

# Vasopressin Antagonists in Polycystic Kidney Disease

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**Summary:** Increased cell proliferation and fluid secretion, probably driven by alterations in intracellular calcium homeostasis and cyclic adenosine 3,5-phosphate, play an important role in the development and progression of polycystic kidney disease. Hormone receptors that affect cyclic adenosine monophosphate and are preferentially expressed in affected tissues are logical treatment targets. There is a sound rationale for considering the arginine vasopressin V2 receptor as a target. The arginine vasopressin V2 receptor antagonists OPC-31260 and tolvaptan inhibit the development of polycystic kidney disease in cpk mice and in three animal orthologs to human autosomal recessive polycystic kidney disease (PCK rat), autosomal dominant polycystic kidney disease (Pkd2/WS25 mice), and nephronophthisis (pcy mouse). PCK rats that are homozygous for an arginine vasopressin mutation and lack circulating vasopressin are markedly protected. Administration of V2 receptor agonist 1-deamino-8-D-arginine vasopressin to these animals completely recovers the cystic phenotype. Administration of 1-deamino-8-D-arginine vasopressin to PCK rats with normal arginine vasopressin aggravates the disease. Suppression of arginine vasopressin release by high water intake is protective. V2 receptor antagonists may have additional beneficial effects on hypertension and chronic kidney disease progression. A number of clinical studies in polycystic kidney disease have been performed or are currently active. The results of phase 2 and phase 2-3 clinical trials suggest that tolvaptan is safe and well tolerated in autosomal dominant polycystic kidney disease. A phase 3, placebo-controlled, double-blind study in 18- to 50-yr-old patients with autosomal dominant polycystic kidney disease and preserved renal function but relatively rapid progression, as indicated by a total kidney volume >750 ml, has been initiated and will determine whether tolvaptan is effective in slowing down the progression of this disease.

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Autosomal-dominant polycystic kidney disease (ADPKD) is the most common of the inherited renal cystic diseases and a leading cause of end-stage renal disease.<sup>1</sup> It is genetically heterogeneous with 2 genes identified: *PKD1* and *PKD2*. Autosomal-recessive polycystic kidney disease (ARPKD) is less common than ADPKD, but together with nephronophthisis is the leading cause of end-stage renal

disease in childhood. It is caused by mutations to *PKHD1*.<sup>2</sup> Currently there is no effective therapy for these diseases. Advances in the understanding of cystogenesis and availability of genetically related animal models provide unique opportunities to develop effective treatments. This article summarizes recent advances, raising the hope that vasopressin V2-receptor antagonists will become a safe and effective therapy for PKD.

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## PATHOGENESIS OF PKD

The cloning of *PKD1* and *PKD2* in 1994 and 1996<sup>3-6</sup> and of *PKHD1* in 2002<sup>7-9</sup> were major steps toward the understanding of PKD. The

proteins encoded by these genes are membrane-associated proteins. Polycystin-2 (PC2 or TRPP2), the protein encoded by *PKD2*, is a TRP channel with high permeability to calcium. Polycystin-1 (PC1) and fibrocystin/polyductin (FC/PD) are thought to be cell surface receptors that directly in the case of PC1<sup>10,11</sup> or indirectly in the case of FC/PD<sup>12</sup> interact with and regulate the channel function of PC2. PC1 and FC/PD also have other functions, some of which are in turn regulated by PC2. For example, PC2 binding to PC1 reduces the ability of PC1 to constitutively activate G proteins.<sup>13</sup>

