Therapeutic potential of vasopressin V2 receptor antagonist in a mouse model for autosomal dominant polycystic kidney disease: optimal timing and dosing of the drug

E. Meijer¹, R.T. Gansevoort¹, P.E. de Jong¹, A.M. van der Wal², W.N. Leonhard³, S.R. de Krey³, J. van den Born¹, G.M. Mulder⁴, H. van Goor⁴, J. Struck⁵, E. de Heer² and D.J.M. Peters³

¹Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, ²Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands, ³Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands, ⁴Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands and ⁵Research Department, B.R.A.H.M.S. AG, Hennigsdorf/Berlin, Germany

Correspondence and offprint requests to: R.T. Gansevoort; E-mail: r.t.gansevoort@int.umcg.nl

Abstract

Background. The renoprotective effect of vasopressin V2 receptor antagonist (V2RA) is currently being tested in a clinical trial in early autosomal dominant polycystic kidney disease (ADPKD). If efficacious, this warrants life-long treatment with V2RA, however, with associated side effects as polydipsia and polyuria. We questioned whether we could reduce the side effects without influencing the renoprotective effect by starting the treatment later in the disease or by lowering drug dosage.

Methods. To investigate this, we administered V2RA OPC-31260 at a high (0.1%) and low (0.05%) dose to a tamoxifen-inducible kidney epithelium-specific Pkd1-deletion mouse model starting treatment at Day 21 (early) or 42 (advanced). After 3 and 6 weeks of treatment, we monitored physiologic and potential renoprotective effects.

Results. Initiation of V2RA treatment at advanced stage of the disease lacked renoprotective effects and had less pronounced physiologic effects than early initiation. After 3 weeks on a high dose, cyst ratio and kidney weight were reduced versus untreated controls (18 versus 25%, P = 0.05, and 0.33 versus 0.45 g, P = 0.03, respectively). After 6 weeks of treatment, however, this did not reach significance anymore, even at a high dose (cyst ratio 24 versus 27%, P = 0.12, and kidney weight 0.55 versus 0.66 g, P = 0.38).

Conclusions. Our results suggest that intervention with V2RA should be instituted early in ADPKD and that it might be necessary to further increase the dosage of this drug later in the disease to decrease cyst growth.

Keywords: ADPKD; disease stage; drug dose; PKD; vasopressin V2 receptor antagonist

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common renal hereditary disease, characterized by progressive cyst formation in both kidneys, often leading to end-stage renal disease (ESRD) [1, 2]. The majority of patients (85%) have a mutation in the PKD1 gene [3, 4]. Current clinical management of ADPKD is mainly focused on managing complications of ADPKD because as yet no proven renoprotective treatments exist that are able to inhibit cyst formation and thus to prevent renal failure.

Over the past 5 years, experimental studies have suggested a central role for vasopressin and 3'-5'-cyclic adenosine monophosphate in promoting cyst growth, kidney enlargement and renal function decline in ADPKD [5–8]. Inhibition of vasopressin by either a pharmacological agent such as vasopressin V2 receptor antagonist (V2RA) [9–11] or by drinking more water [12] led to reduced growth of cysts and renal function preservation in animal models of polycystic kidney disease.

At the moment, a large-scale, randomized controlled trial is being conducted with V2RA at high dosage for ADPKD patients early in the disease course [13]. If proven efficacious, important questions will, however, still remain after completion of this clinical study. First, it will not be known whether V2RA will be effective in ADPKD patients when started later in the disease. Because ADPKD is a progressive condition [14], it seems rational to start an intervention as early in the disease process as possible to delay or prevent long-term consequences as renal failure. Second, it will not be known whether low dosages of V2RA will be able to decrease cyst growth. When given to healthy normally hydrated volunteers, or ADPKD patients, V2RA causes an increase in urinary volume, which is dose dependent up to four times compared with placebo [15]. As a
consequence, polyuria, and especially nocturia causing disturbed night rest, may have a negative influence on quality of life. Given this side effect profile and the fact that potentially life-long treatment is warranted, it could be an option to use lower dosages of V2RA. Third, since 24-h urinary volume decreases in ADPKD patients after multiple doses of V2RA [16], the adverse effects may diminish after prolonged administration. Because of this, we were interested in the time course of the effects.

