"Aquaporin-1: new developments and perspectives for peritoneal dialysis"

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ABSTRACT

Peritoneal dialysis involves diffusive and convective transport and osmosis through the highly vascularized peritoneal membrane. Several lines of evidence have demonstrated that the water channel aquaporin-1 (AQP1) corresponds to the ultrasmall pore predicted by the model of peritoneal transport. Proof-of-principle studies have shown that upregulation of the expression of AQP1 in peritoneal capillaries results in increased water permeability and ultrafiltration, without affecting the osmotic gradient or small solute permeability. Conversely, studies in Aqp1 mice have shown that haplo-insufficiency for AQP1 results in significant attenuation of water transport. Recent studies have demonstrated that AQP1 is involved in the migration of different cell types, including endothelial cells. In parallel, chemical screening has identified lead compounds that could act as antagonists or agonists of AQPs, with description of putative binding sites and potential mechanisms of gating the water channel. By modulating water transport, these pharmacological agents could have clinically relevant effects in targeting specific tissues or disease states.

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AQUAPORIN-1: NEW DEVELOPMENTS AND PERSPECTIVES FOR PERITONEAL DIALYSIS

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The capacity to remove excess water by osmosis across the peritoneal membrane is a major predictor of outcome and mortality in end-stage renal disease patients treated by peritoneal dialysis (PD) (1). Ultrafiltration (UF) failure is a frequent abnormality in long-term PD patients and the main reason for technical failure (2). The identification of aquaporin-1 (AQP1), a 28 kDa protein abundantly expressed in the membrane of human red blood cells, as the first member of the AQP family [see Ref. (3) for review] had a major relevance for understanding the mechanisms of UF in PD (4).

Aquaporin-1 is a water-specific membrane channel, impermeable to urea and glycerol (5), that is distributed in the endothelium lining the peritoneal capillaries and postcapillary venules (6) [Figure 1(a,b)], which are the most important barrier to solute transport during PD (8). Structural information obtained by electron cryomicroscopy (9) shows that the pore formed by AQP1, with its narrow diameter of only 2.8 Å, allows the accommodation of a single water molecule (3) and perfectly fits the postulated size of the ultrasmall pore of the peritoneal membrane. Of interest is the location of the side chain of a cysteine residue (Cys189) in the pore, which explains why the water permeability mediated by AQP1 can be inhibited by mercury compounds (10). The functional relevance of AQP1 for PD was first suggested by ex vivo inhibition of peritoneal water permeability by HgCl₂ in a rat model (11). Initial studies conducted in Aqp1 knockout mice demonstrated that the osmotically driven water transport across the peritoneal membrane was significantly decreased in Aqp1⁻/⁻ mice compared with wild-type littermates (12). Further studies using a standard peritoneal exchange test validated in the
mouse (13) demonstrated that $Aqp1^{-/-}$ mice had an approximately 50% decrease in cumulative UF during PD with a hypertonic dialysate, as predicted by the three-pore model (7) [Figure 1(c)]. Of note, deletion of AQP1 had no effect on the structure of the peritoneal membrane, the transport of small solutes, or the magnitude of the osmotic gradient (7). Furthermore, heterozygous $Aqp1^{+/+}$ mice demonstrated an intermediate phenotype, showing that haplo-insufficiency in AQP1 results in significant attenuation of water flux and intraperitoneal volume curves [Figure 1(c)].

REGULATION OF AQP1 EXPRESSION TO INCREASE UF

The identification of AQP1 as the molecular counterpart of the ultrasmall pore provided a major insight into the debate about the regulation of UF capacity in PD (14). Because they reject solutes but facilitate the transport of water, the ultrasmall pores mediate up to half the UF volume during crystalloid osmosis (15), a fact that was demonstrated by studies in Aqp1 mice (7). These findings suggest that modulating the expression of AQP1 in the peritoneal membrane could be used to treat UF failure. The proof of principle for this concept was provided by a study in a rat model of PD (16). Knowing that the expression of AQP1 in the perinatal rat lung is induced by corticosteroids (17) and that the promoter of the Aqp1 gene contains glucocorticoid response elements (18), Stoenoiu et al. showed that treatment of rats with high doses of corticosteroids was associated with an increased expression of AQP1 in the capillary endothelium, resulting in a significant increase in water transport and net UF across the membrane (16). These modifications were observed in the absence of any effect on osmotic gradient or small solute transport, emphasizing the specific role of AQP1 in water transport during PD. Alternative mechanisms involved in the regulation of AQP1 in different cell types are being actively investigated (19–22).