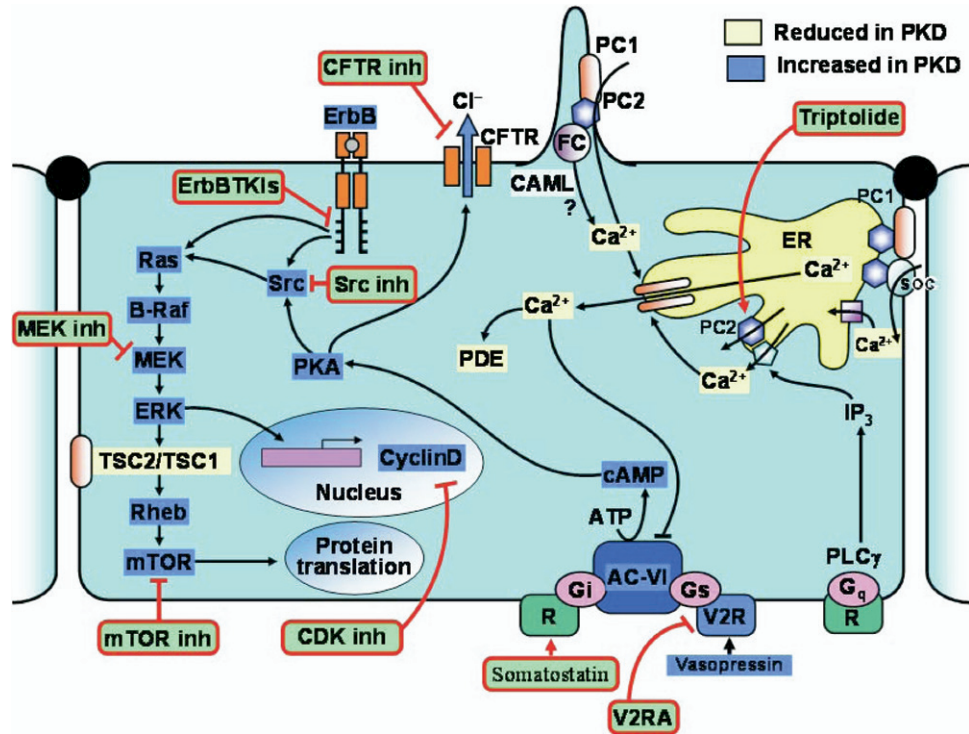
PCs and FC/PD are multifunctional proteins with numerous interacting partners that are essential to maintain the differentiated phenotype of the tubular epithelium.<sup>14</sup> Reduction in one of these proteins below a critical level induces changes in protein trafficking and targeting, cell-matrix and cell-cell interactions, proliferation and apoptosis, planar polarity, and fluid secretion that result in the initiation and growth of cysts.<sup>15</sup> The underlying molecular mechanisms are complex. The PCs and FC/PD participate in kinase cascades that connect interactions at cell-matrix and cell-cell contacts to the regulation of nuclear transcription and cell differentiation.<sup>16-18</sup> PC1 and FC/PD also may undergo regulated intramembrane proteolysis, a process initiated by ligand binding that releases cytoplasmic peptide fragments that migrate to the nucleus and affect transcription.<sup>19-22</sup>

The role of the PKD proteins in primary cilia and regulation of intracellular calcium homeostasis has received the most attention. PC1, PC2, and FC/PD are located in primary cilia.<sup>23-25</sup> In the primary cilia, the PC/FC complex senses and translates mechanical stimulation into calcium entry, which triggers calcium-induced calcium release from the endoplasmic reticulum.<sup>26-28</sup> PC2 also is present in the endoplasmic reticulum, where it interacts with other calcium channels, the IP3R and RR.<sup>29-32</sup> Together, PC2, inositol triphosphate (IP3R), and ryanodine receptor (RR) are responsible for calcium release from intracellular stores. Reductions in the levels of PCs or FC/PD below a critical threshold impair intracellular calcium homeostasis.<sup>33,34</sup> In renal tubular epithelial cells, intracellular calcium limits cyclic adenosine monophosphate (cAMP)

accumulation by inhibiting AC6 and stimulating phosphodiesterase-1.<sup>35-37</sup> This may account for the renal accumulation of cAMP in animal models of ADPKD and ARPKD.<sup>38-41</sup> cAMP stimulates cell proliferation and chloride (cystic fibrosis transmembrane conductance regulator-mediated)-driven fluid secretion.<sup>42-44</sup> Although under normal conditions cAMP inhibits mitogen-activated protein kinase (MAPK) signaling and cell proliferation, in conditions of calcium deprivation such as in PKD it stimulates cell proliferation in an src-, ras-, and b-raf-dependent manner. This proliferative effect may be enhanced further by the stimulation of mislocalized Erb-B receptors by epidermal growth factor-like factors present in cyst fluid.<sup>45</sup> The signaling pathways activated downstream from mutated PC1 converge with those activated in tuberous sclerosis complex, possibly owing to disruption of the physical interaction between PC1 and tuberin or to phosphorylation of tuberin by ERK and Akt, leading to activation of mammalian target of rapamycin.<sup>46</sup>

