Lipids and renal cystic disease

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Summary of key findings

Glucosylceramide (GlcCer) levels were found to be higher in kidney tissue of autosomal dominant PKD/conditional knockout mice and nephronophthisis mice (jck and pcy mice) than in normal kidney tissue. The same was found in renal tissue of human polycystic kidney disease (PKD) as compared with normal kidney tissue. Similarly, independent of different genetic mutations and clinical heterogeneity, common defects of most cystic epithelium include proliferation, apoptosis and activation of growth-regulating pathways. On this background, an inhibitor of GlcCer synthase (Genz-123346) was shown to inhibit cystogenesis in PKD 1, jck and pcy mice. Molecular analysis in vitro and in vivo demonstrated that Genz-123346 inhibits two pathways that are dysregulated in PKD, namely Akt protein kinase–mammalian target of rapamycin (mTOR) signalling [1] and cell cycle machinery [2]. The authors suggest that inhibitors of GlcCer synthesis may represent a potential new treatment for PKD.

Review of the field

Basic science studies have led to several molecular mechanisms in cystogenesis including aberrant signalling of the cilia–cell cycle, calcium–cyclic AMP, Wnt and mTOR pathways [1]. These preclinical results have led to several prospective randomized clinical trials in patients with autosomal dominant polycystic kidney disease (ADPKD). With the effect of cyclic AMP on cell proliferation and fluid secretion, clinical studies have been undertaken to block these effects of cyclic AMP. Since arginine vasopressin stimulates cyclic AMP in the principal cells of the collecting duct, and a safe V2 vasopressin receptor antagonist, tolvaptan, is available [2], the TEMPO clinical trial is underway in a large randomized study in ADPKD [3]. The somatostatin analogue, octreotide, is another inhibitor of cyclic AMP, which has also been studied in 34 patients with ADPKD followed up for 1 year [4]. After 1 year, the mean kidney volume was stable in the octreotide group but increased in placebo group. There was a significant difference in the percent change in kidney volume between the two groups. A similar effect was observed on liver volume. There was no difference in kidney function.

Two major clinical trials examining the effect of mTOR inhibition were recently reported in the New England Journal of Medicine [5,6]. This approach was stimulated by studies in experimental animals which demonstrated a decrease in renal cyst and kidney volume with mTOR suppression by rapamycin (sirolimus) [7,8]. The study by Serra et al. [5] randomly assigned 100 ADPKD patients with a creatinine clearance of at least 70 mL/min to sirolimus (2 mg/day) or standard care for 18 months. In this study, there was no significant difference in the growth of total renal volume or difference in glomerular filtration rate (GFR). The larger study by Walz et al. [6] was a 2-year, double-blind trial of 433 ADPKD patients treated with either placebo or the mTOR inhibitor everolimus. Again, the primary end point was total renal volume as measured by magnetic resonance imaging at 12 and 24 months. The everolimus-treated group exhibited less increase in total kidney volume at 12 (P = 0.02) and 24 months (P = 0.06). There was, however, no significant difference in cyst growth at either 12 or 24 months. The mean decrement in GFR at 24 months was 8.9 mL/min/1.73 m² in the everolimus versus 7.7 mL/min/1.73 m² in the placebo group (P = 0.15). While everolimus showed less increase in total kidney volume during the first 12 months, there was no improvement in GFR. In fact, based on linear regression, there was a greater decline in GFR with everolimus versus placebo (5.4 versus 3.2 mL/min/1.73 m², P = 0.004). Also of concern, the dropout rate in the everolimus group was 32.7% versus 14.7% in the placebo group.

It is on this background that the recent Nature Medicine publication [9] with inhibition of GlcCer accumulation should be considered. Glycosphingolipids have been previously recognized to play an important role in regulation of proliferation, apoptosis and cell signalling [10–12]. The specific inhibitor of the synthesis of GlcCer, Genz-123346, in three mouse models demonstrated a beneficial
effect both histologically and functionally. Of importance, the GlcCer synthase inhibitor blocks two pathways that have been shown to be aberrant in PKD, namely the mTOR and cell cycling pathways. Given the complexity of the pathogenesis of ADPKD, it may be necessary, as with other neoplasms, to block more than one pathway such as proliferation, apoptosis, growth signalling, oxidant injury and fibrosis. Of importance, a similar compound has been shown in phase 1 and 2 clinical trials to be well tolerated in Gaucher’s disease [13]. Since any therapy for ADPKD would probably be life-long, the safety of the intervention is very important. Since humans with ADPKD progress slowly over decades, whereas most rodent models progress more rapidly, the extrapolation to human disease must be done with caution. Thus, ultimately, long-term interventional studies with adequate statistical power in humans with ADPKD must be the gold standard.

