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Original Investigation

Short-term Effects of Tolvaptan in Individuals With Autosomal Dominant Polycystic Kidney Disease at Various Levels of Kidney Function

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Background: A recent study showed that tolvaptan, a vasopressin V_2 receptor antagonist, decreased total kidney volume (TKV) growth and estimated glomerular filtration rate (GFR) loss in autosomal dominant polycystic kidney disease (ADPKD) with creatinine clearance ≥ 60 mL/min. The aim of our study was to determine whether the renal hemodynamic effects and pharmacodynamic efficacy of tolvaptan in ADPKD are dependent on GFR.

Study Design: Clinical trial with comparisons before and after treatment.

Setting & Participants: Patients with ADPKD with a wide range of measured GFRs (mGFRs; 18-148 mL/min) in a hospital setting.

Intervention: Participants were studied at baseline and after 3 weeks of treatment with tolvaptan given in increasing dosages, if tolerated (doses of 60, 90, and 120 mg/d in weeks 1, 2, and 3, respectively).

Outcomes: Change in markers for aquaresis (free-water clearance, urine and plasma osmolality, 24-hour urine volume, and plasma copeptin) and kidney injury (TKV and kidney injury biomarkers).

Measurements: GFR was measured by ^{125}I-iothalamate clearance; TKV, by magnetic resonance imaging; biomarker excretion, by enzyme-linked immunosorbent assay; and osmolality, by freezing point depression.

Results: In 27 participants (52% men; aged 46 ± 10 years; mGFR, 69 ± 39 mL/min; TKV, 2.15 [IQR, 1.10-2.77] L), treatment with tolvaptan led to an increase in urine volume and free-water clearance and a decrease in urine osmolality, TKV, and kidney injury marker excretion. Changes in urine volume and osmolality with treatment were less in participants with lower baseline mGFRs (both $P < 0.01$). However, change in fractional free-water clearance was greater at lower baseline mGFRs ($P = 0.001$), suggesting that participants with decreased GFRs responded more to tolvaptan per functioning nephron.

Limitations: Limited sample size, no control group.

Conclusions: In patients with ADPKD with decreased kidney function, response to tolvaptan is lower for TKV, urinary volume, and osmolality, but larger for fractional free-water clearance. This latter finding suggests that patients with ADPKD with lower GFRs might benefit from long-term treatment with tolvaptan, as has been observed for patients with preserved GFRs.

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INDEX WORDS: Autosomal dominant polycystic kidney disease (ADPKD); tolvaptan; vasopressin V_2 receptor antagonist; drug efficacy; disease progression; kidney function; glomerular filtration rate (GFR).

Editorial, p. 811

Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary disease that leads to cyst formation, especially in the kidneys, resulting in kidney enlargement and function loss. Fifty percent of affected individuals need renal replacement therapy in their sixth decade of life.[1]

Experimental studies have suggested that arginine vasopressin (AVP) may have a central role in the pathophysiology of this disease. Studies of humans have shown that in patients with ADPKD, higher AVP levels are associated with a decrease in kidney

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function and an increase in total kidney volume (TKV) during follow-up.[2] Blocking the AVP V_2 receptors therefore is a promising therapeutic intervention in this disease. Several experimental studies have demonstrated that AVP V_2 receptor antagonists slow the rate of cyst development and kidney growth in various models for cystic kidney disease.[3-7] In a study involving 20 patients with ADPKD, the AVP V_2 receptor antagonist tolvaptan given at low dose (45/15-mg split dose) was reported to cause a decrease in TKV after 1 week of treatment.[8] A study by Higashihara et al[9] suggested that long-term use of this drug is associated with less increase in TKV and less decrease in kidney function when compared with historical control patients with ADPKD who were matched for several patient characteristics. Recently, the TEMPO (Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes) 3:4 Study[10] prospectively showed that use of tolvaptan, given in dosages between 45/15 and 90/30 mg/d as a split dose, slowed the increase in TKV and decline in kidney function over a 3-year period in 1,445 patients with ADPKD.[11] All 3 mentioned studies were performed in patients with ADPKD with relatively preserved kidney function. Studies of animals and humans have suggested that the efficacy of AVP receptor antagonists may be lower when given at later stages in the disease.[6,8]

We recently completed a study of short-term renal hemodynamic effects of tolvaptan in patients with ADPKD (ClinicalTrials.gov identifier NCT01336972).[12] The renal hemodynamic results showed that changes in glomerular filtration rate (GFR), effective renal plasma flow, and filtration fraction were not different between patients with lower compared to higher GFR kidney function. The aim of the present analyses was to determine whether the pharmacodynamic efficacy of tolvaptan in patients with ADPKD is dependent on kidney function, which would give information about the efficacy of tolvaptan in patients with decreased kidney function. For that reason, we investigated short-term responses on various efficacy parameters to target therapeutic doses of this drug in patients with ADPKD with a wide range of kidney function, including those with GFRs < 30 mL/min/1.73 m^2, and we investigated whether therapy-induced changes in these parameters are dependent on baseline kidney function.

METHODS

Study Population

Study participants were eligible when ADPKD was diagnosed based on the Ravine criteria[13] and were aged 18 to 70 years. Patients were given information about this study at the outpatient clinic by their nephrologists. If they were interested, an appointment with the study physician was made. Participants were included by estimated GFR (eGFR; isotope-dilution mass spectrometry–traceable 4-variable MDRD [Modification of Diet in Renal Disease] Study equation[14]) in 3 strata ($>$60, 30-60, and $<$30 mL/min/1.73 m^2) to ensure that inclusion was balanced to cover a wide range of kidney function. We used eGFR only for inclusion. In all analyses, we used measured GFR (mGFR; iothalamate clearance).

