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In Focus

Effect of resveratrol on progression of polycystic kidney disease: a case of cautious optimism

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Autosomal dominant polycystic kidney disease (ADPKD) is a common inherited genetic cause of chronic kidney disease, affecting 1 in 500 individuals in the USA. In addition, ADPKD is the most common genetic cause of end-stage renal disease (ESRD), accounting for ∼7–10% of patients on renal replacement therapy [1]. It is characterized by the development of fluid-filled cysts in the renal tubules and their progressive enlargement over time leading to the distortion of renal tissue and ongoing kidney damage and culminating in ESRD [2, 3]. At the molecular level, ADPKD is caused by mutations in the *PKD1* (85%) or *PKD2* (15%) genes, which encode the cilia-associated polycystin-1 (PC1) and polycystin-2 (PC2) proteins [4]. PC1 and PC2 are membrane proteins that are located in the primary cilia of the tubular epithelial cells, and while the ligand for PC1 has not been identified, PC2 is most likely a non-selective calcium channel [2, 3]. There are multiple mechanisms by which mutations in the *PKD1* and *PKD2* genes result in cystogenesis, including increased cell proliferation, enhanced fluid secretion, abnormal cell–matrix interaction, alterations in cell polarity and abnormal ciliary structure and function [2]. However, there are also several nongenetic pathways that can contribute to the progression and modify the severity of ADPKD. This is evidenced by the fact that patients with ADPKD have large intrafamilial variability in renal function even between monozygotic twins and siblings [5]. In addition, in monozygotic twins, there can be significant differences in the age at which ESRD is reached, thereby highlighting the role of nonhereditary pathways in ADPKD-associated renal injury and progression of disease [5]. These include pathways related to growth factor and cytokine signaling [6]. The importance of inflammation and cytokine signaling in PKD has been established in numerous studies over the past few decades. Gardner *et al.* [7] noted high concentrations of inflammatory chemokines and cytokines in the cyst fluid from patients with ADPKD. Cowley *et al.* [8] subsequently demonstrated that increased expression of monocyte chemoattractant protein-1 (MCP-1) was accompanied by accumulation of macrophages in the kidneys of rats with ADPKD. It is now known that MCP-1 is present in the urine of patients with ADPKD and that high levels of urinary MCP-1 correlate with the rate of cyst growth and progression of disease [9, 10]. In line with these discoveries, large numbers of activated macrophages have been found around renal cysts in animal models of ADPKD whose depletion inhibited epithelial cell proliferation and cyst growth and improved renal function [11]. Furthermore, Swenson-Fields *et al.* [12] demonstrated that increased numbers of macrophages expressing the M2 marker CD163 are also present in the interstitium of kidneys of patients with both AD and autosomal recessive PKD, some of which are in close proximity to the cysts. In turn, the cyst epithelial cells stimulated macrophage differentiation toward a distinct M2-like phenotype that, based on *in vitro* studies, promoted proliferation and microcyst formation. Furthermore, recent studies have demonstrated that macrophage migration inhibitory factor (MIF) is upregulated in the epithelial cells lining renal cysts and that this protein accumulates in the cyst fluid of patients with ADPKD [13]. MIF was found to be a key regulator of cyst growth in ADPKD via several converging mechanisms, including cytokine signaling. It was shown that MIF-dependent macrophage recruitment was associated with increased MCP-1 and tumor necrosis factor α (TNF-α) production, which in turn increased expression of MIF. Hence, a positive feedback loop between inflammation, inflammatory cytokines and MIF was identified, with the end result being progression of the disease [13]. Therefore, besides the genetic abnormalities that instigate the underlying disease in ADPKD, inflammation, macrophage activation, differentiation

and accumulation also play an important role in the pathogenesis and progression of PKD.

