Effects of combined administration of rapamycin, tolvaptan, and AEZ-131 on the progression of polycystic disease in PCK rats

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Sabbatini M, Russo L, Cappellaio F, Troncone G, Bellevicine C, De Falco V, Buonocore P, Riccio E, Bisesti V, Federico S, Pisani A. Effects of combined administration of rapamycin, tolvaptan, and AEZ-131 on the progression of polycystic disease in PCK rats. Am J Physiol Renal Physiol 306:F1243–F1250, 2014. First published March 19, 2014; doi:10.1152/ajprenal.00694.2013.—Both experimental and clinical studies have suggested that any potential treatment of polycystic kidney disease (PKD) should start early and last for a long time to be effective, with unavoidable side reactions and considerable costs. The aim of the present study was to test how low doses of rapamycin (RAPA; 0.15 mg/kg ip for 4 days/wk), tolvaptan (TOLV; 0.005% in diet), or AEZ-131 (AEZ; a novel ERK inhibitor, 30 mg/kg for 3 days/wk by gavage), alone and in association, affect the progression of polycystic renal disease in PCK rats. Rats were treated for 8 wk starting at 4–6 wk of age. The efficacy of low doses of such drugs in inhibiting their respective targets was confirmed by immunoblot experiments. Compared with rats in the control (CON) group, RAPA treatment caused a significant reduction in cyst volume density (CVD; 49% vs. the CON group) and was numerically similar to that in TOLV-treated rats (18%, not significant), whereas AEZ treatment was not effective. RAPA + TOLV treatment resulted in a significantly lower CVD (∼49% vs. the CON group) and was associated with a striking decrease in cAMP response element-binding protein phosphorylation, and similar data were detected in RAPA + AEZ-treated rats (∼42%), whereas TOLV + AEZ treatment had no effect. RAPA administration significantly lessened body weight gain, whereas TOLV administration resulted in a mild increase in diuresis and a significant increase in CAMP urinary excretion. Histological data of tubular proliferation were in full agreement with CVD data. In conclusion, this study demonstrates that the association of low doses of RAPA, TOLV, and AEZ slows the progression of PKD with limited side effects, suggesting the use of combined therapies also in clinical trials.

polycystic kidney disease; rapamycin; tolvaptan; extracellular signal-regulated kinase inhibitors; cyst volume density

AUTOSOMAL DOMINANT polycystic kidney disease (PKD) is the most common monogenic kidney disease and the fourth leading cause of end-stage kidney disease in adults worldwide. Recently, several clinical randomized trials have evaluated the effects of inhibitors of mammalian target of rapamycin (RAPA) (mTOR) (30, 32, 38), tolvaptan (TOLV) (36), and long-acting somatostatin (8), all of which were positively tested in previous animal studies (13, 19, 22, 33, 34, 37), on autosomal dominant PKD progression, and new studies are still in progress (20). Unfortunately, despite some encouraging results (8, 36), these trials have not confirmed the exciting results obtained with the same drugs in rodent models of PKD, which are genetically different and free of the clinical pattern that characterizes human disease.

Nonetheless, taken together, the results of experimental and human studies seem to agree on two concepts: a potential therapy of PKD should start early, when renal function is still preserved, and should be long lasting, with unavoidable problems related to drug side effects, patients’ compliance, and considerable costs for healthcare providers.

As a consequence, at present, a definitely accepted therapeutic protocol for this disease has not been found, since treatment of PKD must necessarily balance benefits and risks; hence, before new intervention studies are started, there is a need for new preclinical models that include different drugs or new therapeutic strategies able to combine efficacy and safety.

A possible approach is represented by the association of two or more drugs acting on different sites of the pathways involved in determining cyst formation and expansion: this could determine a synergistic effect on the clinical target and allow the use of lower dosages of each drug, with reduced side effects.

Therefore, the aim of the present study was to test the association of three different classes of drugs successfully used in rodent PKD: mTOR inhibitors, aquaretics, and inhibitors of the ERK1/2 pathway. These drugs were administered alone and in association, starting with doses 50% lower than those commonly used in previous studies. Our research was carried out in rats of the PCK strain, a model of PKD characterized by progressive cystogenesis, slow impairment of renal function, intrarenal accumulation of cAMP, and abnormal activation of mTOR, adenylyl cyclase, and ERK signaling, which resemble in many clinical and morphological aspects type I human autosomal dominant PKD, although the pattern of inheritance is autosomal recessive (17). Cyst volume density (CVD) and inhibition of the signaling pathways after drug administration were investigated as markers of disease progression and drug biological activity, respectively.