We therefore investigated the effect of different dosages of V2RA in Pkd1-deletion mouse model for ADPKD at different stages of the disease with three main study questions: first, whether treatment with V2RA is equally effective when initiated later in the disease course as compared with early administration; second, whether lower dosages result in less polyuria and are equally effective in reducing cyst formation as higher dosages and third, whether V2RA effects change over time.

Methods

Experimental animals

In this study, a tamoxifen-inducible kidney epithelium-specific Pkd1-deletion mouse model is used. Upon administration of tamoxifen to these mice, a genomic fragment containing exons 2–11 of the Pkd1 gene is specifically deleted in renal epithelial cells and cysts are formed. The inducible Pkd1-deletion mice (tam-KspCad-CreERT2;Pkd1lox2–1l/lox2–11) have been described previously [17]. We administered tamoxifen (0.5 mg dissolved in ethanol at 0.25 μL sunflower oil; Sigma-Aldrich, St Louis, MO) for three consecutive days to mice at postnatal Day 11 per gavage. This young age was chosen because most cysts will arise from the distal tubules as well as in collecting ducts when the gene is knocked out at an early age [18]. Tam-KspCad-CreERT2;Pkd1lox2–1l/lox2–11 mice that received tamoxifen are indicated as iKsp

Study design

At 3 weeks of age, tamoxifen-treated male and female mice were divided into control groups and three treatment groups (Table 1). This time point serves as Week 0 of the experiment. Figure 1 illustrates the time course of the experiment. The vasopressin V2RA OPC 31260 (V2RA; Otsuka Pharmaceutical Co., Tokushima, Japan) was added to ground rodent chow (AB Diets BV, RMH-B starch, 2103) at 0.05% [low dose (LD)] and 0.1% [high dose (HD)]. These dosages were equivalent to previously performed experiments with this medicament [9, 10]. Animals, water and food were weighed each week. Mice were housed with an average of four mice per cage. Water and approximate food intake was divided by the number of mice per cage to obtain an average intake per animal. V2RA intake per cage. Water and approximate food intake was divided by the number of mice per cage to obtain an average intake per animal. V2RA intake per cage, weight of the mice. Animals were sacrificed at Weeks 0, 3 and 6 of the experiment (Table 1 and Figure 1). Group 1, Week 0: one male, two females; Week 3: two males, one female; Week 6: two males, one female. Group 2, Week 0: three males, one female; Week 3: four males, five females; Week 6: four males, three females. Group 3, Week 6: 5 males, 10 females. Group 4, Week 3: three males, four females; Week 6: eight males, six females. Group 5, Week 6: four males, six females. All experiments were approved by the local animal experimental committees of the Leiden and Groningen University Medical Centers and by the Commission on Animal Experimentation Biotechnology in Animals of the Dutch Ministry of Agriculture.

Experimental protocol

Twenty-four-hour urine outputs in individual metabolic cages were obtained before mice were sacrificed. The animals were weighed and anesthetized with isoflurane gas (0.5% isoflurane with a flow of 0.6 L/min). Blood was obtained by cardiac puncture for determination of plasma electrolytes, creatinine and urea. Both kidneys were removed and weight was measured on a precision scale. Half of the right kidney was placed into formaldehyde. The tissues were embedded in paraffin for histomorphometry and immunohistochemistry. The other half of the right kidney and the left kidney were frozen immediately in liquid nitrogen for messenger RNA (mRNA) isolation.