Resveratrol is a natural polyphenol found in high concentrations in red grapes, berries, peanuts and legumes [14–16]. It is produced in plants in response to environmental stress such as mechanical injury, microbial infection and ultraviolet irradiation, leading to fortification of the plants' natural defenses. Resveratrol has been found to have a wide range of positive effects in human and animal studies, including cardioprotective, anticancer, neuroprotective, antiaging, antioxidant and anti-inflammatory properties [14–16]. Considering that kidney disease is associated with oxidative stress and inflammation, which are both the cause and consequence of kidney injury, resveratrol has emerged as a potential therapeutic agent in a range of kidney disorders [17, 18]. There are several mechanisms through which resveratrol confers antioxidant and anti-inflammatory effects. It has been shown that administration of resveratrol leads to activation of nuclear factor erythroid 2–related factor 2 (Nrf2), which increases expression of antioxidant enzymes [superoxide dismutase (SOD), catalase and glutathione peroxidase], reduces markers of inflammation including TNF-α and IL-6 and improves renal function in rats with diabetic nephropathy [19]. In addition, by increasing the expression and activity of adenosine monophosphate–activated protein kinase (AMPK) and the AMPK pathway, resveratrol exerts antioxidant, antifibrotic and anti-inflammatory effects [20]. Moreover, AMPK activation can slow cyst progression in ADPKD by inhibiting the cystic fibrosis transmembrane conductance regulator chloride channel expression, which has been shown to be involved in cystogenesis and cyst enlargement [20, 21]. Another important target of resveratrol is sirtuin (SIRT), whose activation has many different effects, including prevention of inflammation by reducing nuclear factor-κB (NF-κB) expression [14]. SIRT is a nicotinamide adenine dinucleotide-dependent deacetylase with seven isoforms in mammals (SIRT1–7) [22]. The most well-studied isoform is SIRT1, which is a crucial molecule in glucose, lipid and energy metabolism and has known antioxidant, antiapoptotic and anti-inflammatory effects. The renoprotective properties of SIRT1 are mediated via its regulation of several factors including those related to apoptosis, fibrosis and inflammation, such as p53, the NF-κB p65 subunit and matrix metalloproteinase 14 (MMP-14) [22, 23]. These salutary effects have been confirmed in various models of renal disease, including acute kidney injury and diabetic nephropathy. SIRT1 deacetylates and inactivates the p65 subunit of NF-κB, thereby exerting significant anti-inflammatory effects [22]. Furthermore, there is evidence that SIRT1 activation may lead to increased nuclear content and transcriptional activity of Nrf2, thus indicating that SIRT1 antioxidant effects are mediated via Nrf2-dependent and -independent mechanisms [24, 25]. While resveratrol mediates antioxidant and anti-inflammatory effects via multiple mechanisms, there is also evidence that it causes inhibition of the mammalian target of rapamycin (mTOR) signaling pathway independent of its effect on SIRT1 and Nrf2. Given that mTOR complexes (mTORC1 and mTORC2) are involved in cell growth and survival and play a deleterious role in PKD, resveratrol-mediated inhibition of the mTOR pathway may provide an additional protective effect [14].

In light of the evidence linking cytokine signaling and inflammation with cystogenesis and progression of renal disease in ADPKD and the pleiotropic pathways by which resveratrol may remediate these effects, examination of this compound in PKD has significant therapeutic potential and value (Figure 1).

In an article published in the current issue of *Nephrology Dialysis Transplantation*, Ming Wu *et al.* [26] examined the

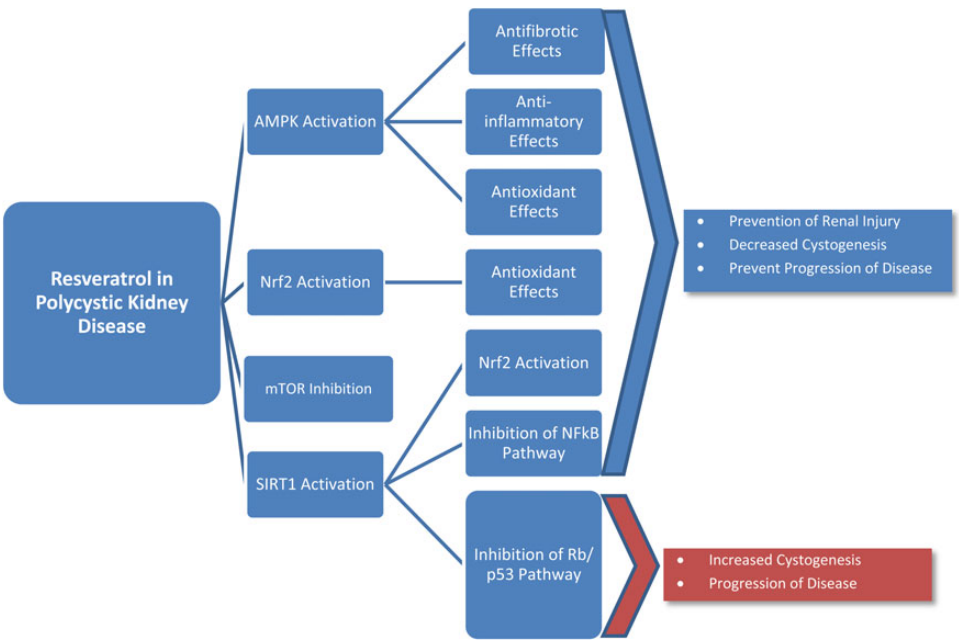

FIGURE 1: Some of the effects that resveratrol treatement may have on the progression of PKD.