Main exclusion criteria were as follows: diuretic use, pregnancy or breast-feeding, previous exposure to tolvaptan, risk factors for decreased kidney function other than ADPKD (eg, renal cancer, single kidney, active glomerular nephritides, and nephrotoxic drugs), recent renal surgery, diabetes mellitus, contraindications to magnetic resonance imaging (MRI; ferromagnetic prostheses, aneurysm clips, severe claustrophobia, and body mass index > 35 kg/m^2), critical electrolyte imbalances, and uncontrolled hypertension. Participants with hypertension were treated with an angiotensin I–converting enzyme inhibitor or angiotensin II receptor blocker, with the addition of any other antihypertensive drug if needed (except diuretics).

This study was approved by the ethics board at the University Medical Center Groningen (METc 2010.187) and performed in adherence to the ICH-GCP (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use−Good Clinical Practice) guidelines. Written informed consent was obtained from all participants.

Study Design

Participants were screened 2 to 42 days before tolvaptan was administered. They were instructed to collect urine for 24 hours before every kidney function measurement test and not to drink alcohol or use food or beverages containing methyl xanthines within 24 hours of kidney function testing to avoid effects on AVP signaling. Because tolvaptan is a weak cytochrome P450 3A4 (CYP3A4) substrate, participants were instructed not to consume grapefruit or Seville oranges within 72 hours prior to receiving tolvaptan.

One day before initiating tolvaptan treatment, participants visited the clinic for kidney function and TKV measurements (baseline visit). The day after the baseline visit, participants initiated tolvaptan treatment in a split-dose regimen with 45 mg in the morning and 15 mg approximately 8 hours after the first dose. After 1 week of treatment, if this low dosage was tolerated, participants started an intermediate dosage (60/30-mg/d split dose), which after another week, if tolerated, was uptitrated to a split-dose regimen with 90/30 mg/d. On the last day of this 3-week treatment period, as well as 3 weeks after the last dose of tolvaptan, kidney function and TKV were measured again. On the last day of treatment, the highest tolerated dose of tolvaptan was administered 30 minutes after the start of kidney function tracer infusion.

Because of the large number of variables measured in this intensive study protocol, the present study focuses on effects of tolvaptan on efficacy variables, whereas the effects on renal hemodynamics, adverse events, and safety are reported in detail elsewhere.[12]

Measurements and Calculations

On kidney function and TKV measurement days, participants visited our clinic at about 7:45 AM, by which time they had been fasting for 4 hours (but drinking water ad libitum). Blood samples were drawn at around 8:00 AM, in which creatinine (Roche enzymatic assay), effective plasma osmolality (2 × (plasma sodium + plasma potassium) + plasma glucose), plasma and urine osmolality (freezing point depression), and copeptin were measured. Copeptin is a surrogate for AVP and was measured using a chemiluminescence immunoassay (CT-proAVP LIA; Thermo Fisher Scientific Inc) as described previously.[15] Free-water clearance was calculated as urine flow minus osmolar clearance. Osmolar

clearance was calculated by the formula (urine osmolality × urine volume)/plasma osmolality. Fractional free-water clearance was calculated by dividing free-water clearance by GFR.

Kidney function measurements used the constant infusion method of ^{125}I-iothalamate and ^{131}I-hippuran.[16-18] After drawing a time-0 blood sample at about 8:00 AM, a priming solution containing 20 mL of infusion solution (0.04 MBq of ^{125}I-iothalamate and 0.03 MBq of ^{131}I-hippuran) was given, followed by a constant infusion of 6 to 12 mL/h, with the lowest infusion rates in participants with decreased kidney function on the basis of serum creatinine level at screening. Plasma concentrations of both tracers were allowed to stabilize during 1.5 hours, which was followed by two 2-hour periods for simultaneous assessment of ^{125}I-iothalamate and ^{131}I-hippuran clearances. Clearances were calculated as urinary concentration of ^{125}I-iothalamate × urine volume divided by plasma concentration of ^{125}I-iothalamate at the end of each clearance period and infusion rate of ^{131}I-hippuran × plasma volume divided by plasma concentration of ^{131}I-hippuran at the end of each clearance period. Because urinary clearance of ^{131}I-hippuran equals plasma clearance in case of perfect urine collection, we routinely use the ratio of plasma to urinary clearance of ^{131}I-hippuran to correct urinary clearance of ^{125}I-iothalamate as a measure of GFR for voiding errors. The mean of the 2 GFR values is used for analyses.

Immediately after completing the kidney function test, participants underwent a standardized abdominal MRI protocol without the use of intravenous contrast to measure TKV. Scanning was performed on a 3-Tesla (T) research magnetic resonance scanner (Intera; Philips) or a 1.5-T magnetic resonance scanner (MAGNETOM Avanto; Siemens) in case of contraindications for the 3-T scanner that are not an issue on a 1.5-T scanner (n = 8). Cardio matrix coils were used for the 3-T scanner and body matrix and spine matrix coils were used for the 1.5-T MRI. Slice thickness was 4.0 mm. Alice software (Perceptive Informatics) is used to measure TKV by calculating the volume of serial renal outlines that have been verified by independent radiologists familiar with ADPKD.