AQUAPORIN-1 AND CELL MIGRATION

Acute peritonitis is the most frequent complication of PD. It is consistently associated with an increased transport of small solutes due to microvascular proliferation and/or recruitment (23,24). An increased effective peritoneal surface area ensues, leading to a faster reabsorption of glucose and an early dissipation of the osmotic gradient and consequent decreased UF. Our studies in human and mouse tissues have shown that peritoneal inflammation is associated with a marked increase in nitric oxide synthase (NOS) activity due to upregulated endothelial NOS (eNOS) and inducible NOS

Figure 1 — Distribution and role of aquaporin-1 (AQP1) in the peritoneal membrane. In a cross-section of the human parietal peritoneum stained for AQP1, AQP1 is detected in the endothelium lining peritoneal capillaries, venules, and small veins (A) ($m =$ mesothelium; bar represents 40 μm). Immunogold electron microscopy on mouse visceral peritoneum unicyl sections shows a very strong signal for AQP1 in the plasma membrane and plasma membrane infoldings of capillary endothelial cells (B) (bar represents 500 nm). Effect of AQP1 deletion on the transport of water across the peritoneal membrane (C): mice with a targeted deletion of Aqp1 were investigated using a peritoneal equilibration test essentially similar to that performed in patients. The initial ultrafiltration (UF) rates were determined in $Aqp1^{+/+}$ mice (black squares), $Aqp1^{+/+}$ mice (green triangles), and $Aqp1^{-/-}$ mice (pink circles) during a 2-hour exchange with hypertonic dialysate. In comparison with $Aqp1^{+/+}$ mice, mice lacking AQP1 show a significantly lower initial UF rate, whereas intermediate values are observed in $Aqp1^{-/-}$ mice. [Modified from Ni et al. (7).]
(iNOS) isoform, whereas AQP1 expression is unchanged (6,23,25). Studies using NOS inhibitors or eNOS knock-out mice revealed that the loss of UF in acute peritonitis is due to increased peritoneal transport of small solutes, mediated primarily by the increased eNOS, which appears to be instrumental in vascular proliferation (24,26,27). Of interest, recent studies by Verkman and colleagues have established a role for AQP1 in cell migration, wound healing, and tumor growth and dissemination [see Ref. (28) for review]. In fact, AQP1 deficiency appears to impair the migration of endothelial cells, with reduced blood vessel formation in tumor beds (29). More generally, various AQPs may be involved in the migration of different cell types, including astrocytes, renal proximal tubule cells, cancerous cells, and fibroblasts, probably by facilitating rapid changes in cell volume, contributing to the formation of localized cell membrane protrusions during migration (28).

Using a model of catheter-induced peritonitis, we recently showed that mice lacking AQP1 demonstrated decreased angiogenesis and a significantly lower increase in small solute transport compared with wild-type littermates. Furthermore, Aqp1−/− mice had a lower number of infiltrating macrophages and a lower number of marginating cells, resulting in significant attenuation of the inflammatory infiltrate (30). These changes, which are similar to those observed in the eNOS knockout mice (24), suggest that AQP1 may play a role in vascular proliferation and leukocyte recruitment during acute inflammation. Taken together, these studies suggest that pharmacoregulation of AQP1 may yield distinct benefits, depending on the clinical circumstances, for patients treated by PD.