## OPPORTUNITIES FOR INTERVENTION

The increased understanding of the molecular mechanisms of PKD has provided a number of targets for therapeutic intervention (Fig. 1). Triptolide binds to PC2, induces calcium release by a PC2-dependent mechanism, and ameliorates cystic disease in a *Pkd1* animal model.<sup>47</sup> Consistent with observations of milder disease in patients who have ADPKD and cystic fibrosis,<sup>48,49</sup> cystic fibrosis transmembrane conductance regulator inhibitors inhibit the development of cysts by Madin-Darby canine kidney (MDCK) cells in collagen gels<sup>50</sup> and in metanephric organ cultures<sup>51</sup> by inhibiting chloride secretion. How to apply this strategy without inducing cystic fibrosis will be challenging. Erb-B tyrosine kinase inhibitors have been used successfully in a variety of models, but different Erb-B receptors seem to be important in different animal models.<sup>45,52-55</sup> These drugs have significant toxicity, which may limit their use for extended periods of time. The same can be said for src, mek, and cdk inhibitors.<sup>56-58</sup> This concern is less for mammalian target of rapamycin inhibitors thanks to the extensive experience with this drug in transplantation.<sup>46,59,60</sup>



**Figure 1.** Diagram depicting hypothetical pathways up-regulated or down-regulated in PKD and rationale for treatment with triptolide, cystic fibrosis transmembrane conductance regulator inhibitors, ErbB tyrosine kinase, src, extracellularly regulated kinase (ERK) or cyclin-dependent kinase inhibitors, mammalian target of rapamycin inhibitors, somatostatin, and V2-receptor antagonists. Dysregulation of  $[Ca^{2+}]_i$ ; increased concentrations of cAMP, and mislocalization of ErbB receptors occur in cells/kidneys bearing PKD mutations. Increased accumulation of cAMP in polycystic kidneys may result from: (1) disruption of the polycystin complex because PC1 may act as a Gi-protein-coupled receptor; (2) stimulation of  $Ca^{2+}$  inhibitable AC6 and/or inhibition of  $Ca^{2+}$ -dependent PDE1 by a reduction in  $[Ca^{2+}]_i$ ; (3) increased levels of circulating vasopressin owing to an intrinsic concentrating defect; and (4) up-regulation of vasopressin V2 receptors. Increased cAMP levels contribute to cystogenesis by stimulating chloride and fluid secretion. In addition, cAMP stimulates MAPK/ERK signaling and cell proliferation in a Src- and Ras-dependent manner in cyst-derived cells or in wild-type tubular epithelial cells treated with  $Ca^{2+}$  channel blockers or in a low  $Ca^{2+}$  medium. Activation of mislocalized ErbB receptors by ligands present in cystic fluid also contributes to the stimulation of MAPK/ERK signaling and cell proliferation. Phosphorylation of tuberin by ERK and Akt (or inadequate targeting to the plasma membrane caused by defective interaction with polycystin 1) may lead to the dissociation of tuberin and hamartin and lead to the activation of Rheb and mammalian target of rapamycin. AC-VI, adenylate cyclase 6; CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; mTOR, mammalian target of rapamycin; PDE, phosphodiesterase; PKA, protein kinase A; R, somatostatin sst2 receptor; TSC, tuberous sclerosis proteins tuberin (TSC2) and hamartin (TSC1); V2R, vasopressin V2 receptor; V2RA, vasopressin V2-receptor antagonists. Modified with permission from Torres et al.<sup>1</sup>

### RATIONALE FOR THERAPIES TARGETING THE ARGININE VASOPRESSIN–V2-RECEPTOR AXIS AND RENAL cAMP

Targeting strategies that minimize the effects of a medication on normal cells are essential in chronic diseases that require long-term treatments. The central role of cAMP in the pathogenesis of PKD and the ability to hormonally modulate cAMP in a cell-specific manner provide opportunities for such strategies in PKD.

Among various hormonal systems that may influence the development of PKD, a combination of favorable factors make the arginine vasopressin (AVP)-V2-receptor axis a particularly attractive target.