MATERIALS AND METHODS

This study was carried out using 54 male rats of the Sprague-Dawley or PCK strain (both from Charles River Laboratories). Rats were 4–6 wk old when the study began, fed a standard protein content diet (Altromin, Rieper) and tap water ad libitum, and kept at constant temperature and humidity in a 12:12-h dark-light cycle.

In the present study, three different drugs were used alone or in association: RAPA (Sigma), TOLV (Otsuka Pharmaceutical), and AEZ-131 (AEZ), a new specific inhibitor of ERK phosphorylation (AEterna-Zentaris). A preliminary study was performed in six normal Sprague-Dawley rats to test the safety and efficacy of 2 mo of
treatment with AEZ (30 mg/kg by gavage, 3 times/wk), since the unique chronic study with this drug lasted 30 days, although at higher doses (30 mg·kg⁻¹·day⁻¹ by gavage) (I. Seipelt, unpublished observations). Since TOLV was incorporated in the normal diet (Rieper), a preliminary study was also performed to ascertain the daily ingestion of food in adult PCK rats (n = 6) to establish the correct drug concentration in the diet.

According to our experimental design, PCK rats were divided into seven experimental groups (n = 6 rats/group) that were followed up in individual cages for 8 wk. Untreated PCK rats represented the control (CON) group. The other groups (treated with doses in individual cages for 8 wk. Untreated PCK rats represented the control (CON) group. The other groups (treated with doses of RAPA (FD-RAPA group; 0.2 mg·kg⁻¹·day⁻¹) are also reported for comparison with rats treated with lower doses of the drug.

During the followup period, rats in the CON group and all rats treated with TOLV underwent two 24-h urine collections in metabolic cages to evaluate urinary output and urinary cAMP excretion under basal conditions and after 6 – 7 wk of treatment.

At the end of the followup period, 4 h after the last administration of the drugs under study, rats were anesthetized [zoletil (30 mg/kg) + xilazine (10 mg/kg)], and a blood sample was obtained by cardiac puncture to evaluate blood parameters. After a median laparotomy, both kidneys were gently dissected, excised, and weighed under sterile conditions, and small samples of the left kidney were cut and immediately frozen (−80°C) for immunoblot experiments. The right kidney was used for histological experiments.

This experimental protocol was submitted to and approved by our local Veterinary Authority, according to Italian law. The health status of the animal colony was monitored in accordance with Italian Veterinary Board guidelines.

### Histological Experiments

**CVD estimation.** Kidney tissue was fixed in buffered formalin solution (10%), with the time monitored to avoid overfixation. Fixed kidneys were stabilized by embedding in agar gel to facilitate and homogenize the cutting (4). To minimize sampling bias and to avoid field selection variation, areas of the cortex at hilum and 45° and 75° from the hilum were evaluated in each sample. Hematoxylin and eosin-stained sections were used to estimate CVD by a blinded pathologist. All slides were digitalized using the “macro” function of a LEICA DMD108 photomicroscope. The acquired images were then analyzed by open-source software (ImageJ) for standard point counting stereology (11, 33). Each point had a known associated area of 0.5 mm², and a total count of ~200 points collected over 7–10 sections was achieved (4, 16).

**Immunohistochemistry.** Immunohistochemical detection of proliferating cells was performed by the enzyme-labeled antibody method on 4-µm-thick serial sections in selected groups of rats (see RESULTS). Paraffin sections were dewaxed and rehydrated. Heat-induced antigen retrieval was carried out by microwaving the slides at 650 W with a 0.01 M sodium-citrate solution. Sections were incubated with antibodies against PCNA (dilution: 1:1,000, Santa Cruz Biotechnology, Santa Cruz, CA) and processed by Benchmark Autostainer (Ventana, Roche) using an UltraView Polymer Detection kit. Negative controls were obtained by omitting the primary antibody. PCNA is a protein synthesized in the early G1 and S phases of the cell cycle and is useful as a marker of proliferating cells. Cell proliferation was estimated by evaluating the percentage of PCNA-positive nuclei in the cystic tubular epithelium, normal tubules, and collecting ducts in 10 high-power fields (magnification: ×400). The number of positive cells was expressed as a semi-quantitative value (+: reactivity < 10%, ++: reactivity > 10% and < 20%, + + : reactivity > 20% and < 50%, + + + : reactivity > 50%).

