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New therapeutic prospects in autosoma dominant polycystic kidney disease

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a common cause of ESRD in adults. There is currently no specific treatment for ADPKD, but great progress has been made in recent years in understanding of the cystogenesis process and the pathogenesis of the disease. There is clear evidence supporting a predominant role of proliferation of epithelial cells, transepithelial fluid secretion, and extracellular matrix remodelling. Various therapeutic approaches to ADPKD are being tested based on this evidence and using renal volume progression as efficacy parameter (table I).

PHENOTYPIC CHANGES IN THE POLYCYSTIC EPITHELIAL CELL REPRESENTING NEW THERAPEUTIC TARGETS

Multiple changes have been reported in polycystic epithelial cells, but there is still much to be known. We will only address changes providing therapeutic possibilities.

Polycystin-1 and polycystin-2 (PC1, PC2) have been located, among other sites, in primary cilia. It has been suggested that these cilia act as flow sensors in the renal tubule and, in response to flow, cause calcium influx to the cell mediated by P-2, which acts as a cation channel. An abnormal PC2 (or an abnormal PC1, due to interaction of both) would cause a reduction in intracellular calcium. On the other, it is evident that cAMP levels are increased in this disease. Low intracellular calcium may cause a proliferative response to high levels of cAMP, which behaves as an antiproliferative in a normal epithelial cell. High cAMP levels have also been shown to have a significant role in fluid secretion. PC1 binds to G proteins, normally acting on protein-bound receptors whose function is cAMP inhibition. A deficient or abnormal PC1 therefore results in increased cAMP levels. This cAMP facilitates through various mechanisms migration of aquaporin-2 to the apical membrane, increasing osmotic permeability of the membrane[1] (fig. 1).

In ADPKD there is an early concentration defect, occurring before kidney destructuration by cysts, that is thought to be due to inadequate translocation of aquaporin-2 into the apical membrane, most likely secondary to a defect in F-actin depolymerisation. Wang X et al. have recently shown the deleterious effect of vasopressin on polycystic kidneys. Using knockout rats for the vasopressin gene and crossing them with polycystic rats, these authors showed that polycystic rats with no vasopressin did not virtually develop the disease, while they developed polycystic disease if they were administered endogenous vasopressin.[2] This observation supports the key role of vasopressin in cystogenesis.

An interaction has also been shown between PC1 and tuberin. Tuberin is the protein encoded by the *TSC2* gene, responsible for one of the two forms of tuberous sclerosis. PC1 apparently regulates mTOR (mammalian target of rapamycin) through the MAP and Ser kinases.[3] mTOR stimulates cell growth and proliferation, and its inhibition promotes apoptosis. The complex formed by tuberin and hamartin (a protein encoded by the *TSC1* gene) maintains

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Table I. Current therapeutic proposals for autosomal dominant polycystic kidney disease