The cysts in PKD derive predominantly from vasopressin-sensitive tubular segments expressing V2 receptors (ie, the collecting duct and the distal nephron). Although there is general agreement that the cysts in ARPKD and nephronophthisis derive from collecting ducts, a careful

review of the literature indicates that cysts in ADPKD and in slowly progressive Pkd1 and Pkd2 animal models derive predominantly from collecting duct and distal nephron.<sup>61</sup> The expression of V2 receptors is strong in the medullary thick ascending limb, macula densa, and medullary collecting duct, intermediate in connecting tubule and cortical collecting duct, and low in cortical thick ascending limb and distal convoluted tubule.<sup>62</sup>

Vasopressin acting on V2 receptors is the main hormonal regulator of adenylyl cyclase activity in freshly dissected collecting ducts.<sup>63</sup> The V2-induced effects on cAMP and water permeability may be limited by the action of AVP on V1a receptors on apical and basolateral membranes, stimulating phospholipase C, phosphoinositide hydrolysis, and Ca<sup>2+</sup> release from the endoplasmic reticulum.<sup>64</sup>

To avoid dehydration mammals live under the constant tonic action of AVP on the distal nephron and collecting duct.<sup>65-68</sup> Only after drinking large volumes of liquid do plasma AVP levels decrease enough to render the urine more dilute than plasma. Thus, during most of the day, cyst epithelial cells are stimulated persistently to proliferate and secrete fluid.

The circulating levels of AVP are increased in human ADPKD and in all animal models in which it has been ascertained.<sup>69-71</sup> This may be the result of a central defect<sup>72</sup> or, more likely, to compensate for the reduced concentrating capacity of the polycystic kidneys. This concentrating defect may be owing to the disruption of the corticomedullary architecture by the cysts, early development of tubulointerstitial disease, or directly linked to the PKD cellular phenotype.<sup>73</sup> The up-regulation of aquaporin 2 (AQP2) in polycystic kidneys,<sup>38-40,73</sup> in sharp contrast to other forms of nephrogenic diabetes insipidus, suggest enhanced vasopressin activity and a defect distal to the production of AQP2.

In contrast to the V2-receptor down-regulation in other conditions with persistently increased AVP,<sup>74</sup> the V2 receptor is overexpressed in polycystic kidneys.<sup>38-40,73</sup> This is probably owing to the up-regulation of the V2-receptor promoting activity by cAMP.<sup>75</sup>

The restricted expression of V2 receptors to

epithelial cells of the distal nephron and collecting duct<sup>62</sup> and on endothelial cells, where it has been implicated in the secretion of von Willebrand factor,<sup>76</sup> suggest that V2 antagonists are likely to be well tolerated. Indeed, there is already considerable experience with these compounds in clinical trials for congestive heart failure and hyponatremia. These have as main side effects an expected mild to moderate thirst and dry mouth, and increased urination that are all generally well tolerated.<sup>77-81</sup>

## PRECLINICAL TRIALS OF VASOPRESSIN V2-RECEPTOR ANTAGONISTS

Gattone et al<sup>73</sup> initially reported that administration of an AVP-receptor antagonist ameliorated the cystic enlargement and azotemia in a mouse model of rapidly progressive renal cystic disease. To test the effects of AVP V2-receptor antagonists in animal models orthologous to human diseases, we used 2 compounds: OPC-31260 and tolvaptan. OPC-31260, a strong antagonist of the V2 receptor in rodents, is 82 times more selective for rat V2 receptors than for rat V1a receptors.<sup>82</sup> Because OPC-31260 is a relatively weak antagonist of the human V2 receptor, we also used tolvaptan, a stronger antagonist for the human receptor (*K<sub>i</sub>* value 22 times higher than that for OPC-31260), which is 29 times more selective for human V2 receptors than for human V1a receptors.<sup>82</sup>