Plasma samples for measurement of tolvaptan were collected at the final treatment visit (highest dose of tolvaptan) at 6 time points: 8:00 AM (before the start of kidney function measurement), 9:30 AM (1 hour after taking 90 mg of tolvaptan), 10:30 AM, 11:30 AM; 12:30 PM, and 1:30 PM. Median tolvaptan levels per eGFR group at the different measurement time points are shown in Fig S1 (provided as online supplementary material). Tolvaptan was measured in these samples using a reverse-phase high-performance liquid chromatography system with tandem mass spectrophotometric detection, as described previously.[19] The lower limit of quantitation was 5.00 ng/mL.

Samples from 24-hour urine collections were used to measure concentrations of urinary markers representing damage to different nephron segments. As glomerular damage markers, we measured immunoglobulin G (IgG) and albumin; as proximal tubular damage markers, neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 (KIM-1)[20]; as distal tubular damage marker, heart-type fatty acid binding protein (H-FABP) [21]; and as inflammatory marker, monocyte chemotactic protein 1 (MCP-1).[22] Urine was stored at −80°C and thawed before measurement. All biomarkers were measured by enzyme-linked immunosorbent assay. For KIM-1, MCP-1, and NGAL, antibodies were obtained from R&D Systems Inc. H-FABP and IgG antibodies were obtained from HyTest Ltd. If the measured value was below the lower limit of detection, we used the lower limit of detection value divided by 2. To calculate 24-hour excretion, concentrations were multiplied by 24-hour urine volume. In case participants had macroscopic hematuria (possibly due to cyst rupture), they were excluded from the analysis of urinary biomarkers because blood contamination may interfere with the assays measuring these biomarkers (n = 2).

Statistical Analyses

Analyses were performed at the study center with SPSS, version 20.0 (SPSS Inc). Parametric-distributed variables are given as mean ± standard deviation, whereas nonparametric-distributed variables are given as median with interquartile range. For all analyses, 2-sided $P < 0.05$ was considered to indicate statistical significance. For Pearson correlation tests, all variables with a skewed distribution were logarithmically transformed to fulfil the requirement of normal distribution of residuals. Pearson, or Spearman in case a variable had no normal distribution even after log-transformation, correlation test was performed to assess associations between baseline GFR and (changes in) various variables. Differences between baseline and final treatment variables were tested with a paired *t* test or, in case of nonparametric distributed variables, Wilcoxon signed rank test.

RESULTS

Twenty-nine individuals were included, of whom 27 completed the study (n = 9 per eGFR stratum); 2 patients withdrew because of adverse events (one after 3 days of treatment because of polyuria and another after 13 days because of xerostomia). Characteristics of the 27 participants who completed the study are given in Table 1. All participants completed the study on the 90/30-mg/d tolvaptan split-dose regimen, except one participant who did not tolerate the highest dose and completed the study on 60/30 mg. Mean baseline mGFR was 69.1 ± 38.6 (range, 18-148) mL/min.

The effects after 3 weeks of treatment with tolvaptan are given in Table 2. A significant decrease in TKV and mGFR was observed, as well as in all other measured variables. This decrease was not seen in urinary MCP-1 and KIM-1 concentrations, which were essentially unchanged. By contrast, plasma osmolality, copeptin concentration, and urine volume increased significantly. All these changes were reversible after 3 weeks' withdrawal of tolvaptan; only TKV remained slightly but significantly lower than the baseline value (median volume, 2,103 [interquartile range, 1,080-2,737] mL; change, −1.7% ± 2.9%; $P = 0.006$).

At baseline, mGFR was negatively associated significantly with absolute values of TKV, plasma copeptin, 24-hour urine volume, free-water clearance, and fractional free-water clearance and positively associated significantly with urine osmolality (Table 3). This indicates that participants with lower mGFRs had larger kidneys; higher plasma copeptin level, 24-hour urine volume, and free-water clearance; and lower urine osmolality. We also found significant associations between mGFR and excretion of several urinary biomarkers. Lower mGFR was associated with higher urinary excretion of albumin, IgG, NGAL, and H-FABP, whereas we found no association between mGFR and urinary excretion of KIM-1 and MCP-1. As an extra analysis, we also investigated the association between study variables and effective renal plasma flow (measured by hippuran clearance). This analysis showed essentially the same results as the association with mGFR.

Table 1. Baseline Characteristics of Study Participants, Stratified by eGFR at Inclusion