**Immunoblot Experiments**

Western blot analysis was used to test the biological activity of the drugs used in this study according to our standardized procedures (12, 27). We tested the inhibition of phosphorylation of either ribosomal S6 kinase (RSK), 40S ribosomal protein S6 (S6), and CAMP response element-binding protein (CREB), downstream effectors of ERK, mTOR, and adenyl cyclase activity, respectively, i.e., three signaling pathways involved in cyst formation and progression (35).

Briefly, samples were harvested in lysis buffer [containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1 Triton X-100, 1 mM EGTA, 1.5 mM MgCl₂, 10 mM NaF, 10 mM sodium pyrophosphate, 1 mM Na₂VO₃, 10 µg/ml aprotinin, and 10 µg/ml leupeptin] and clarified by centrifugation at 10,000 g. Protein concentration was estimated with a modified Bradford assay (Bio-Rad Laboratories, Berkeley, CA). Antigens were revealed by an ECL detection kit (Amersham Pharmacia Biotech). Signal intensity was quantified with a Phosphorimager (Typhoon 8600, Amersham Pharmacia Biotech) interfaced with ImageQuant software. Anti-phosphorylated (p)p90 ribosomal protein S6 kinase (RSK), 40S ribosomal protein S6 (S6), and cAMP response element-binding protein (CREB), downstream effectors of ERK, mTOR, and adenylyl cyclase activity, respectively, i.e., three signal pathways involved in cyst formation and progression (35).

### Statistics

Considering the heterogeneity of treatments, the single parameters of the different groups under study were compared with those of the CON group by a two-tailed Student’s t-test for unpaired data. Student’s t-test for paired data and one-way ANOVA (followed by
Bonferroni’s correction) were performed for selected parameters where appropriate. Data are expressed as means ± SD. * values of <0.05 were considered statistically significant.

RESULTS

Three rats died throughout the study (two rats during gavage for AEZ administration and one rat under RAPA administration). Judging by the physical appearance of the rats, the treatment with all drugs was well tolerated.

Body and Kidney Weights

Compared with the CON group, a significant reduction in body weight gain was observed in all experimental groups with the exception of rats in TOLV group. This was more evident in rats treated with RAPA (−63%, −48%, −40%, and −40% vs. the CON group in the FD-RAPA, RAPA, RAPA + TOLV, and RAPA + AEZ groups, respectively; Table 1). The two-kidney weight was also significantly reduced in all groups versus the

Fig. 1. Cyst volume density (CVD) in the groups under study. A: data from the groups treated with tolvaptan (TOLV) compared with the control (CON) group. B: data from the groups treated with rapamycin (RAPA) compared with the CON group. *Significantly different vs. the CON group (*P < 0.05, minimum value, by one way-ANOVA with a Bonferroni’s post test).

Fig. 2. Representative histological images (stained with hematoxilin-eosin) of equatorial kidney sections in the groups under study. A: CON group; B: TOLV group; C: RAPA group; D: TOLV + RAPA group; E: TOLV + AEZ group; F: RAPA + AEZ group. An image from the AEZ group is not shown since images did not differ from those from the CON group.
CON group, but the body weight-to-kidney weight ratio was significantly lower only in the FD-RAPA (−9.6% vs. the CON group), TOLV (−8.7%), RAPA (−7.8%), and TOLV + RAPA (−5.2%) group, whereas negligible changes were detected in the other groups.

CVD

CVD averaged 24.7% in rats from the CON group and, as expected, was consistently decreased in rats from the FD-RAPA group, our reference group (−34%, P < 0.001 vs. the CON group; Table 1).

Low-dose TOLV and RAPA treatment resulted in only minor reductions in CVD (−18% and −19% vs. the CON group, respectively), which, conversely, was not affected at all by AEZ treatment.

When the drugs were administered in association, a striking reduction in CVD was detected in the TOLV + RAPA group (−49% vs. the CON group) and RAPA + AEZ group (−42% vs. the CON group), whereas the association of AEZ with TOLV had no additional benefit compared with TOLV alone (−19% vs. the CON group).