The PCK rat is a model of human ARPKD caused by a splicing mutation (IVS35-2A→T) that skips exon 36 and leads to a frameshift in *Pkhd1*. Administration of OPC-31260 to PCK rats between 3 and 10 weeks of age reduced the renal accumulation of cAMP and Ras and ERK activation, and inhibited disease development, as reflected by lower kidney weights, plasma creatinine and blood urea nitrogen (BUN) concentrations, renal cyst volumes, and mitotic and apoptotic indices.<sup>38,40</sup> By comparing the kidney weights of the treated and untreated PCK rats with those of 10-week-old wild-type Sprague-Dawley rats, the estimated degree of protection was 60% to 75%, depending on the dose. Administration of OPC-31260 from 10 to 18 weeks of age reduced the renal accumulation of cAMP and inhibited disease progression, as reflected by lower kidney

weights, plasma creatinine and BUN concentrations, renal cyst and fibrosis volumes, mitotic and apoptotic indices, and systolic blood pressures. The weights of the kidneys at 18 weeks of age in the treated rats were identical to those of the control PCK rats at 10 weeks of age, indicating that the administration of OPC-31260 completely halted disease progression. OPC-31260 did not have a significant effect on fibropolycystic liver disease, consistent with the absence of AVP-V2 receptors in the liver.

The *Pkd*<sup>-/WS25</sup> mouse is a double heterozygote for a *Pkd2* null allele and an unstable *Pkd2* WS25 mutation. It is a model of human ADPKD (*PKD2*) that reliably develops renal cysts within 3 months. OPC-31260, administered in the diet to *Pkd2*<sup>-/WS25</sup> mice between 3 and 16 weeks of age, reduced the renal accumulation of cAMP and inhibited disease development, as reflected by lower kidney weights, plasma BUN concentrations, renal cyst and fibrosis volumes, and mitotic and apoptotic indices.<sup>39</sup> The kidney weights of treated *Pkd2*<sup>-/WS25</sup> mice were similar to wild-type, indicating that renal enlargement was prevented. OPC-31260 did not have a significant effect on polycystic liver disease.

The *pcy* mouse is a model of nephronophthisis caused by a missense mutation in *NPHP3*, the gene mutated in adolescent nephronophthisis. Administration of OPC-31260 to CD1/*pcy* mice between 4 and 30 weeks of age inhibited the renal accumulation of cAMP and disease development, as reflected by lower kidney weights, plasma BUN concentrations, renal cyst and fibrosis volumes, and mitotic and apoptotic indices.<sup>38</sup> Administration of OPC-31260 to CD1/*pcy* mice between 15 and 30 weeks of age also inhibited the renal accumulation of cAMP levels and disease progression, as reflected by the lower kidney weights, plasma BUN concentrations, renal cyst and fibrosis volumes, and mitotic and apoptotic indices. Kidney weights of mice started on treatment at 15 weeks and killed at 30 weeks of age were significantly lower than those of untreated mice at 15 weeks of age. This suggests that OPC-31260 not only halted disease progression but also induced disease regression.

To confirm that tolvaptan, a V2-receptor antagonist used in clinical trials for hyponatremia

and congestive heart failure, also is capable of inhibiting the development of PKD, this compound was administered to the same animal models of PKD. In the 3 models, the administration of tolvaptan reduced the renal enlargement and cystic pathology.<sup>40,83,84</sup>

## MODULATION OF RENAL CYSTOGENESIS BY CIRCULATING VASOPRESSIN

To confirm that the protective effect of V2-receptor antagonists is indeed owing to vasopressin V2-receptor antagonism, we generated PCK *AVP*<sup>+/+</sup>, PCK *AVP*<sup>+/-</sup>, and PCK *AVP*<sup>-/-</sup> rats, as well as wild-type and Brattleboro controls, by breeding F1 rats resulting from PCK (*Pkhd1*<sup>-/-</sup>) and Brattleboro (*AVP*<sup>-/-</sup>) crosses. Brattleboro rats are homozygous for a 1-base pair deletion of a guanine nucleotide in the second exon of the *AVP* gene and lack circulating AVP. At 10 and 20 weeks of age PCK *AVP*<sup>-/-</sup> rats showed polyuria and reduced renal cAMP compared with the PCK *AVP*<sup>+/+</sup> rats.<sup>85</sup> This was accompanied by a marked reduction in kidney weight and renal cyst and fibrosis volumes.