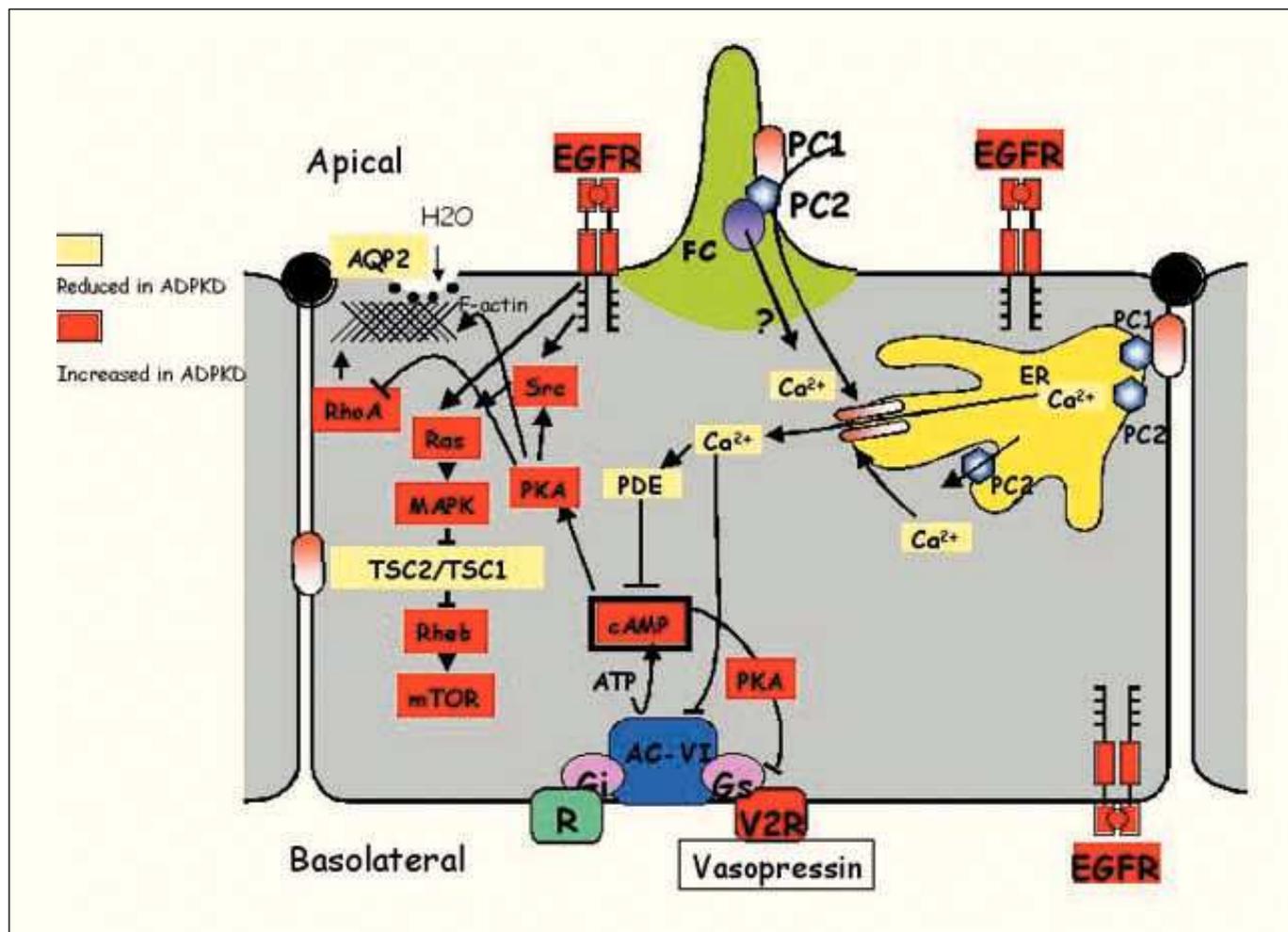| Tested in murine models only	Some evidence in humans
– Roscovitine	– Vasopressin V2 antagonists
– Triptolide	– Rapamycin
– Small molecules	– Somatostatin analogues
– VEGF inhibitors	– ACEIs/ARBs
– EGFR inhibitors	

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Figure 1. Proposed phenotypic changes in a polycystic epithelial cell (Modified from Torres VE, *Nature Clin Pract* 2005). The PC1/PC2 complex mediates calcium influx to the cell and in turn Ca release from endoplasmic reticulum. Disruption of this complex reduces intracellular Ca. Adenylcyclase 6 (AC-VI) is the amin AC in the collecting tubule. It is stimulated through G proteins and inhibited by Ca, and results in increased cAMP levels. Activation of RhoA causes F-actin to remain polymerised and prevents insertion of AQP2 in the apical membrane. On the other hand, changes in the TSC1/TSC2 complex caused by changes in PC1 keep mTOR activated. PC1: polycystin-1, PC2: polycystin-2, FC: fibrocystin, EGFR: epidermal growth factor receptor, AQP2: aquaporin-2, AC-VI: acetyl choline IV, TSC1/TSC2 tuberous sclerosis 1 and 2, mTOR: mammalian target of Rapamycin, V2R: vasopressin V2 receptor, ER: endoplasmic reticulum, G1/Gs: G proteins.