Plasma and urine analysis

Creatinine, urea, potassium and sodium were measured in plasma and urine, using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY). Copeptin was measured using a new sandwich immunoassay (B.R.A.H.M.S. AG, Hennigsdorf/Berlin, Germany), which was based on the assay described previously [19] to detect murine copeptin, the capture and detection antibodies were made in sheep and directed to amino acids 139–148 and 156–168 of proAVP. Urinary osmolarity was calculated as 2 × (Urinary sodium concentration + Urinary potassium concentration) + Urinary urea concentration [20]. Copeptine clearance in milliliters per minute was calculated as the (urinary excretion of creatinine in 24 h/serum creatinine concentration)/1440. Fractional urea excretion (FE Urea) was calculated as ([Plasmaurea × Ucrea]/[Plasmacreatinine × Ucreatinine]) × 100.

Immunohistochemistry and histomorphometric analysis

Transverse tissue sections (4 μm), including cortex, medulla and papilla, were stained for periodic acid Schiff and hematoxylin–eosin to measure total area of cysts [9] and with Sirius red collagen stain to measure fibrosis [21]. Two transverse tissue sections were randomly selected per animal. The total area of the cysts and of fibrosis was quantified using Aperio Image Scope software (version 9.1.772.1570; Aperio Technologies Inc., Vista, CA) in these two sections and average values were taken. Total area of cysts (corrected for tubular lumina) was divided by total area of the tissue and multiplied by 100% to obtain a cyst ratio, expressed as percentage. Total intensity of fibrosis staining was divided by total intensity on the slide (both positive and negative) and multiplied by 100% to obtain a cyst ratio, expressed as percentage. Total intensity of fibrosis staining was divided by total intensity on the slide (both positive and negative) and multiplied by 100% to obtain a cyst ratio, expressed as percentage. Total intensity of fibrosis staining was divided by total intensity on the slide (both positive and negative) and multiplied by 100% to obtain a cyst ratio, expressed as percentage.

mRNA expression analysis

Kidneys snap frozen in liquid nitrogen were stored at –80°C until further processing. Renal tissues were homogenized in phosphate-buffered saline containing 1% 2-mercapto-ethanol using a MagNa Lyser, Instrument

Table 1. Experimental groups. Early treatment was initiated at Week 0 and late treatment started at Week 3 (see also Figure 1); HD (0.1%); LD (0.05%)

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Fig. 1. Study design.
Total RNA was isolated from renal tissue homogenates using TRI-Reagent (Sigma-Aldrich) according to the manufacturer’s protocol. cDNA was synthesized with Superscript III (Invitrogen). Quantitative gene expression analysis of Avpr2 and Aqp2 was performed on a Light Cycler 480 (Roche Applied Science) using FastStart Universal SYBR Green Master (ROX) (Roche Applied Science) according to the manufacturer’s protocol. Hprt (hypoxanthine phosphoribosyl transferase) was used as a housekeeping gene. Primer sequences are available upon request.

Data were analysed according to the Pfaffl DD\textsuperscript{CT} method, taking polymerase chain reaction efficiencies into account [24]. Expression was calculated relative to the median of untreated iKsp-Pkd1del mice.

**Results**

**Week 0**

Non-treated ADPKD versus wild-type mice. At the start of the experiment (Week 0), iKsp-Pkd1\textsuperscript{del} mice were not different from wild-type mice regarding cyst ratio. Total renal weight (iKsp-Pkd1\textsuperscript{del} mice 0.18 g versus wild types 0.19 g, P = 0.7), plasma creatinine (13 versus 15 mmol/L, P = 0.3) and urea (11 versus 12 mmol/L, P = 0.7) were also not different.

The iKsp-Pkd1\textsuperscript{del} mice were divided into controls and different treatment groups (Table 1), i.e. a non-treated control group, a group treated with LD V2RA early start, a group treated with HD V2RA early start and a group treated with HD V2RA late start. Formation of these groups and start of the experiment took place at Day 21 after birth. Figure 1 depicts the time course of the experiment. There were no differences between the different groups of iKsp-Pkd1\textsuperscript{del} mice regarding body weight, food intake (Figure 2) nor plasma sodium concentration at any time point during the study.