**What does this mean for the practising nephrologist?**

At this stage, this basic science paper does not impact clinical practice. However, the involvement of renal lipids in ADPKD might emphasize the potential importance of the use of statins in these patients. Currently, there is a randomized ADPKD children’s study examining the effect of angiotensin-converting enzyme (ACE) inhibition with and without a statin [14]. Since many of the cysts originate very early including in utero, future studies in ADPKD children may be the most critical in slowing renal and cardiac disease progression. A recent randomized study in ADPKD children with borderline hypertension (75–95th percentile) has demonstrated a decrease in loss of renal function and less cardiac enlargement over 5 years with an ACE inhibitor compared with placebo [15]. Since cardiovascular complications are the most common cause of death in ADPKD [16], early detection and treatment of hypertension, probably initially with an ACE inhibitor, are advised. Of interest, the HALT PKD randomized study [17] is based primarily on observations, not in rodents, but in humans. This study examines an ACE inhibitor plus angiotensin blocker (ARB) versus an ACE inhibitor alone in patients with early versus advanced ADPKD. An earlier randomized study demonstrated that ADPKD patients have increased circulating plasma renin activity and aldosterone compared with patients with essential hypertension at comparable blood pressures, kidney function and sodium intake [18]. Also, ADPKD kidneys from patients have been demonstrated to have all the components of the RAAS in contrast with normal kidneys [19]. A 7-year prospective randomized study in ADPKD patients with hypertension and left ventricular hypertrophy (LVH) demonstrated the optimal reversal of LVH occurred with ACE inhibition versus a calcium channel blocker and a blood pressure goal of 120/80 versus 140/90 mmHg [20]. Thus, this study has important implications for cardiovascular complications and mortality in patients with ADPKD.

**Take-home message**

Glycosphingolipids may be involved in the renal disease of ADPKD, but more preclinical studies need to be performed prior to examining this possibility in randomized studies in patients with ADPKD.

**Conflict of interest statement.** None declared.

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Unravelling fibrosis: two newcomers and an old foe

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Novel pathways to fibrosis

Acute tubular cell with disrupted repair mechanisms or persistent injury causes interstitial inflammation and may lead to renal fibrosis and chronic kidney disease (CKD). Both progress even when the injurious stimulus has been removed. Tubular epithelial cells and fibroblasts are key players in kidney fibrosis. Two new studies have addressed the question of what molecular events determine between physiological repair and pathological fibrosis of the kidney. Two novel mechanisms, cell cycle G2/M arrest of proximal tubular epithelial cells and epigenetic modifications of fibroblasts, were identified as key events in kidney fibrosis. An old acquaintance, transforming growth factor-$\beta$1 (TGF-$\beta$1), is one of several potential common links between these novel pathways for fibrosis [1,2].

Tubular epithelium cell cycle arrest

Tubular cells participate in kidney fibrosis. Early evidence that tubular epithelial cells themselves may give rise to interstitial fibroblasts through epithelial–mesenchymal transition (EMT) has been recently disputed [3,4]. In addition, injured tubular cells may die and secrete cytokines that may promote inflammation, recruitment of circulating fibrocytes (such as CCL21), or fibrosis (such as TGF-$\beta$1) [5,6]. Bonventre et al. recently suggested that repair of acute kidney injury (AKI) is dependent on proliferation of surviving cells that replace damaged cells leading to recovery (as opposed to recruitment of stem cells from the bone marrow) [7]. They now suggest that the incapacity of tubular epithelial cells to proliferate (caused by G2 arrest) is a root of fibrosis in the kidney. Tubular cell proliferation requires entry into the cell cycle, a sequential occurrence of molecular and cellular events termed the G1, S, G2 and M phases. Cell cycle checkpoints monitor the structural integrity of chromosomes before progression through crucial cell cycle stages. Checkpoints occur at entry into the S phase of DNA replication (the G1/S checkpoint) and at entry into mitosis (the G2/M checkpoint), as well as during replication (intra/S checkpoints) [8].

In ischaemic, toxic and obstructive AKI models, Yang et al. identified a causal association between proximal tubular cell G2/M cell cycle arrest and interstitial fibrosis [1]. G2/M-arrested proximal tubular cells activate c-jun NH2-terminal kinase (JNK) signalling, which upregulates the release of profibrotic cytokines such as TGF-$\beta$1 and connective tissue growth factor (CTGF), which in turn stimulated the proliferation and collagen synthesis by fibroblasts. JNK inhibition or bypassing the G2/M arrest by administration of a p53 inhibitor or the removal of the contralateral kidney rescued fibrosis. Pharmacological induction of epithelial G2/M cell cycle arrest in vivo increased the expression of genes that induce fibroblast activation and kidney fibrosis, while reversal of epithelial G2/M arrest protected from fibrosis.

The nephrotoxin responsible for Chinese herb nephropathy, aristolochic acid, induced G2/M arrest in cultured tubular cells. The candidate factors responsible for cell cycle arrest in ischaemic or obstructive AKI not identified include oxidative stress, endogenous and exogenous nephrotoxins, TNF superfamily cytokines, activated neuro-