When rats from the CON group were pooled together with those treated with either TOLV (CON, TOLV, TOLV + RAPA, and TOLV + AEZ group) or RAPA (CON, RAPA, TOLV + RAPA, and RAPA + AEZ group), significant differences were observed by ANOVA (Fig. 1). The association of TOLV and RAPA, in fact, resulted in significantly lower values of CVD compared with either the CON, TOLV, or TOLV + AEZ groups (Fig. 1A); in contrast, for RAPA treatment, all groups treated with RAPA showed a slower progression of cyst enlargement compared with rats in the CON group and was considerably high in RAPA + TOLV and RAPA + AEZ groups (Fig. 1B).

Representative histological images of the groups under study are shown in Fig. 2.

Immunohistochemical Experiments

Tubular cell proliferation was evaluated in rats from the CON, TOLV + RAPA, and RAPA + AEZ group, in which the greatest reduction in CVD was observed (Fig. 3). In both these latter groups, the intensity of staining was almost halved compared with the CON group (Table 2), whereas minor changes were observed in the other groups under study (data not shown).

Immunoblot Experiments

Inhibition of RSK phosphorylation was evidenced only in rats treated with AEZ, with no synergistic action when the drug was administered in association with TOLV or RAPA (Fig. 4, top). A significant inhibition of S6 phosphorylation was observed in all groups of rats treated with RAPA; both AEZ and TOLV showed no interference with the mTOR pathway, and no synergistic effect was observed when RAPA was associated with TOLV or AEZ (Fig. 4, middle). Finally, CREB phosphorylation was significantly inhibited by TOLV but was not influenced by RAPA or by AEZ. Interestingly, the association of TOLV + RAPA induced a further, striking reduction in CREB activity compared with TOLV alone (Fig. 4, bottom). Representative immunoblots of each quantified protein are shown in Fig. 5.
Effects of TOLV on Urinary Volume and Urinary cAMP

Administration of low-dose TOLV resulted in a significant increase in urinary volume compared with basal values in all treated groups, compensated for by a concomitant rise in water ingestion; in rats from the TOLV + AEZ group, in which no change in cyst volume was detected, this increase was milder (Fig. 6). Unexpectedly, urinary cAMP excretion was doubled in rats from the TOLV and TOLV + RAPA groups and only slightly increased in the TOLV + AEZ group.

Renal Function and Laboratory Data

Plasma levels of both creatinine and urea were similar in all groups under study. Slight differences were detected in aspartate aminotransferase values in rats from the TOLV group (+38% vs. the CON group, \( P < 0.05 \)) and in rats from the TOLV + RAPA group (+49% vs. the CON group, not significant) and in total plasma \( \text{Ca}^{2+} \) levels, which were significantly lower in all groups of rats treated with RAPA (Table 3). No other changes were detected in the remaining laboratory data, including plasma \( \text{Na}^{+} \) concentration, in any group.

DISCUSSION

Our working hypothesis was to test whether the combined administration of low doses of two drugs active on the pathways involved in cyst formation could delay the evolution of PKD in PCK rats. The peculiarity of the study was in drug dosages: in fact, weekly RAPA doses in our treated rats were 57% lower than those used in the FD-RAPA group or in a previous study (33), and both TOLV and AEZ dosage in our rats were reduced by 50% compared, with, respectively, the lowest dose used in the same animal model (34) or the dose tested in normal rats (I. Seipelt, unpublished observations).
cells of polycystic kidneys that integrates the signaling from different growth factors, beyond cAMP, which could be potentially modified by mTOR inhibition.

The AEZ data were even more interesting since the drug had no efficacy on cyst progression when administered alone, despite a 60% inhibition of pRSK, but resulted in a consistent effect on CVD when associated with RAPA. This occurred with no evident changes in phosphorylation of either pS6 or pRSK in immunoblot experiments. Probably, the double concomitant inhibition of mTOR and ERK1/2 avoided the “escape” phenomenon that occurs when only one pathway is inhibited. Indeed, both in vitro and in vivo data have suggested that the phosphatidylinositol 3-kinase/Akt/mTOR and Raf/MEK/ERK pathways are strictly interconnected with multiple feedback loops (9); the inhibition of one pathway can result in maintained signaling via the reciprocal pathway. This implies that the dual targeting of these systems may lead to superior efficacy and better clinical outcome than single inhibition (29).