impact of resveratrol treatment on the progression of PKD using a series of *in vivo* and *in vitro* experiments. They treated male Han:SPRD (Cy/+) rats, a nonorthologous model of ADPKD, with 200 mg/kg/day of resveratrol or vehicle by gavage for 5 weeks and found that resveratrol treatment significantly reduced renal tissue TNF-α, MCP-1 and complement factor B (CFB) levels. This was associated with a significant reduction in macrophage infiltration in the renal tissue of PKD animals. In addition, resveratrol treatment significantly reduced cyst volume density and the proliferation index. Animals treated with resveratrol also had a modest but statistically significant improvement in their serum creatinine and blood urea nitrogen levels, indicating improvement in renal function.

In order to study the mechanisms by which resveratrol reduces inflammation and attenuates renal damage, the authors conducted a series of studies employing cell culture and zebra fish models of ADPKD in addition to the animal model. They found that resveratrol treatment decreased phosphorylation of the p65 subunit of NF-κB without altering its protein abundance while reducing the p50 subunit expression in animals with PKD. Furthermore, resveratrol reduced the phosphorylation of S6K, a downstream target of the mTOR pathway. Interestingly, resveratrol treatment of animals with PKD did not restore renal levels of the antioxidant enzyme SOD2 or reduce renal markers of oxidative stress, nitrotyrosine and 8-hydroxy-2′–deoxyguanosine. Given that resveratrol is capable of activating the Nrf2 pathway, these findings are somewhat unexpected. However, in the cell culture experiments, the authors did find that resveratrol concentrations at 2–10 μM increased SOD2 levels in OX161 cells (a human ADPKD cell line) while at a concentration of 50 μM it decreased SOD2 levels. This is not surprising since Nrf2 activators attenuate oxidative stress and inflammation at low concentrations and intensify oxidative stress and inflammation at high levels [27]. Therefore, the unexpected findings regarding markers of oxidative stress may have been related to the dose of resveratrol employed in the *in vivo* experiments. In addition, in a series of studies in OX161 cells, the authors demonstrated that resveratrol treatment resulted in reduced NF-κB activation via reduced p65 phosphorylation and reduced p50 expression, causing decreased production of inflammatory cytokines such as MCP-1 and CFB. The deleterious role of NF-κB activation was further confirmed via use of an NF-κB-specific inhibitor, QNZ. However, the fact that resveratrol can reduce cystogenesis and inflammatory cytokine production above and beyond QNZ treatment demonstrates that it must also be acting via NF-κB-independent mechanisms. In fact, the authors showed that resveratrol also downregulated components of the mTOR pathway, which may add an extra dimension to this compound's impact on PKD beyond the NF-κB pathway. Furthermore, as noted earlier, resveratrol is an activator of the AMPK and SIRT pathways, both of which can have pleiotropic properties including anti-inflammatory, antioxidant, antifibrotic and antiapoptotic effects. Therefore, resveratrol may act through a range of mechanisms whose effects may hold major therapeutic potential in ADPKD. While some of these pathways were explored in this study, several other areas, including AMPK, Nrf2 and p53 pathways, await investigation in the future.

It should be noted that resveratrol-mediated activation of the SIRT pathway may have a deleterious effect in PKD. Zhou *et al.* [28] found that mice lacking both *Pkd1* and *SIRT1* genes had decreased cyst formation and SIRT1 inhibition in Pkd1 knockout mice resulted in a decreased rate of cyst development. These instigators subsequently showed that SIRT1 activation caused renal epithelial cell proliferation through deacetylation and phosphorylation of the retinoblastoma (Rb) protein (which inactivates this protein) and dysregulated cell death via deacetylation of the p53 protein, leading to continuous epithelial cell growth and cyst formation. Therefore, the results of the study by Zhou *et al.* [28] should be taken into account when studying compounds that can act through activation of SIRT1.

In summary, the study by Ming Wu *et al.* [26] demonstrated the salutary effects of resveratrol in attenuating inflammation and retarding progression of ADPKD in experimental animals. While the use of resveratrol provides an intriguing therapeutic option that addresses many of the pathogenic processes involved in PKD progression, its improper use can have potential adverse consequences. For instance, via overactivation of SIRT1, it may enhance formation and enlargement of cysts, and at high doses it may intensify inflammation and accelerate progression of renal disease. Future studies are needed to address these important issues and identify the safe and effective dosage of this natural medicinal product.

CONFLICT OF INTEREST STATEMENT

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