To confirm that the protective effect of AVP deficiency on the development of PKD is owing to the lack of stimulation of the renal V2 receptors, PCK *AVP*<sup>-/-</sup>, PCK *AVP*<sup>+/+</sup>, and wild-type rats were treated with the V2 agonist 1-deamino-8-D-arginine vasopressin (DDAVP), administered via osmotic minipumps at a dose of 10 ng/h/100-g body weight between 10 and 20 weeks of age.<sup>85</sup> This dose is the minimal dose necessary to achieve urine osmolalities in Brattleboro rats similar to those observed in wild-type Sprague-Dawley rats. Administration of DDAVP to PCK *AVP*<sup>-/-</sup> corrected the polyuria, increased the renal concentration of cAMP, recovered the full cystic PCK phenotype as reflected by the kidney weights and cyst and fibrosis indices, and significantly increased the plasma BUN concentrations. Administration of DDAVP to PCK *AVP*<sup>+/+</sup> rats increased the severity of PKD, as reflected by significantly higher kidney weights, cyst and fibrosis indices, and plasma BUN concentrations. Administration of DDAVP to wild-type rats at the dose used in this study caused a slight but significant increase in renal mass per unit of body weight

without inducing cystic changes or fibrosis. This is consistent with previous reports of selective AVP-induced hypertrophy of the medullary thick ascending limb in Brattleboro rats.<sup>86,87</sup>

A nongenetic approach to suppress vasopressin action also supports the importance of vasopressin in the modulation of renal cystogenesis. Addition of 5% glucose in the drinking water increased fluid intake and urine output 3.5-fold, reduced urinary AVP excretion, AVP V2-receptor expression, and ERK activation, inhibited proliferation, reduced the severity of the cystic disease, and improved renal function.<sup>88</sup>

#### OTHER OBSERVATIONS SUPPORTING AN EFFECT OF VASOPRESSIN ON RENAL CYSTOGENESIS

The long-acting somatostatin analogue octreotide and the endothelin ET<sub>B</sub> receptor antagonist A-192621 have been reported to have opposing effects in animal models orthologous to human PKD that may be mediated by opposing effects on vasopressin-stimulated cAMP accumulation in the kidney. The administration of octreotide to PCK rats lowers cAMP levels and inhibits the development of PKD, whereas the administration of A-192621 to *Pkd2*<sup>-fws25</sup> mice increases urine osmolarity and renal cAMP and aggravates the severity of the cystic disease.<sup>89,90</sup> At physiologic concentrations somatostatin inhibits vasopressin-induced cAMP generation and water permeability via Gi-coupled somatostatin receptor (SSTR)1 and SSTR2 receptors, which are located predominantly in the distal nephron and collecting tubule.<sup>91-93</sup> On the other hand, endothelin-1 acting via ETB receptors, the predominant endothelin-receptor subtype in the collecting tubules, inhibits vasopressin action and promotes diuresis.<sup>94</sup>

#### OTHER POTENTIAL BENEFITS OF AVP V2-RECEPTOR ANTAGONISTS IN PKD

In addition to its effects on cystogenesis, AVP may have effects on blood pressure and renal function that may be relevant to the progression of PKD.

#### Effects on Blood Pressure

The inverse correlation between urine concentrating capacity and average 24-hour blood pressures in children with ADPKD<sup>71</sup> and the correlation between urine volume and mean arterial blood pressure in Modification of Diet in Renal Disease study participants with ADPKD<sup>95</sup> suggest that the increased circulating levels of AVP observed in ADPKD may contribute to the development of hypertension, one of the most common manifestations of this disease. AVP effects on blood pressure are mediated by V1a and V2 receptors. V1a receptor activation may increase blood pressure by a direct effect on vascular smooth muscle and by reducing medullary renal blood flow and pressure natriuresis.<sup>96</sup> V2-receptor activation enhances  $\beta$  and  $\gamma$  epithelial sodium channel expression and function and acts synergistically with aldosterone in the cortical collecting duct.<sup>97-99</sup> On the other hand, V2-receptor activation also may exert an antihypertensive effect by inducing nitric oxide synthesis in collecting ducts and increasing medullary blood flow.<sup>100</sup> Impaired nitric oxide synthesis, which has been reported in human ADPKD and in animal models of PKD,<sup>101,102</sup> may be a prerequisite for the prohypertensive effect of vasopressin.