mTOR inhibited. There is evidence showing that PC1 interacts with tuberin, but also directly with mTOR. Thus, PC1 dysfunction would result in mTOR activation. Renal epithelial cells show a high mTOR activity in postnatal development, while mTOR is virtually inactive in adults, and is only activated in cases of renal «repair» (e.g. compensatory renal hypertrophy, renal obstruction, …). Demonstration of mTOR activation in polycystic kidneys suggests that this is a «futile» continuous repair mechanism promoting extracellular matrix deposition, proliferation, and fibrosis.[4]

RENAL VOLUME AS A MEASURE OF THE EFFICACY OF A TREATMENT FOR ADPKD

The slow impairment of kidney function in ADPKD, as well as the probable ineffectiveness of treatment in advanced renal failure stages, prompted the need for validating an objective measure of disease progression. The NIH sponsored a large US multicentre study for this purpose, CRISP (Consortium for Radiologic Imaging Studies of PKD). The most relevant result was the evidence that MRI is the best procedure for estimating changes in cystic and renal volume in this disease over short follow-up periods. The study found that the renal «growth» rate remains constant for a given patient, that at a given age patients with bulkier kidneys progress more rapidly, and that a renal volume higher than 750 mL is a poor prognostic factor for disease progression.[5,6]

THERAPEUTIC PROPOSALS

Vasopressin receptor antagonists

V2 receptors are located in the basolateral membrane of principal cells in the

final part of the distal tubule and along the collecting tubule. These receptors are bound to a G protein that stimulates cAMP production.

The effect of vasopressin, through V2 receptors, on cAMP levels in epithelial cells from collecting tubules, together with the cystogenic effect of cAMP, suggested the idea of treating animal models of polycystic disease with antagonists of vasopressin V2 receptors. The V2 antagonist OPC-31260 was initially used in animal models of dominant and recessive polycystic disease and nephronophthisis with encouraging results.[7] Antagonist OPC-41061 (Tolvaptan) was subsequently used in PKD rats because it had the greatest affinity for human V2 receptors. This agent was greatly effective for reducing cAMP levels, renal weight, cystic volume, fibrosis, and apoptotic and mitotic indices. As there are no V2 receptors in the liver, no improvement was seen in hepatic cystic disease.[8]

A phase IIb study in polycystic patients with normal kidney function showed an excellent tolerability and efficacy at doses ranging from 60 mg and 480 mg daily.[9] Urine output, frequency of nocturia, 24-hour urine osmolarity, and natremia correlated to the Tolvaptan dose administered.

The clinical trial TEMPO III/IV (Tolvaptan Efficay and safety in Management of Polycystic kidney disease and its Outcomes) is ongoing. The purpose of this trial is to assess the effect on renal volume, as measured by MRI, after 3 years of treatment with titrated doses of 60 mg to 120 mg of Tolvaptan. This is a multicentre study (125 hospitals from Europe, North and South America, Australia, and Japan) planned to enrol 1500 adult patients (aged 18-50 years) with GFR higher than 60 mL/min and evidence of rapid disease progression (renal volume > 750 mL). Recruitment started in March 2007 and is expected to be completed during 2011. Spain was excluded from this clinical trial due to disagreement of the sponsoring pharmaceutical company (OTSUKA) with the regulations for clinical trials in our country.

Somatostatin analogues

Somatostatin is a 14-amino acid cyclic peptide secreted by D-cells of the pancreatic islets, gastrointestinal tract, nervous system, and thyroid gland. Evidence that somatostatin decreases cAMP levels, combined with the observation of a decreased kidney size in a polycystic patient with a pituitary adenoma administered somatostatin suggested its potential value in ADPKD.[10] Unlike vasopressin antagonists, somatostatin analogues have an action on polycystic liver disease.

A study conducted by Ruggeneti et al. in 12 patients showed that treatment with octreotide was clearly effective for reducing renal volume.[10]

There are two clinical trials ongoing with octreotide: a phase III trial at the Mario Negri Institute to assess its effectiveness for slowing renal impairment, and a phase II/III trial at the Mayo Clinic to assess its effectiveness in massive polycystic liver disease.

mTOR inhibitors

Based on evidence of mTOR activation in polycystic cells, mTOR inhibitors have become an attractive therapeutic option for ADPKD.