**Week 3**

Non-treated ADPKD versus wild-type mice. After 3 weeks of the experiment, iKsp-Pkd1\textsuperscript{del} mice had higher 24-h urinary volumes and lower 24-h urinary osmolarity than wild-type mice (P = 0.02), suggesting a decreased urinary concentrating capacity (Figure 3). iKsp-Pkd1\textsuperscript{del} mice had significantly higher cyst ratios and larger kidneys than wild-type mice (P < 0.001; Figure 4). Cysts were never megalin positive, suggesting that the cysts did not
derive from proximal tubules (Figure 5). Most cysts were uromodulin positive, indicating the majority of cysts arose from distal parts of the nephron. Approximately 20% of the cysts, generally smaller cysts, were positive for aquaporin-2, indicating cysts derived from principal cells of the collecting ducts in a minority of cases. Sixty percent of the cysts were positive for lectin DBA, identifying cysts derived from the collecting duct and late distal tubules. A large proportion of the cysts stained for uromodulin as well as DBA, which is in agreement with a previously published expression pattern for DBA and uromodulin in mouse kidneys [25]. Creatinine clearance was not different between the treated (median 1%) and the untreated iKsp-Pkd1del mice (median 0.7%). Creatinine clearance was not changed by V2RA administration. Median creatinine clearance was 119 μL/min in the treated animals and 150 μL/min in the untreated animals (P = 0.6).

**Week 6**

**Non-treated ADPKD versus wild-type mice.** After 6 weeks of the experiment, untreated iKsp-Pkd1del mice had a higher 24-h urinary volume and a lower 24-h urinary osmolarity (P < 0.05) compared with wild types (Figure 3), suggesting a decreased concentrating capacity. Untreated iKsp-Pkd1del mice also had cysts (difference in cyst ratios P = 0.007) and larger kidneys (P = 0.03) than wild types (Figure 4). Creatinine clearance was not different between iKsp-Pkd1del mice (141 μL/min) and wild types (179 μL/min), P = 0.37.

**Early start LD versus early start HD versus non-treated ADPKD mice.** Treatment with V2RA for 6 weeks resulted in larger 24-h urinary volume (P < 0.04) and lower 24-h urinary osmolarity (P = 0.003) with the higher dose inducing more effects than the lower dose (Figure 3). Fractional urea excretion was not different between the HD and LD group (P = 0.16; Figure 3). No significant renoprotective effects of V2RA were observed at this time point. Although cyst ratios and kidney weights were slightly different between the treated and non-treated iKsp-Pkd1del mice, the differences were not statistically significant (P = 0.4 and P = 0.3, respectively LD versus untreated; Figure 4). Also the amount of fibrosis (median fibrosis on HD was 3%, on LD 5 versus 6% in untreated mice and wild types) and creatinine clearance (median 192 μL/min on HD, 246 μL/min on LD and 141 μL/min in the group without treatment) were not different (P = 0.7 and P = 0.8, respectively) between the treated (either with high dosage or with low dosage of V2RA) and non-treated iKsp-Pkd1del mice.

**Later start HD versus early start HD and non-treated ADPKD.** Later initiation of treatment with HD V2RA, started in Week 3 of the experiment, induced less diuresis compared with early treatment with HD V2RA (P = 0.01; Figure 3). Although there was a trend toward a higher urinary osmolarity when treatment was initiated later in the disease course, this was not significant (P = 0.16). There was no renoprotective effect of V2RA treatment when initiated later in the disease course: both cyst ratios and kidney weights were not different (P = 0.9 and P = 0.8, respectively) from non-treated iKsp-Pkd1del mice (Figure 4).
Comparison of HD V2RA effects at Week 3 and at Week 6

Intake of V2RA per gram body weight was not different for iKsp-Pkd1<sup>det</sup> mice receiving the HD of V2RA after 3 weeks of treatment compared with iKsp-Pkd1<sup>det</sup> mice after 6 weeks of treatment (Figure 6). At Week 3 as well as at Week 6 of the experiment treatment with V2RA resulted in significant physiologic effects (Figure 6). Treated animals showed a higher water intake, lower urine osmolarity and a higher fractional urea excretion compared to untreated iKsp-Pkd1<sup>det</sup> mice. Mice at 6 weeks of treatment had less physiologic effects of V2RA when compared to mice at 3 weeks of treatment: 24-h urinary osmolarity was higher (321 versus 450 mOsm/L, P < 0.001), whereas water intake (14 versus 18 mL, P = 0.005) and fractional urea excretion (39 versus 67%, P < 0.001) were lower (Figure 6).