#### CKD Progression

AVP levels are increased in CKD.<sup>103</sup> Bankir et al<sup>104</sup> proposed that this contributes importantly to disease progression.<sup>105-107</sup> AVP (or exogenous DDAVP) increases urea and decreases NaCl concentrations in the thick ascending limb of Henle and at the macula densa by increasing intrarenal urea recycling. This results in a suppression of tubuloglomerular feedback and a stimulation of renin secretion that may lead to glomerular hyperfiltration, albuminuria, renal hypertrophy, and tubulointerstitial disease. In support of this hypothesis, suppression of circulating AVP in five-sixths nephrectomized rats by doubling the daily water ingestion has been shown to reduce proteinuria, blood pressure, renal hypertrophy, glomerulosclerosis, and tubulointerstitial fibrosis.<sup>106,108</sup> The attenuation of renal disease progression in five-sixths nephrectomized Brattleboro rats is

reversed by the administration of DDAVP, suggesting that V2 receptors play a major role in the deleterious influence of vasopressin on disease progression.<sup>105</sup> Contrary to these observations, a retrospective analysis of Modification of Diet in Renal Disease patients with baseline glomerular filtration rates (GFRs) of 25 to 55 mL/min/1.73 m<sup>2</sup> raised the possibility that a high fluid intake could be detrimental to patients with chronic renal insufficiency, particularly to those with ADPKD.<sup>95</sup> The patients with the greater urine volumes and the lowest urine osmolalities experienced the fastest GFR declines. Because they tended to have lower serum sodium concentrations and had urines hypotonic to plasma, the investigators concluded that excess water intake and not a renal concentrating defect caused the high urine volume. Further studies will be necessary to elucidate the potential beneficial or detrimental effects of high fluid intake in ADPKD patients with renal insufficiency.

### CLINICAL TRIALS OF VASOPRESSIN V2-RECEPTOR ANTAGONISTS

The observations in animal models of PKD strongly suggest that AVP is a powerful modulator of cystogenesis and provide support for clinical trials of V2-receptor antagonists in this disease. The Tolvaptan Efficacy and Safety in Management of PKD and Outcomes trial (TEMPO) consists of several studies. Two phase 2 studies on the safety, pharmacokinetics, and pharmacodynamics of tolvaptan tablets in ADPKD included 11 and 37 volunteers, 18 to 60 years old, with a serum creatinine level of less than 1.8 mg/dL, randomized to oral placebo or tolvaptan.<sup>109-111</sup> Each study began with a 1-day baseline. Patients drank ad libitum and recorded fluid intake and output.

Study A was a randomized, placebo-controlled (8 treatment, 3 placebo), ascending dose (0, 15, 30, 60, and 120 mg administered 72 hours apart) study. Urine was collected at 0 to 4, 4 to 8, 8 to 12, 12 to 16, and 16 to 24 hours postdosing. Tolvaptan caused dose-dependent increases in urine output and reductions in urine osmolality and AQP2 excretion, without significant changes in cAMP excretion. AQP2 excretion changes paralleled those in urine out-

put. A significant increase in plasma AVP of 2- to 3-fold was seen at the highest dose of tolvaptan (<0.03 at 24 h post-120 mg) when compared with its own baseline, although the difference from the group taking placebo did not reach statistical significance ( $P = .06$ ). The maximum means observed at any time were 4.1 (tolvaptan) and 3.2 ng/L (placebo). Hyposthenuria was sustained during 4 to 16 hours postdosing, but urine output increased to greater than 300 mOsm/L in 5 (15 mg), 2 (30 mg), and 1 (60 mg) patient 16 to 24 hours postdosing. These results indicate that cAMP production may not be inhibited beyond 16 hours postdosing, AQP2 excretion is not superior to urine output to monitor the response, and cAMP excretion is not a good marker of cAMP production in the renal medulla.

In study B, subjects took tolvaptan in doses of 15/15, 30/0, 30/15, or 30/30 mg twice daily (8 AM and 4 PM) for 5 days. The mean urine output increased on average from between 2,974 and 4,586 mL/d by a further 2,974 to 4,586 mL on day 1, declining to a further 1,764 to 2,274 mL on day 5. A negative fluid balance was seen on acute introduction of tolvaptan (-708 to -901 mL), however, this equilibrated by day 5 of study B (-99 to +558 mL). AVP increased dose-dependently with variable significance compared with baseline. For the highest dose, the mean level at day 5 remained in the midnormal range of 1 to 3 ng/L. Twice-daily administration was necessary for adequate suppression of the vasopressin effect reflected by persistent urine hypotonicity and the best result was obtained with the administration of 30 mg twice daily.