Our data suggest a possible role for low doses of ERK1/2 inhibitors in PKD treatment. Indeed, the concept that PKD may be considered a slowly progressive benign neoplasia is commonly accepted (7, 15, 23) and justifies the scientific interest in the Ras/Raf/ERK pathway, which is deeply activated in PKD (21). The results of previous studies, however, are conflicting: prolonged administration of PD-184352, a selective MEK inhibitor, induced significant beneficial effects in a mouse model of nephronophisis (22), whereas administration of PLX-5568, a new specific inhibitor of Raf, attenuated the proliferation of either human autosomal dominant PKD and canine Madin-Darby canine kidney (MDCK) cells, which express endogenous polycystin-1, but failed to influence renal function and proteinuria in Han:SPRD rats (5). In these latter rats, moreover, increased renal and hepatic fibrosis were detected after treatment (5). Indeed, most of the Raf inhibitors tested for clinical use are effective only in tumors that contain B-Raf mutations, which is not the case in PKD.

AEZ was well tolerated, as determined the slight decrease in body weight gain, partially explained by its administration route, and was not associated with peculiar side effects nor with changes in main laboratory data or renal function. Probably, the low doses and alternate-day administration prevented the onset of the adverse reaction commonly associated with antineoplastic drugs. Such a dose, however, undoubtedly resulted in a synergistic action with RAPA: both drugs, in fact, act on cellular targets strictly linked in regulating translation initiation, as recently demonstrated in embryonic fibroblasts from Pkd1 mice (26). Positive results with combined inhibition

Our hypothesis was fully confirmed since, despite the fact that single drugs had limited or even any effect on CVD, the association of low doses of TOLV + RAPA and RAPA + AEZ were able to induce markedly reduced cyst growth (−49% and −42% vs. the CON group, respectively), with no modifications in renal function, mild side effects, and no increased mortality.

In the TOLV + RAPA group, in fact, beyond the expected inhibition of pS6, RAPA caused a further reduction of CREB in the TOLV group, in fact, beyond the expected inhibition of pS6, RAPA caused a further reduction of CREB dephosphorylation. CREB is a transcription factor particularly expressed in tubular cells of polycystic kidneys that integrates the signaling from different growth factors, beyond cAMP, which could be potentially modified by mTOR inhibition.

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Table 3. Main laboratory data of the groups under study

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose, mg/dl</th>
<th>Urea, mg/dl</th>
<th>Creatinine, mg/dl</th>
<th>Ca2+, mg/dl</th>
<th>Na+, meq/l</th>
<th>K+, meq/l</th>
<th>Aspartate Aminotransferase, IU/dl</th>
<th>Alanine Transaminase, IU/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>152 ± 25</td>
<td>39.6 ± 10.1</td>
<td>0.40 ± 0.11</td>
<td>9.6 ± 0.2</td>
<td>141 ± 3.7</td>
<td>4.13 ± 0.4</td>
<td>150 ± 52</td>
<td>55 ± 16</td>
</tr>
<tr>
<td>TOLV</td>
<td>161 ± 25</td>
<td>41.0 ± 4.6</td>
<td>0.34 ± 0.05</td>
<td>9.4 ± 0.2</td>
<td>141 ± 4.2</td>
<td>3.97 ± 0.2</td>
<td>207 ± 32*</td>
<td>69 ± 14</td>
</tr>
<tr>
<td>RAPA</td>
<td>130 ± 14</td>
<td>39.4 ± 1.3</td>
<td>0.53 ± 0.14</td>
<td>9.0 ± 0.5**</td>
<td>144 ± 4</td>
<td>4.17 ± 0.1</td>
<td>145 ± 18</td>
<td>56 ± 20</td>
</tr>
<tr>
<td>AEZ</td>
<td>140 ± 10</td>
<td>42.5 ± 3.6</td>
<td>0.40 ± 0.15</td>
<td>9.4 ± 0.3</td>
<td>142 ± 1.1</td>
<td>3.95 ± 0.05</td>
<td>176 ± 41</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>TOLV + RAPA</td>
<td>153 ± 36</td>
<td>41.7 ± 15.7</td>
<td>0.32 ± 0.10</td>
<td>9.0 ± 0.4*</td>
<td>144 ± 5.1</td>
<td>4.07 ± 0.28</td>
<td>223 ± 88</td>
<td>63 ± 16</td>
</tr>
<tr>
<td>TOLV + AEZ</td>
<td>176 ± 14</td>
<td>38.2 ± 6.2</td>
<td>0.38 ± 0.10</td>
<td>9.4 ± 0.5</td>
<td>144 ± 3.6</td>
<td>4.02 ± 0.23</td>
<td>149 ± 56</td>
<td>76 ± 11</td>
</tr>
<tr>
<td>RAPA + AEZ</td>
<td>154 ± 32</td>
<td>38.7 ± 59</td>
<td>0.38 ± 0.08</td>
<td>9.1 ± 0.4*</td>
<td>142 ± 5.1</td>
<td>3.60 ± 0.42</td>
<td>164 ± 49</td>
<td>60 ± 23</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; n = 6 rats/group. *P < 0.05 vs. the CON group (minimum value, Student’s t-test for unpaired data; see MATERIALS AND METHODS).
of ERK and mTOR have already been demonstrated in some experimental models of human cancers, where concomitant administration of PD-0325901 (an ERK inhibitor) and RAPA was significantly superior to the single drugs, even at the maximum tolerated doses, in mice bearing human lung tumor xenografts (18).