	eGFR > 60 mL/min/1.73 m^2 (n = 9)	eGFR = 30-60 mL/min/1.73 m^2 (n = 9)	eGFR < 30 mL/min/1.73 m^2 (n = 9)	*P*[a]
eGFR (mL/min/1.73 m^2)	83 [75 to 95]	47 [39 to 60]	18 [16 to 25]	<0.001
Age (y)	37 [35 to 45]	47 [38 to 57]	52 [48 to 58]	0.02
Male sex	4 (44)	3 (33)	7 (78)	0.2
BMI (kg/m^2)	24.5 [22.0 to 26.9]	23.9 [21.7 to 27.9]	27.2 [24.2 to 30.6]	0.3
Mean arterial pressure (mm Hg)[b]	88 [85 to 95]	86 [84 to 92]	94 [89 to 109]	0.06
Antihypertensive drug use	7 (78)	8 (89)	9 (100)	0.3
ACEi/ARB use	7 (78)	8 (89)	8 (89)	0.8
Serum creatinine (μmol/L)	76 [69 to 80]	135 [89 to 143]	280 [242 to 319]	<0.001
Plasma copeptin (pmol/L)	6.4 [3.6 to 7.6]	9.2 [3.8 to 18.5]	30.0 [13.0 to 45.2]	0.001
Effective plasma osmolality (mOsm/kg)[c]	293 [291 to 296]	296 [294 to 299]	294 [293 to 299]	0.3
24-hour urine				
Volume (mL/24 h)	1,720 [1,530 to 2,575]	2,790 [2,358 to 3,325]	3,050 [1,935 to 3,200]	0.02
Osmolality (mOsm/kg)	499 [379 to 632]	291 [200 to 381]	303 [281 to 346]	0.002
Albumin (mg/24 h)	26 [17 to 42]	28 [18 to 133]	144 [53 to 480]	0.01
IgG (mg/24 h)	6.1 [3.6 to 8.9]	3.9 [1.7 to 18.7]	18.6 [10.9 to 73.2]	0.02
NGAL (μg/24 h)	40.4 [24.4 to 73.5]	56.5 [46.9 to 92.9]	304.5 [172.6 to 552.2]	0.002
H-FABP (μg/24 h)	1.0 [0.5 to 1.4]	0.9 [0.3 to 2.7]	11.3 [4.5 to 17.0]	0.001
MCP-1 (μg/24 h)	1.0 [0.7 to 1.4]	0.9 [0.8 to 2.2]	0.8 [0.7 to 1.1]	0.6
KIM-1 (μg/24 h)	1.7 [0.9 to 2.0]	1.5 [0.8 to 2.1]	1.6 [1.5 to 2.1]	0.8
Free-water clearance (L/24 h)	−1.3 [−2.1 to −0.8]	−0.0 [−0.7 to 1.1]	−0.1 [−0.5 to 0.1]	0.002
Fractional free-water clearance (%)	−0.9 [−1.1 to −0.5]	−0.1 [−0.7 to 1.1]	−0.2 [−1.1 to 0.3]	0.06
mGFR (mL/min)	110 [96 to 128]	57 [48 to 88]	28 [20 to 40]	<0.001
TKV (L)	1.1 [0.7 to 2.1]	1.3 [0.9 to 2.7]	2.8 [2.4 to 6.7]	0.005

Note: Values for categorical variables are given as number (percentage); values for continuous variables are given as median [interquartile range]. Conversion factor for serum creatinine in μmol/L to mg/dL, ×1/88.4.

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate; H-FABP, heart-type fatty acid binding protein; IgG, immunoglobulin G; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemotactic protein 1; mGFR, measured glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; TKV, total kidney volume.

[a]*P* values were calculated with Kruskal-Wallis test (comparison among 3 groups).

[b]Mean arterial pressure (calculated with the mean of 3 blood pressure measurements after 10 minutes of rest) was measured 10 minutes after the start of tracer infusion.

[c]Plasma osmolality was measured before tracer infusion started.

Table 2. Changes in Study Variables During Tolvaptan Treatment

	Baseline	Final Treatment	Absolute Change	Percentage Change	*P*[a]
Effective plasma osmolality (mOsm/kg)	295.2 ± 3.6	299.6 ± 4.6	4.4 ± 4.2	1.5% ± 1.4%	<0.001
Plasma copeptin (pmol/L)	9.6 [4.8 to 25.5]	28.4 [16.6 to 41.1]	15.8 [11.4 to 20.7]	182% [109% to 288%]	<0.001
Urine volume (mL/24 h)	2,584 ± 839	5,930 ± 1,790	3,347 ± 1,689	146.0% ± 99.9%	<0.001
Urine osmolality (mOsm/kg)	359 [289 to 425]	139 [126 to 173]	−208 [−254 to −136]	−55% ± 17%	<0.001
Albuminuria (mg/24 h)	45 [20 to 133]	43 [21 to 105]	−7 [−13 to 8]	−10% ± 39%	0.03
IgG (mg/24 h)	9.1 [3.7 to 21.9]	1.3 [0.1 to 12.4]	−4 [−9 to −1]	−74% [−98% to −18%]	0.001
NGAL (μg/24 h)	72.8 [44.7 to 200.7]	56.0 [24.8 to 167.5]	−10 [−20 to 4]	−21% ± 33%	0.02
H-FABP (μg/24 h)	1.8 [0.6 to 5.9]	0.5 [0.2 to 4.4]	−0.8 ± 3.0	−57% [−79% to 14%]	0.009
MCP-1 (μg/24 h)	0.9 [0.7 to 1.4]	0.8 [0.6 to 1.3]	−0.2 [−0.5 to 0.3]	−15% [−73% to 19%]	0.8
KIM-1 (μg/24 h)	1.5 ± 0.8	1.6 ± 0.8	0.0 ± 0.7	10% ± 52%	0.9
Free-water clearance (L/24 h)	−0.5 ± 1.0	3.0 ± 1.3	3.5 ± 1.7		<0.001
Fractional free-water clearance (%)	−0.2 ± 1.0	4.2 ± 2.4	4.4 ± 2.3		<0.001
mGFR (mL/min)	69.1 ± 38.6	64.4 ± 35.7	−3.0 [−9.0 to −0.3]	−5.4% ± 8.0%	0.002
TKV (mL)	2,147 [1,100 to 2,767]	2,052 [1,040 to 2,690]	−60 [−103 to −16]	−3.7% ± 3.0%	<0.001

Note: Values are given as mean ± standard deviation or median [interquartile range]. The 24-hour urine was collected the day before kidney function measurement. Plasma samples were collected in the morning before kidney function measurement. TKV was measured on day of kidney function measurement.