In both studies, tolvaptan dose-dependently induced modest increases in serum sodium and osmolality, without changes in other electrolytes. No appreciable changes in vital signs were noted. Thirst appropriately maintained fluid intake. No serious adverse events were reported and no one discontinued tolvaptan in either study. In study A, 21 mild and 3 moderate side effects were reported in the tolvaptan group ( $n = 8$ ) and 4 mild and 1 moderate side effect was reported in the placebo group ( $n = 3$ ). In study B a total of 35 mild and 6 moderate side effects were reported in 21 of 37 subjects.

Dry mouth was the most frequently reported side effect and was not clearly dose dependent.

The pharmacokinetic profile of oral tolvaptan in ADPKD individuals was similar to a healthy control population. In summary, tolvaptan was well tolerated throughout a range of doses and when administered once or twice a day in ADPKD individuals with normal renal function. Twice-daily administration was necessary for adequate suppression of the vasopressin effect reflected by persistent urine hypotonicity.

Forty-six of the 48 participants in the previous phase 2 tolvaptan studies with a GFR of greater than 30 mL/min were enrolled in a 3-year, open-label, phase 2 clinical trial to acquire tolerability, long-term safety, and pilot efficacy data.<sup>112</sup> Initially, tolvaptan was administered in ascending doses of 15/15, 30/15, 45/15, 60/30, or 90/30 mg orally twice daily (8 AM and 4 PM) beginning at 30/15 mg to establish maximal tolerated and minimum effective doses (titration phase); 96%, 61%, and 46% of subjects said they could tolerate 45/15, 60/30, and 90/30 doses for the rest of their life. Subjects then were randomized to a low (45/15, n = 22) or a high (60/30, n = 24) dose extended therapy. Sixteen of the planned 36-month follow-up evaluations had been completed at the time of the last report. Average daily doses have been 59.7 and 82.5 mg. Polyuria has been well tolerated. The median urine osmolalities have ranged from 165 to 253, 123 to 154, and 108 to 152 mOsm/L before AM and PM doses and at bedtime. The serum creatinine level increased from 1.20 and 1.36 mg/dL at baseline to 1.36 and 1.49 mg/dL at 2 months, but had returned toward the baseline level at 16 months (1.27 and 1.39 mg/dL) in the low- and high-dose groups, respectively. The administration of tolvaptan was accompanied by a slight, but sustained, reduction in serum BUN and an increase in serum uric acid. Serum sodium concentrations at 2 and 16 months were unchanged from baseline. Serious adverse events led to discontinuation of tolvaptan in 4 subjects. These included a reversible increase in serum creatinine level from 1.4 to 1.7 mg/d, left periorbital swelling, atrial fibrillation with transient ischemic episode, and pituitary microadenoma. In sum-

mary, these preliminary results from this open-label study suggest that a split dose regimen of tolvaptan is well tolerated, appears to be safe, and is able to sustain urine hypotonicity.

A phase 3, multicenter, double-blind, placebo-controlled, parallel-arm trial of split-dose regimens of tolvaptan has been initiated in 18- to 50-year-old patients, with relatively rapid progression, as indicated by a total kidney volume (TKV) of greater than 750 mL, and relatively preserved renal function as reflected by an estimated GFR of greater than 60 mL/min. The primary outcome measure is renal volume change by magnetic resonance (MR). This clinical trial is expected to enroll 1,200 to 1,500 participants with 3 years' duration of treatment (<http://www.clinicaltrials.gov/ct/show/NCT00428948?order=7>).

In summary, extensive animal studies suggest that AVP is a powerful modulator of cystogenesis, that inhibition of renal cAMP production accounts for the protective effect of V2 receptor antagonists, and that these drugs may afford additional benefits on hypertension and CKD progression. These studies have provided a strong rationale for clinical trials using V2 receptor antagonists in ADPKD which are currently in progress.

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