A potential use of ERK inhibitors in clinical trials, however, should be considered with caution due to their considerable side effects. In fact, the cyclin kinase inhibitor roscovitine, a downstream effector of ERK and a powerful inhibitor of cyst growth in mouse models of PKD (6), which is currently being tested in several clinical cancer trials, can induce hyponatremia, hypokalemia, and acute renal failure, which represent worrying features in long-term treatments (2). Therefore, reduced doses of such drugs, peculiar schedules of administration, and association with different drugs could be considered in future trials.

It is interesting to note that the association TOLV + AEZ did not affect cyst progression, despite the fact that TOLV is also effective in inhibiting ERK (24); a negative interaction between the two drugs at the level of the intracellular targets of TOLV probably accounts for these results, as evidenced by the milder diuresis and lower cAMP excretion in rats from the TOLV + AEZ group compared with the other TOLV-treated groups.

The increased cAMP excretion in TOLV-treated rats represents a new finding. Experimental studies in PCK rats have clearly shown that TOLV reduces intracellular levels of cAMP through adenylyl cyclase inhibition, but no studies have reported its concentration in urine. Renal handling of cAMP is complex since this substance is freely filtered at the glomerular level and is secreted at the tubular level (3). It has been shown that increasing doses of TOLV determine the proportional rises in antidiuretic hormone levels (40), which, in turn, determine the rise in cAMP excretion (1). We hypothesize that in our rats, TOLV enhances cAMP excretion by raising antidiuretic hormone concentrations. Whether urinary excretion of cAMP may become, in the future, a biological marker of TOLV activity, however, needs further experimental and clinical evidence.

Finally, the results from our study confirm the efficacy of RAPA also in PCK rats, as previously shown in different mouse models (14, 31) and in Han:SPRD rats (33) but not in the PCK strain (25). Indeed, in contrast with Renken et al. (25), who found no effect of RAPA (2 mg·kg\(^{-1}\)·day\(^{-1}\) orally) on PKD progression, we (28) have previously observed that 0.2 mg·kg\(^{-1}\)·day\(^{-1}\) RAPA by an intraperitoneal route resulted in consistent pS6 inhibition and resulted in a significant decrease in CVD. Here, we show that even a lower dose of RAPA (0.15 mg/kg, 4 days/wk) was still effective in terms of CVD and associated with 40% higher weight gain compared with FD-RAPA rats, with no change in the main laboratory data.

In conclusion, the data of the present study demonstrate that the association of low doses of different classes of drugs commonly tested in rodent models of PKD may result in significant effects on cyst progression in the PCK rat, with minor side effects.

Although animal data must be interpreted with caution and cannot be extrapolated to clinical settings as no single animal model can truly replicate human autosomal dominant PKD in its complexity, our results highlight the possibility of using combined therapies also in clinical trials to exploit their synergestic effects while reducing potential adverse reactions, as recently tested in human polycystic liver disease (10).

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: M.S. and A.P. conception and design of research; M.S., L.R., F.C., G.T., C.B., and A.P. performed experiments; M.S., V.D.F., P.B., and E.R. analyzed data; M.S., G.T., and C.B. interpreted results of experiments; M.S. and S.F. drafted manuscript; M.S., S.F., and A.P. edited and revised manuscript; M.S., L.R., F.C., G.T., C.B., and A.P. approved final version of manuscript; V.D.F. prepared figures.

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