus upregulation of adipocyte AQP7 expression may provide a novel therapy to reduce fat mass in obesity.

AQP1 as a novel target of anti-angiogenesis therapy for tumors

AQP1 is expressed in endothelial cells of microvessels in various types of tumors[57,58], suggesting a possible role of AQP1 water channels in the angiogenesis, growth and metastasis of tumors. Indeed, recent studies have shown markedly impaired tumor angiogenesis and endothelial cell migration in AQP1-deficient mice[59,60]. In a tumor-bearing model with melanoma cells implanted subcutaneously into wild-type and AQP1 knockout mice, the AQP1-deficient mice showed significantly slowed tumor growth and improved survival. Histological studies clearly indicated a dramatically lower density of microvessels and larger necrotic tissues in the tumors from AQP1-deficient mice. Further studies using primary cultures of aortic endothelial cells indicated that AQP1-deficient endothelial cells exhibited marked defective migration and tubule formation, whereas other intrinsic endothelial cell functions associated with angiogenesis, such as proliferation and adhesion, did not differ between wild-type and AQP1 null mice.

Therefore, inhibition of AQP1 function and expression may have potential applications in tumor therapy by inhibiting tumor angiogenesis and thereby limiting tumor growth and spreading.

Perspectives

Facilitated water transport across the cell membrane is a fundamental mechanism associated with a variety of processes in human physiology and pathophysiology. Although the importance of AQP in mammalian physiology and diseases involving water and solute transport has been established[61] and potential clinical applications targeting various AQP have been proposed, potent pharmacological modulators of AQP are still lacking. In particular, drug discovery targeting AQP function presents a challenge in developing feasible assays for high-throughput screening to identify potent small-molecule inhibitors of AQP-mediated water transport. The real therapeutic potential of AQP as drug targets will depend upon successful development of such novel methodologies to identify high-affinity and highly selective modulators that target a particular subtype of AQP of choice from the available large collections of combinatorial small molecules and natural compounds, and the evaluation of their *in vivo* efficacy.

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