Since the physiologic effects were lower after 6 compared to 3 weeks of treatment, we analysed serum levels of copeptin, a stable precursor of and a marker for endogenous vasopressin, as well as renal mRNA levels of vasopressin V2 receptor and aquaporin 2.

There was no difference in copeptin concentration between mice treated for 6 weeks when compared to mice treated for 3 weeks (P = 0.7; Figure 6). Expression of both the vasopressin V2 receptor and aquaporin-2 was lower in mice after 6 weeks of treatment compared with mice at 3 weeks of treatment (P = 0.004 and P = 0.006, respectively).
Discussion

Vasopressin V2RA, when given at HD early in the disease, reduced cyst formation and kidney weight in a Pkd1-deletion mouse model for ADPKD. When the same dosage was administered for a longer period of time, cyst ratio and kidney weight were lower, but this difference did not reach statistical significance anymore. The direct physiologic effects of vasopressin V2 receptor inhibition decreased after prolonged administration of the vasopressin V2RA (i.e. urinary osmolarity was higher and water intake was lower after prolonged administration compared with the earlier time point). Initiation of V2RA treatment at a more advanced stage in the disease had less pronounced physiologic effects compared with early start of treatment with the same dosage and lacked renoprotective effects.

The beneficial effect of the vasopressin V2RA at Week 3 of the experiment is in line with literature. Also in other models for polycystic kidney disease (autosomal recessive polycystic kidney disease, nephronophthisis and a Pkd2-model of ADPKD), this beneficial effect has been described previously [9–11]. Of note, the effects of vasopressin V2RA has, as far as we know, never been investigated in a Pkd1 model for ADPKD. This is consistent with the observed dose-dependent polyuria seen in healthy volunteers [15]. Initiation of V2RA treatment at HD in a more advanced disease stage unexpectedly induced also less pronounced physiologic effects when compared with HD treatment earlier initiated in the disease. Both lower dosage and initiation later in the disease could therefore partly overcome the physiologic side effects. In this study, however, treatment with a low dosage as well as later initiation of treatment did not decrease cyst growth.

After 6 weeks of treatment, V2RA exerted physiologic effects and decreased cyst growth when compared to untreated animals. However, when compared to 3 weeks of treatment, these effects were diminished and did not reach statistical significance in all cases. The decreased efficacy of the drug after prolonged administration could be caused

Fig. 5. Tubular segment identity of cysts. Panels (A–D) show kidney sections from an untreated iKsp-Pkd1del mouse, sacrificed at 3 weeks. Panels (E–H) show kidney sections from a mouse treated with a HD V2RA for 3 weeks. Sections are stained for the proximal marker megalin (A and E), the distal markers Tamm–Horsfall protein (THP; B and F), aquaporin-2 (AQ2; C and G) and lectin DBA (D and H). *Indicates positive staining of the cyst.
by inadequate intake of V2RA. Mice almost doubled in bodyweight throughout the experiment. However, the study drug was administered as a percentage of food, and food intake also increased throughout the experiment. Consequently, the intake of V2RA per gram body weight was not different at 6 weeks of treatment when compared with the earlier time point (Figure 6). This does not, however, exclude the possibility that there are differences in pharmacokinetic parameters of the V2RA at Week 6 compared to Week 3 of treatment. For instance, it could be that in these growing mice, metabolism of the V2RA increases, thereby decreasing the serum concentration of the V2RA at the later time point, despite an equal intake of the drug. Unfortunately, the low blood volume obtained after sacrificing the animals did not allow the measurement of drug concentration. Another cause for the decreased efficacy of V2RA could be the result of incomplete vasopressin V2 receptor antagonism. This could be caused by an increase in vasopressin levels or changes in vasopressin V2 receptor expression. Copeptin, the stable precursor of vasopressin [29, 30], was not, however, increased after prolonged administration. Vasopressin V2 receptor mRNA expression was decreased at 6 weeks of the experiment compared to the situation at 3 weeks. This means that there is more vasopressin per receptor present, which may lead to decreased efficacy of V2RA and may explain the observation that physiologic effects decreased in the mice after prolonged administration of the drug. The phenomenon of decreased diuresis after multiple dosages of a V2RA is also observed in humans with ADPKD [16]. If this is indeed due to incomplete vasopressin V2 receptor blockade, this line of reasoning leads to the hypothesis that the dosage