Abbreviations: H-FABP, heart-type fatty acid binding protein; IgG, immunoglobulin G; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemotactic protein 1; mGFR, measured glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; TKV, total kidney volume.

[a]*P* value represents significance of difference between baseline and treatment variables.

Table 3. Associations Between Baseline mGFR and Absolute Study Variables at Baseline and During Tolvaptan Treatment

	Baseline		Treatment	
	β[a]	*P*	β[a]	*P*
Plasma				
ln(copeptin)	−0.764	<0.001	−0.632	0.001
Effective osmolality	−0.146	0.5	0.073	0.7
C_{max}	NA	NA	−0.025	0.9
24-hour urine				
Volume	−0.374	0.05	0.380	0.05
ln(osmolality)	0.654	<0.001	0.073	0.7
ln(albumin)	−0.557	0.004	−0.561	0.004
IgG	−0.507	0.01	−0.671	<0.001
ln(NGAL)	−0.649	<0.001	−0.726	<0.001
ln(H-FABP)	−0.540	0.005	−0.647	<0.001
ln(MCP-1)	0.082	0.7	−0.461	0.02
KIM-1	0.068	0.7	−0.119	0.6
FWC	−0.668	<0.001	0.228	0.3
FFWC	−0.415	0.03	−0.757	<0.001
ln(TKV)	−0.566	0.002	−0.574	0.002

Note: The 24-hour urine was collected the day before kidney function measurement. Plasma samples were collected in the morning, before kidney function measurement. TKV was measured on the day of kidney function measurement.

Abbreviations: C_{max}, maximum tolvaptan concentration; FFWC, fractional free-water clearance; FWC, free-water clearance; H-FABP, heart-type fatty acid binding protein; IgG, immunoglobulin G; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemotactic protein 1; mGFR, measured glomerular filtration rate; NA, not applicable; NGAL, neutrophil gelatinase-associated lipocalin; TKV, total kidney volume.

[a]β is the correlation coefficient (Pearson coefficient; except for IgG, Spearman coefficient was given).

Table 3 also shows associations between baseline mGFR and the mentioned absolute variables measured during tolvaptan use. In general, results were similar, but 3 exceptions were noted. The associations of baseline mGFR with urine osmolality and free-water clearance lost significance, and the association with 24-hour urine volume reversed; that is, participants with lower mGFRs had lower 24-hour urine volumes during tolvaptan use.

Some of the changes in study variables during tolvaptan treatment were associated with baseline mGFR (Tables 4 and 5). For instance, the lower the baseline mGFR, the lower the increase in urine volume and the lower the decrease in urine osmolality and mGFR. Lower mGFR furthermore was associated with less decrease in TKV when expressed as percentage ($P = 0.06$), but not when expressed as absolute change (Fig 1). The same held true for change in copeptin levels. Free-water clearance also increased less in participants with lower mGFRs, but when free-water clearance is expressed normalized for mGFR (fractional free-water clearance), it shows that the change in free-water clearance per single nephron was stronger in those with lower mGFRs (Fig 2). Changes in excretion of urinary biomarkers during tolvaptan use were not associated with baseline mGFR, except for IgG and MCP-1.

Of note, there was no significant association between baseline mGFR and maximal tolvaptan concentration (C_{max}; mean, 745 ± 305 ng/mL; $P = 0.7$) or area under the concentration curve ($AUC_{0\text{-}5h}$; mean, 2,673 ± 956 h·ng/mL; $P = 0.5$). In general, C_{max} and AUC_t (where t = time) were not associated with absolute or percentage changes in the study variables listed in Table S1. Similarly, baseline and on-treatment copeptin concentrations were not associated with changes in these study variables, except for absolute change in fractional free-water clearance. The higher the copeptin level at baseline, the more the increase in fractional free-water clearance (Table S2).

DISCUSSION

In this study, we found that 3-week treatment with tolvaptan caused an increase in urine volume, free-water clearance, and plasma copeptin level and a decrease in urine osmolality. Furthermore, tolvaptan induced a decrease in TKV and urinary excretion of biomarkers that are associated with damage to various nephron segments. In this study, we investigated whether the pharmacodynamic effects of tolvaptan were dependent on kidney function, which is important to know because if tolvaptan is effective only in patients with preserved kidney function, early diagnosis and lifelong treatment (with side effects) would be preferable. The changes in urine volume, free-water clearance, urine osmolality, and TKV were less pronounced in participants with lower baseline mGFRs. However, when using absolute change in TKV, this effect was similar in participants with lower compared with higher mGFRs. However, in participants with lower mGFRs, the increase in fractional free-water clearance was greater than in participants with higher GFRs. This latter finding suggests that tolvaptan also is effective in patients with decreased kidney function.

