FO006

THE NRF2 ACTIVATOR BARDOXOLONE METHYL INHIBITS CYST FORMATION, REDUCES INFLAMMATION, AND IMPROVES MITOCHONDRIAL FUNCTION IN CELLULAR MODELS OF POLYCYSTIC KIDNEY DISEASE

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INTRODUCTION: Autosomal dominant polycystic kidney disease (ADPKD), caused by heterozygous loss-of-function mutations in genes that encode polycystin-1 (*PKD1*) or polycystin-2 (*PKD2*), is the most common inherited cause of chronic kidney disease. As ADPKD progresses, normal renal tissue is disrupted by fluid-filled cysts, leading to end-stage kidney disease in many patients. Hallmarks of ADPKD include inflammation, fibrosis, oxidative stress, and alterations in cellular metabolism. Bardoxolone methyl (Bard) potently activates Nrf2, a transcription factor that inhibits the production of pro-inflammatory mediators, restores redox balance, and improves mitochondrial function. In a recent Phase 2 clinical trial (PHOENIX, NCT03366337), Bard significantly improved eGFR in patients with ADPKD. To further explore the mechanism of action that may contribute to this effect, we asked whether Bard inhibits inflammation, restores redox balance, and improves mitochondrial function in proximal tubule cells derived from patients with ADPKD. We also assessed its activity in the Madin-Darby Canine Kidney (MDCK) cell model of renal cyst formation.

METHODS: We used primary single-cyst derived cells from two different ADPKD donors, an SV40-immortalized single-cyst culture from a single ADPKD donor, two additional immortalized ADPKD cell lines, WT 9-7 and WT 9-12, MDCK cells, and a normal human proximal tubule cell line, HK-2, to evaluate the effects of Bard. To assess Nrf2 activation, *NQO1* and *GCLM* mRNA levels were measured by qPCR, and total glutathione levels were measured using the GSH-Glo assay. MCP-1 levels were measured by ELISA and reactive oxygen species were assessed by CM-H2DCFDA fluorescence. Mitochondrial function was monitored using an XFe96 Analyzer (Seahorse Biosciences). MDCK cells were grown in a 3D matrix and treated with forskolin to stimulate cyst formation.

RESULTS: We found that Bard increased Nrf2 activity in both primary and immortalized cells. Consistent with the important role of inflammation in ADPKD, we observed higher levels of pro-inflammatory mediators in the WT 9-7 and WT 9-12 cell lines than in the HK-2 control cell line. Treatment with Bard significantly reduced the levels of C-C Motif Chemokine Ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1), and suppressed inflammation-induced reactive oxygen species. Bard also dose-dependently improved parameters of mitochondrial function, including spare respiratory capacity and maximal respiration, in the ADPKD cell lines. In these cells, pro-inflammatory stimuli suppressed mitochondrial function and this was partially restored by Bard treatment. In the MDCK cyst model, Bard reduced the number of mature cysts grown in a 3D matrix, but did not inhibit cell growth or increase cell death.

CONCLUSIONS: In summary, Bard increased Nrf2 activity, reduced inflammation and reactive oxygen species, and improved mitochondrial function in primary and immortalized renal cells derived from patients with ADPKD. Bard also inhibited cyst formation in the MDCK cyst model without increasing cellular toxicity. Together, these results suggest that activation of Nrf2 by Bard may have the potential to improve the molecular features that are hallmarks of ADPKD. A Phase 3 clinical trial (FALCON) is planned to evaluate the safety and efficacy of Bard in patients with ADPKD.