Sirolimus is a macrocyclic lactone isolated from *Streptomyces hygroscopicus*, developed as an immunosuppressant agent for prophylaxis of organ rejection in adult patients with a low to moderate immunological risk receiving a kidney transplant.

Everolimus is a macrolide antibiotic that binds to the intracellular protein FKBP, inhibiting mTOR. It is an active oral derivative of sirolimus with a shorter half-life and a greater availability.

Both sirolimus and everolimus inhibit cell growth and proliferation while enhancing apoptosis by inhibiting the signal cascade mediated by mTOR. These drugs, initially used to prevent rejection, are being introduced for the treatment of some glomerulonephritis, tumours, refractory uveitis, and as stent coating to prevent coronary re-stenosis. The effectiveness of mTOR inhibitors to reduce renal volume in murine mo-

dels of polycystic disease has recently been reported.[11,12] A reduction in the volume of native kidneys has also been shown in polycystic patients undergoing transplant treated with sirolimus.[3] Studies with animal models have shown weight loss with high sirolimus doses. As somatostatin analogues, mTOR inhibitors also act upon the liver. Qian Q et al. recently showed sirolimus to be effective for reducing hepatic volume in transplanted polycystic patients treated with the drug, but found no significant differences in renal volume.[13]

There are currently three clinical trials ongoing with sirolimus to slow progression of renal disease: a phase I-II trial at the Cleveland Clinic (30 patients), a phase III trial at the Zurich University (100 patients), and a phase II trial at the Mario Negri Institute (16 patients). Everolimus is being tested in a trial sponsored by Novartis in Germany (430 patients).

Roscovitine

As there is evidence that primary cilium dysfunction appears to be involved in cell cycle regulation, abnormalities in proteins located in the cilium or centriole may affect cell cycle and proliferation resulting in polycystic disease.[20-22] Therapeutic intervention at this cell cycle level may be effective in polycystic disease. Bukanov NO et al. showed that roscovitine (CYC202), a cyclin-dependent kinase inhibitor, slows progression of polycystic disease in JCK and CPK mice.[23] Treatment with pulses of this drug achieves a long-lasting effect, arresting cell cycle, inhibiting transcription, and decreasing apoptosis. Roscovitine has also been shown to be effective in cysts arising from any nephron segment, unlike V2 antagonists, which act upon the collecting tubule.

Interestingly, roscovitine has been shown to decrease cAMP and aquaporin-2, thus enhancing its action in polycystic kidney disease.

This drug is currently being used in clinical trials as an anti-cancer agent at higher doses than those recommended for polycystic disease.

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Small molecules

These are highly promising new drugs which are able to treat some diseases for which there is no current treatment.

O. Ibraghimov-Beskrovnaya et al. showed the role of PC1 in intercellular binding and used cultures of renal tubular cells to identify small molecules selectively inhibiting cystogenesis without inhibiting tubulogenesis. The actual value of these small molecules, candidates to improve prognosis of ADPKD, should be studied.[20]

Triptolide

Leuenroth SJ et al. recently showed the value of triptolide for the treatment of ADPKD.[24] Triptolide is the active ingredient in a traditional Chinese herb used for centuries for neoplastic and autoimmune conditions. Triptolide has been shown to induce apoptosis and to arrest cell growth depending on the concentration reached in the desired cell line. It also acts by inhibiting many proteins involved in inflammatory processes and cell growth. Authors showed the efficacy of triptolide for increasing calcium release through PC2, arresting cell growth, and reducing progression of polycystic disease in an animal model.

Two promising aspects of this study should be emphasised: the potential use of triptolide as a well tolerated treatment in ADPKD, and the evidence that PC2-mediated calcium release may be a therapeutic target, particularly for small molecules.