Experimental research in a murine model has suggested that in a more advanced stage of ADPKD, V_2 receptor antagonists may be less effective.[6] One possible reason is that AVP is upregulated to make up for the impaired urinary concentrating capacity in later-stage disease. This led to the hypothesis that when the agonist (AVP) is increased, the dose of the antagonist (tolvaptan) should be increased.[6] However, our data show that higher plasma tolvaptan levels did not result in more effects, and there was no association between baseline (or on-treatment) copeptin level and most effects of tolvaptan. However, we cannot be sure if higher tolvaptan dosages lead to more effects in patients with lower GFRs because we did not give higher dosages (to our knowledge, this is the first study using this drug in decreased kidney function).

Table 4. Absolute Change From Baseline After 3 Weeks of Tolvaptan Treatment (by eGFR group) and the Association Between Baseline mGFR and Absolute Change in the Variable Under Study

	eGFR at Inclusion			Association With Baseline mGFR	
	>60 mL/min/1.73 m^2	30-60 mL/min/1.73 m^2	<30 mL/min/1.73 m^2	β	*P*
Plasma copeptin (pmol/L)	14.8 [9.5 to 20.7]	17.0 [12.2 to 25.9]	16.3 [12.4 to 20.0]	−0.305	0.1
Effective plasma osmolality (mOsm/kg)	4.6 [2.1 to 7.5]	4.3 [0.6 to 8.8]	6.5 [0.3 to 8.8]	0.175	0.4
Urine volume (mL/24 h)	5,475 [2,518 to 6,113]	3,435 [2,437 to 4,015]	1,860 [1,460 to 3,078]	0.588	0.01
Urine osmolality (mOsm/kg)	−284 [−458 to −252]	−165 [−240 to −75]	−145 [−184 to −107]	−0.716	<0.001
Albuminuria (mg/24 h)	−8 [−13 to 1]	−7 [−28 to 2]	3 [−32 to 34]	−0.064	0.8
IgG (mg/24 h)	−5 [−9 to −3]	−3.8 [−11.1 to −1.0]	−0.5 [−14.5 to 2.1]	−0.202	0.3
NGAL (μg/24 h)	−12 [−20 to −8]	−12 [−21 to 2]	10 [−45 to 43]	−0.373	0.07
H-FABP (μg/24 h)	−0.3 [−1.3 to −0.1]	−0.7 [−2.2 to −0.1]	−1.4 [−5.4 to 2.7]	0.120	0.6
MCP-1 (μg/24 h)	−0.3 [−0.6 to −0.0]	−0.3 [−0.8 to −0.2]	0.5 [0.2 to 1.1]	−0.387	0.06
KIM-1 (μg/24 h)	0.1 [−0.4 to 0.4]	0.0 [−0.5 to 0.4]	0.1 [−0.1 to 1.0]	−0.181	0.4
Free-water clearance (L/24 h)	5.2 [2.8 to 6.2]	3.4 [2.8 to 4.3]	2.3 [1.9 to 3.2]	0.566	0.002
Fractional free-water clearance (%)	3.2 [2.1 to 4.2]	4.2 [2.8 to 4.8]	6.7 [3.4 to 7.8]	−0.584	0.001
mGFR (mL/min)	−8 [−14 to −2]	−4 [−12 to −1]	−1 [−1 to 2]	−0.500	0.008
TKV (mL)	−61 [−82 to −10]	−99 [−120 to −30]	−47 [−110 to −8]	−0.053	0.8

Note: Values are given as median [interquartile range]. Data are absolute change from baseline after 3 weeks of tolvaptan treatment in 3 study groups stratified by eGFR at inclusion and association between baseline mGFR and absolute change in variable under study in 3 groups combined. Spearman correlation coefficients (β) are given for absolute change in urine osmolality, albuminuria, TKV, GFR, IgG, and NGAL. For all other associations, Pearson correlation coefficients are shown. The 24-hour urine was collected the day before kidney function measurement. Plasma samples were collected in the morning before kidney function measurement. TKV was measured on the day of kidney function measurement. Interpretation: higher GFR is associated with an increase in urine volume (positive association) and decrease in urine osmolality (negative association).

Abbreviations: eGFR, estimated glomerular filtration rate; H-FABP, heart-type fatty acid binding protein; IgG, immunoglobulin G; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemotactic protein 1; mGFR, measured glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; TKV, total kidney volume.

Table 5. Percentage Change From Baseline After 3 Weeks of Tolvaptan Treatment (by eGFR group) and Association Between Baseline mGFR and Percentage Change in Variable

	eGFR at Inclusion			Association With Baseline mGFR	
	>60 mL/min/1.73 m^2	30-60 mL/min/1.73 m^2	<30 mL/min/1.73 m^2	β	*P*
Plasma copeptin	237% [192% to 353%]	187% [104% to 413%]	94% [42% to 134%]	0.611	0.001
Effective plasma osmolality	1.6% [0.7% to 2.6%]	1.5% [0.2% to 2.9%]	2.2% [0.1% to 3.0%]	0.176	0.4
Urine volume	216% [162% to 310%]	128% [75% to 151%]	75% [48% to 110%]	0.633	<0.001
Urine osmolality	−66% [−80% to −60%]	−58% [−66% to −38%]	−49% [−54% to −38%]	−0.546	0.003
Albuminuria	−30% [−39% to 6%]	−28% [−43% to 4%]	−1% [−19% to 40%]	−0.190	0.4
IgG	−98% [−98% to −94%]	−74% [−96% to −48%]	0% [−35% to 19%]	−0.684	>0.001
NGAL	−37% [−55% to −18%]	−17% [−49% to 3%]	2% [−14% to 20%]	−0.386	0.06
H-FABP	−47% [−89% to −17%]	−74% [−79% to 114%]	−8% [−52% to 50%]	−0.261	0.2
MCP-1	−36% [−71% to −2%]	−32% [−195% to −13%]	50% [14% to 82%]	−0.467	0.02
KIM-1	10% [−20% to 36%]	3% [−45% to 28%]	4% [−6% to 46%]	−0.047	0.8
mGFR	−7% [−15% to −1%]	−7% [−15% to −2%]	−3% [−6% to 6%]	−0.336	0.09
TKV	−5% [−8% to −1%]	−4% [−7% to −2%]	−2% [−4% to 0%]	−0.361	0.06