PHARMACOLOGICAL ANTAGONISTS OF AQPs

Pharmacological inhibitors of AQPs have been highly anticipated as tools for research on the physiological roles of AQP channels and for intervention in clinical conditions involving alterations in fluid homeostasis (31,32). Until recently, the agents available for experimental AQP inhibition were limited. Metal ions [such as mercury (9), silver, and gold (33)], tetraethylammonium (TEA) (34–36), and acetazolamide (37,38) have been valuable as indicators that pharmacological modification of AQP activity is possible. However, these agents show toxicity, lack of specificity, or variability in effectiveness across experimental preparations, which make their potential translational value debatable. Recently, lead compounds have been identified as potential novel pharmacological agents capable of acting on AQP channels (Figure 2), thus opening new perspectives in the field.

Figure 2 — Structures of pharmacological agents that block aquaporin (AQP)-1 and AQP4 water channels. Loop diuretic compounds bumetanide (A) and furosemide (B) in the aryl sulfonamide class block AQP4 more effectively when delivered intracellularly than when applied extracellularly, an observation that prompted the development of the membrane-permeable AqB.n library of compounds (39). NSC670229 is 1 of 4 compounds from the National Cancer Institute’s Small Molecule Resource shown to block AQP1 and AQP4 water channels (40) (C). Structural illustrations for (A–C) were modified from images available at NCBI PubChem (pubchem.ncbi.nlm.nih.gov). Extracellular tetraethylammonium (TEA) ion is a weak inhibitor of AQP1 and AQP4 (D) (34,36).

Our prior work identified the first non-mercurial AQP1 blocker TEA ion (TEA•), which decreases AQP1-mediated water fluxes in the oocyte expression system (34) and in the kidney (35). TEA sensitivity of human AQP1 was removed by site-directed mutagenesis of tyrosine 186 to phenylalanine (Y186F) in the outer vestibule of the water pore, confirming that the inhibition of TEA has a direct effect on the AQP1 channel (34). The blockade of AQP1 by TEA has been confirmed, extended to other classes of AQPs that carry a homologous tyrosine residue (36), and supported by molecular dynamic modeling (41), although one study failed to confirm blocking activity of
either acetazolamide or TEA (42). TEA is not an ideal AQP inhibitor since it also blocks a variety of K⁺ channels as well as other membrane proteins but, nonetheless, it has successfully been used as an AQP inhibitor in other preparations (43,44).

The chemical class of aryl sulfonamides has yielded attractive candidates as pharmacological agents for AQPs, discovered as clues from side effects of known clinical drug compounds. Development of a novel antagonist (AqB013) that blocks AQP1 and AQP4 (IC₅₀, approximately 20 µmol/L) was accomplished by screening a chemical library structurally related to the loop diuretic bumetanide (45), which was selected as a lead scaffold based on its small but significant inhibition of AQP4 water channel function (39). Biological assays and modeling supported a putative binding site for AqB013 in the intracellular vestibule of the rat AQP4 water channel pore (39). Mutagenesis of residues that were predicted by docking simulations to coordinate AqB013 binding increased the efficacy of the blockade (IC₅₀, approximately 8 µmol/L) and suggested that the inhibition by AqB013 occurs by occlusion of the cytoplasmic vestibule of the water pore. Anti-epileptic aryl sulfonamide drugs such as topiramate and zonisamide also have been suggested to block AQP4 channels (46). Targets for testing the validity of these new AQP blockers include in vivo models of edema in brain, lung and heart, glaucoma, cancer, renal dysfunction, and other conditions involving alterations in fluid transport and homeostasis in AQP-expressing tissues (31).

In a parallel approach, a method for detecting fluorescence changes in calcein-loaded cells was used to screen more than 3500 compounds from the National Cancer Institute Small Molecule Collection. Four compounds were confirmed to inhibit AQP1 and AQP4 water permeability: NSC164914, NSC670229, NSC168597, and NSC301460. They are diverse in chemical structure but are similar in having a record of causing growth inhibition patterns in a panel of human cancer cell lines (40). The structure–activity relationships of these candidate lead compounds remain to be determined for their potential use as anticancer or anti-angiogenesis agents (47).