VEGF inhibitors

The vascular endothelial growth factor (VEGF) was initially described as a specific endothelial growth factor promoting vasculogenesis and angiogenesis and increasing vascular permeability. Existence of receptors for this factor in tubular epithelial cells has recently been reported. Tao Y et al. showed that VEGF inhibition with ribozymes in a murine model of ADPKD (Han:SPRD) slowed polycystic disease.[25]

EGFR inhibitors

Activation of the epidermal growth factor receptor (EGFR) promoting cell proliferation and cystogenesis has been demonstrated in ADPKD. A same group of researchers has reported discordant results with EGFR inhibitors depending on the rat model used. EGFR were effective in Han/SPRD rats,[26] while their effect was somewhat deleterious in CPK rats.[27] It appears that cAMP levels increase in the cells of the collecting tubule in the latter rats. This therapeutic approach is therefore in an early research phase yet.

ACEIs/ARBs

While the RAS (renin-angiotensin system) is activated in ADPKD, and there is evidence of effective reduction of proteinuria and left ventricular hypertrophy with RAS blockade,[14-16] there is no evidence of a renal protective effect of ACEIs or ARBs in ADPKD. In 222 polycystic patients participating in the MDRD study, no therapeutic benefit was found with these drugs, with an impairment rate of glomerular filtration rate of 5.9 mL/min per year.[17] However, this study was not designed to randomly treat patient with ACEIs. Another study of ramipril versus placebo in 64 patients with ADPKD showed no reduction in the time required to double creatinine levels.[18] A 7-year randomised clinical trial (ACEIs versus calcium channel blockers in 72 patients with ADPKD, hypertension, left ventricular hypertrophy, and GFR higher than 30 mL/min) showed no benefit of ACEIs.[19] All these studies assessed a relatively small number of patients for short time periods and when renal failure was already established. A double-blind, randomised clinical trial sponsored by the NIH is ongoing on 1200 patients with early (GFR > 60 mL/min) or advanced polycystic kidney disease (GFR ranging from 30 and 60 mL/min) in an attempt to assess the potential benefit of RAS inhibition in ADPKD. The trial objective is to ascertain whether strict blood pressure control using dual RAS blockade provides advantages as regards progression of polycystic disease (measured as an increase in renal volume in MRI) over ACEIs alone. This is the HALT study.

CONCLUSIONS

Increased understanding of ADPKD allows for proposing new drugs that may improve the natural course of the disease. Anyway, in addition to the still unproved effectiveness of such drugs, there are many unanswered questions: Will all drugs be useful in both PKD1 and PKD2 patients? Should only patients showing signs of rapid renal growth be treated? Must lifetime treatment be administered, or may a short treatment be able to protect for a long time from cyst growth and proliferation? At what age or in what stage of disease should treatment be started? What will be the adequate drug combination to treat the systemic signs of disease? May V2 antagonists lose efficacy over time due to downregulation of V2 receptors? May the toxicity of some drugs be assumed in young patients treated indefinitely?

All these questions should be answered before we start treating patients with ADPKD. Prospects for treatment of such a devastating renal disease as ADPKD are therefore encouraging, but caution is required, and treatments with drugs whose effectiveness, indication, and safety in this particular disease have not been proven should not be started.

Finally, our specialty should congratulate on such a fantastic example of translation of basic research into clinical trials and into a very likely effective therapy for ADPKD.

Roser Torra is a member of the Independent Data Monitoring Committee of the TEMPO ¾ study and REDINREN (ISCIII).

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KEY CONCEPTS

• Changes in the PC1-PC2 complex cause cell dedifferentiation, fluid secretion and proliferation resulting in cyst occurrence.

• Polycystic cell changes include increases in cAMP and mTOR, among other abnormalities.

Vasopressin receptor antagonists and sustained release somatostatin have been shown to be effective for slowing disease progression in murine models by decreasing cAMP levels.

• mTOR inhibitors are good candidates for treatment of the disease because this molecule is continuously activated in the polycystic cell, which leads to a constant «futile» remodelling.

• Other potential treatments for polycystic disease include roscovitine, triptolide, small molecules, VEGF inhibitors, EGFR inhibitors, ACEIs, and ARBs.

• Prospects for treatment of such a devastating renal disease as ADPKD are encouraging, but caution is required, and treatments with drugs whose effectiveness, indication, and safety in this particular disease have not been proven should not be started.

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