Note: Values are given as median [interquartile range]. Data are percentage change from baseline after 3 weeks of tolvaptan treatment in 3 study groups stratified by eGFR at inclusion, and association between baseline mGFR and percentage change in variable under study in 3 groups combined. In the right columns, Spearman correlation coefficient (β) with *P* value is given for percentage change in MCP; for all other associations, Pearson correlation coefficients are shown. The 24-hour urine was collected the day before kidney function measurement. Plasma samples were collected in the morning before kidney function measurement. TKV was measured on the day of kidney function measurement. Interpretation: higher GFR is associated with an increase in urine volume (positive association) and decrease in urine osmolality (negative association).

Abbreviations: eGFR, estimated glomerular filtration rate; H-FABP, heart-type fatty acid binding protein; IgG, immunoglobulin G; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemotactic protein 1; mGFR, measured glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; TKV, total kidney volume.

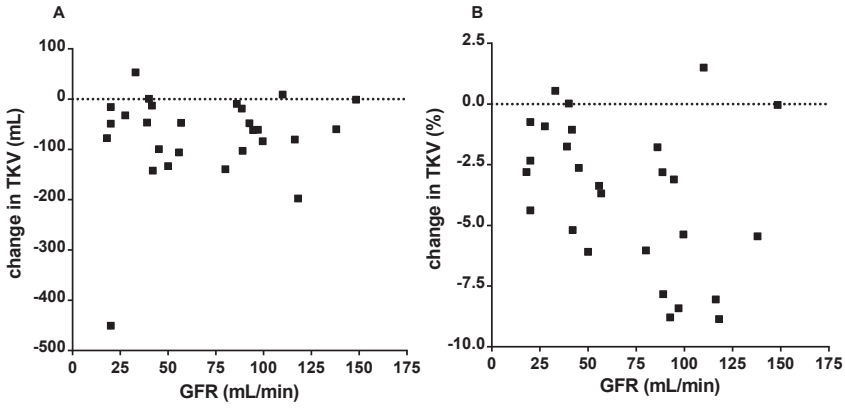

Figure 1. Association between baseline measured glomerular filtration rate (GFR) and (A) absolute (correlation coefficient = −0.053; $P = 0.8$) and (B) percentage (correlation coefficient = −0.361; $P = 0.06$) change in total kidney volume (TKV) during tolvaptan treatment. (Left) Measured GFR is not associated with change in TKV when expressed as absolute value. (Right) In contrast, lower mGFR is associated with less decrease in TKV when expressed as percentage.

Based on results of a study in which patients were administered low-dose tolvaptan (45/15-mg split-dose regimen) for 1 week, Irazabal et al[8] also suggested that tolvaptan may be less effective in lowering TKV in later-stage ADPKD. We similarly observed that in patients with lower GFRs, tolvaptan tended to have less effect on TKV when expressed as percentage change. Moreover, these patients also had less increase in urinary volume and less decrease in urinary osmolality. These results at first sight can be interpreted as participants with lower GFRs showing less responsiveness to tolvaptan.

However, in the present data are several indications that tolvaptan might have similar efficacy in patients with ADPKD with lower GFRs. First, we found that absolute change in TKV with treatment is not associated with baseline mGFR. It may be that it is not possible to achieve more than the 60-mL decrease in kidney volume that we observed during the limited period of 3 weeks' treatment with tolvaptan. Because patients with lower GFRs have higher TKV at baseline, this similar absolute change results in a percentage less change in TKV in patients with low mGFRs at baseline, but that does not automatically indicate less efficacy of the drug. However, changes in TKV during 1 and 3 weeks' use of tolvaptan reflect fluid secretion probably more than cyst growth. Studies that look at longer term effects on cyst growth have yet to be conducted. Second, patients with lower GFRs showed less decrease in urine osmolality during treatment. However, it should be taken into account that their urine osmolality was already lower at baseline, probably because patients with lower GFRs have less urine concentration capacity. The urine osmolality that was reached during treatment (mean, 149 ± 35 mOsm/kg) was not dependent on baseline mGFR ($P = 0.5$). Also, in the group of participants with decreased kidney function (eGFR < 30 mL/min/1.73 m^2) at study inclusion, urine osmolality decreased significantly to this low level, which is approximately the maximal urine-diluting capacity. In all participants, urine osmolality was lower than plasma osmolality during treatment, suggesting that the AVP V_2 receptor was adequately blocked in all participants independent of mGFR. Third, during tolvaptan use, urinary excretion of all biomarkers indicating damage to various nephron segments (except KIM-1) decreased. For nearly all markers, absolute and percentage reductions were independent of mGFR, suggesting that tolvaptan reduced damage to the various nephron segments also in participants with lower mGFRs. Fourth and most important, to reliably assess the renal physiologic effects of tolvaptan, it may be advisable to adjust for functioning