![Graphs showing comparison of V2RA intake, physiologic effects, copeptin concentration, and mRNA expression between Weeks 3 and 6 of the experiment in untreated animals and in animals treated with HD (0.1%) of V2RA.](https://academic.oup.com/ndt/article-abstract/26/8/2445/1917006)
of vasopressin V2RA should be increased as the disease progresses to effectively block the hormonal activity of vasopressin.

An alternative explanation for the decreased sensitivity to V2RA after prolonged administration could be that vasopressin is effectively blocked, but other processes driving cyst formation become more important. It has been described that many signalling pathways are altered in cystic epithelial cells, directly or indirectly regulated by the PKD proteins, and V2RA treatment may not block all of these pathways [2]. In our model, ~20% of cysts are positive for aquaporin-2 (AQP2). These AQP2-positive cysts are the cysts that will be targeted by a vasopressin V2RA. Of note, the percentage of AQP2-positive cysts seems to be less than in the Pkd2 model that has been described in literature, where AQP2 expression is massively increased in the kidney [10]. The majority of cysts in our model appear to derive from other tubular segments that most likely are not inhibited by V2RA. In case this hypothesis is true, this might implicate that institution of combination therapy, targeting inhibition of cyst formation via various signalling pathways, may overcome the decreased efficacy of V2RA after prolonged administration. Examples of such medications could be somatostatin analogues [31–33] and mammalian target of rapamycin (mTOR) inhibitors [34], although two recent clinical trials did not show a clear beneficial effect of mTOR inhibitors in ADPKD, either early [35] or later [36] in the disease.

We acknowledge that this study has limitations. First, unlike in humans, in our model, gene inactivation occurs at a single time point. This could have an effect on the ability of V2RAs to affect the initiation and development of cysts. This may account for the limited benefit from OPC-31260 administration observed in the study. Second, since mice eat mostly at night and OPC-31260 has a short half-life, it is likely that the tubular epithelial cells are exposed to increased concentrations of circulating vasopressin without the protection of the V2 receptor blockade for significant amounts of time. This may account for the limited benefit from OPC-31260 administration observed in the study. In addition, severity of the renal cystic disease in this model is variable. This may have limited the ability of finding significant differences.

In conclusion, V2RA has beneficial effects on cyst growth in a Pkd1-deletion mouse model when initiated at HD early in the disease in the short term. Initiation of V2RA treatment at lower dose or at HD later in the disease had less pronounced physiologic effects but did not decrease cyst growth. After prolonged administration, V2RA induced lower cyst ratio and kidney weight compared to untreated animals, but this did not reach statistical significance. Physiologic side effects, though still significant, were also less pronounced at this later time point. This decreased responsiveness to V2RA could be due to incomplete V2R antagonism or increased importance of other non-vasopressin-related cystogenic pathways. Our results suggest that intervention with V2RA should be instituted early in the disease course of ADPKD and at HD and that it might be necessary to either increase the dosage of this drug later in the disease to decrease cyst growth or to switch to combinational treatment with other drugs.

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Conflict of interest statement. J.S. is an employee of Brahms, the manufacturer of the copeptin assay. None of the other authors has anything to declare.

(See related article by Devuyst et al. Vasopressin-2 receptor antagonists in autosomal dominant polycystic kidney disease: from man to mouse and back; Nephrol Dial Transplant 2011; 26: 2423–2425.)

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