**PHARMACOLOGICAL AGONISTS OF AQPs**

Beyond the current focus on finding AQP antagonists, an equally exciting opportunity is found in the search for pharmacological agonists of AQP channels that would act by binding reversibly to a site on the AQP protein to increase water channel activity (Figure 3). If identified, an AQP agonist could be valuable for enhancing fluid transport across barrier membranes. For instance, an agonist of AQP4 in the astroglial cells at the blood–brain barrier interface could enhance export of water from the brain in the later phase of brain edema, speeding resolution of edematous conditions. Similarly, based on the critical role of AQP1 in mediating UF, an agonist of AQP1 channels in vascular endothelial cells could improve the efficiency of PD (7,11,12).

Consideration of the possibility of an AQP agonist presumes that AQP water channels are not constitutively locked into a rigid open state. This concept is supported by growing evidence that some if not all classes of AQPs are gated. For instance, the lens water channel AQP0 and plant AQPs show changes in water permeability as a function of pH (48–51). A capacity for rapid gating of water permeability implies that the relevant classes of AQPs have both open and closed state conformations, and so overall water permeability at a given time could depend on the relative proportion of the populations of channels that are in the open state. Stabilization of the open state conformation by ligand binding would be a logical basis for a pharmacological agonist activity.

Multiple sites for ligand binding on AQP channels have been proposed based on simulations of molecular dock-
opening of bumetanide-related compounds (39). Docking simulations have identified three putative binding sites, labeled Sites 1 and 2 on the intracellular side of the AQP4 subunit and Site 3, which is located in the extracellular vestibule of the water pore. Site 1 is positioned in the intracellular water pore vestibule and Site 2 is a separate intracellular pocket involving residues from the carboxyl terminal and intracellular loop sequences. The enhanced efficacy of intracellularly administered bumetanide in blocking AQP4 water channels provides evidence that an internal site was the target of action; further analysis with site-directed mutagenesis implicated Site 1 in a block by the membrane-permeable bumetanide derivative AqB013 (39). The existence of a cytoplasmic binding pocket that modulates water permeability in AQP channels is of interest and prompts the speculation that AQPs might also be regulated in vivo by cytoplasmic ligands yet to be identified. An allosteric modulatory role for Site 2 would be consistent with its location outside the direct water pore pathway, but other binding sites that have not yet been identified by modeling are also possible. Of note, the model of Site 2 involves the C-terminus, which in other work has also been suggested to be a candidate regulatory domain in AQP1 for Ca2+ (52) and to be involved in modulation of an ionic conductance sensitive to signaling by cGMP (53–56) and protein kinase C (57).

Mechanisms of gating of AQP channels are being defined. Intracellular domains have been proposed to serve in gating AQP channel functions in mammalian AQP1 (56,57), AQP4 (58), and plant AQPs (59). With structural modeling, Tornroth–Horsefield and colleagues outlined a detailed mechanism of gating in a plant AQP (59). In their model, loop D is a hydrophobic barrier that caps and occludes the intracellular side of the water pore, creating the closed state. Interaction with the N-terminal domain and Ca2+ binding help to anchor loop D into the cytoplasmic pore vestibule decreases the probability of channel opening. Channel opening in response to phosphorylation of conserved serine residues in loop B and C-terminal domains is proposed based on molecular dynamic simulation to involve rearrangement of residues that coordinate Ca2+ ion binding, disrupting the stabilizing interaction between loop D and the N-terminus and opening the hydrophobic gate.

Oclusion of the water pore by a ligand or an intracellular protein domain is appropriate for a process of inhibition. In contrast, a pharmacological agonist might be expected to bind at a separate site, enabling an allosteric modulation that enhances the probability of channel opening without physically interfering with water flux. Current work has suggested that compounds in the chemical AqB.n library of aryl sulfonamide-related derivatives can serve as AQP agonists (Devuyst and Yool, unpublished data). Thus, advances in aquaporin pharmacology may yield new insights into the physiological relevance and clinical roles for these channels as drug targets.

DISCLOSURES

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REFERENCES

10. Preston GM, Jung JS, Guggino WB, Agre P. The mercury-