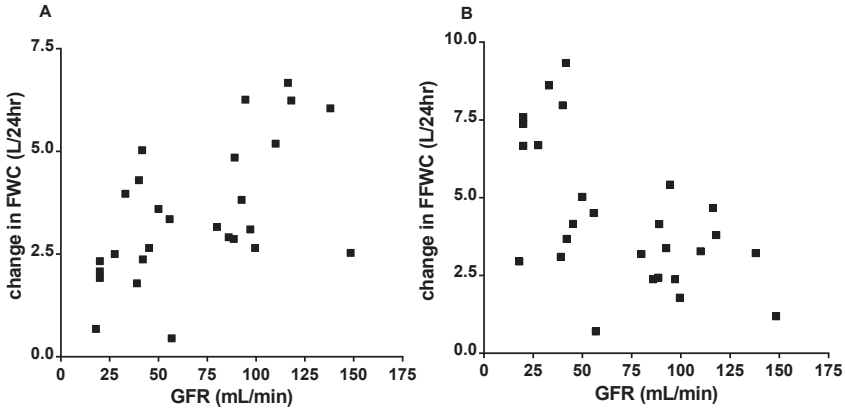

Figure 2. Association between baseline measured glomerular filtration rate (GFR) and absolute change in (A) free-water clearance (FWC; correlation coefficient = 0.566; $P = 0.002$) and (B) fractional FWC (FFWC; correlation coefficient = −0.584; $P = 0.001$) during tolvaptan treatment. In participants with lower measured GFR, FWC increased less (left), but when FWC is expressed normalized for mGFR (FFWC), it shows that the FWC per functioning nephron was higher in those with lower measured GFRs (right).

nephron mass. This is done by studying free-water clearance normalized for GFR, that is, fractional free-water clearance. The data with respect to this latter variable suggest that per functioning nephron, most effect was observed in participants with low baseline mGFRs.

Besides these findings, we found a significant association between mGFR at baseline and markers for proximal (NGAL) and distal tubular damage (H-FABP), and also with albuminuria (marker for glomerular and tubular damage) and IgG (marker for glomerular damage). These data corroborate previous findings in patients with ADPKD[23] and indicate that this disease is associated not only with tubular damage, the nephron segment where cysts are formed, but also with glomerular damage. More importantly, to our knowledge, this study is the first to investigate the effects of tolvaptan on these damage markers in ADPKD. After 3 weeks of treatment, we found a significant decrease in urinary excretion of all biomarkers that were associated with mGFR at baseline, suggesting that tolvaptan reduces tubular and glomerular kidney damage in patients with ADPKD.

Taking all the mentioned points into consideration, we hypothesize that patients with ADPKD with lower GFRs also might benefit from long-term treatment with tolvaptan, as has been observed for patients with relatively preserved GFRs.[11] These data should be considered hypothesis generating, and the renoprotective effect of tolvaptan in patients with ADPKD with decreased GFR can be proved only in long-term, large-scale, randomized, controlled trials.

We acknowledge that this study has limitations. First, the number of participants is relatively small, which increases the chance of type I errors. It should be acknowledged that it is difficult to include large numbers of participants in an intensive study such as the present one. Furthermore, we found significant associations between baseline mGFR and changes in most study variables, suggesting that the number of included participants is sufficient to reach conclusions. Second, patients took tolvaptan for only 3 weeks. Assessing long-term effects of this drug needs additional study. Strengths of this study are the inclusion of participants with a wide range of GFRs and the measurement of GFR with a gold-standard method at 3 time points, instead of using eGFR. In addition, we measured kidney volume with the reference method (MRI) at these 3 time points.

In conclusion, response to tolvaptan on urinary volume, urinary osmolality, and TKV is lower in patients with ADPKD with decreased kidney function when using relative changes. In contrast, in patients with lower kidney function, the effect of tolvaptan on fractional free-water clearance is more distinct. Based on these latter results, we hypothesize that the lower relative response on urinary volume, urinary osmolality, and TKV in patients with decreased kidney function is not due to decreased sensitivity for tolvaptan, but could be due to less functioning renal mass or structural renal abnormalities. In addition, we found that absolute changes in TKV, plasma copeptin, and urinary biomarker excretion were not associated with baseline GFR, suggesting that patients with ADPKD with lower GFRs also might benefit from long-term treatment with tolvaptan, as has been observed for those with relatively preserved GFRs.

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Contributions: Research idea and study design: WEB, EM, PEdJ, FSC, HBK, DO, JO, RTG; data acquisition: WEB, RTG, GJtH, RJR, EJvdJ, PK, GEE, WvO, JS; data analysis/interpretation: WEB, EM, PEdJ, RTG; statistical analysis: WEB, EM, RTG; supervision or mentorship: PEdJ, RTG, WvO. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. RTG takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and registered) have been explained.

SUPPLEMENTARY MATERIAL

Table S1: Association of C_{max} and AUC_t with percentage and absolute change in efficacy variables.

Table S2: Association of baseline and on-treatment plasma copeptin concentration and percentage and absolute change in efficacy variables.

Figure S1: Median tolvaptan concentrations during treatment in 3 study groups.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2014.11.010) is available at www.ajkd.org

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