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Steviol slows renal cyst growth by reducing AQP2 expression and promoting AQP2 degradation

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ABSTRACT

Overexpression of aquaporin 2 (AQP2) was observed and suggested to be involved in fluid secretion leading to cyst enlargement in polycystic kidney disease (PKD). The cyst expansion deteriorates the renal function and, therefore, therapies targeting cyst enlargement are of clinical interest. Of note, inhibition of vasopressin function using vasopressin 2 receptor (V2R) antagonist which decreased cAMP production along with AQP2 production and function can slow cyst growth in ADPKD. This finding supports the role of AQP2 in cyst enlargement. Steviol, a major metabolite of the sweetening compound stevioside, was reported to retard MDCK cyst growth and enlargement by inhibiting CFTR activity. Interestingly, its efficacy was found to be higher than that of $CFTR_{inh}$-172. Since steviol was also found to produce diuresis in rodent, it is likely that steviol might have an additional effect in retarding cyst progression, such as inhibition of AQP2 expression and function. Here, we investigated the effect of steviol on AQP2 function and on cyst growth using an *in vitro* cyst model (MDCK and *Pkd1*$^{-/-}$ cells). We found that steviol could markedly inhibit cyst growth by reducing AQP2 expression in both *Pkd1*$^{-/-}$ and MDCK cells. Real-time PCR also revealed that steviol decreased AQP2 mRNA expression level as well. Moreover, a proteasome inhibitor, MG-132, and the lysosomotropic agent, hydroxychloroquine (HCQ) were found to abolish the inhibitory effect of steviol in *Pkd1*$^{-/-}$ cells. Increased lysosomal enzyme marker (LAMP2) expression following steviol treatment clearly confirmed the involvement of lysosomes in steviol action. In conclusion, our finding showed for the first time that steviol slowed cyst growth, in part, by reducing AQP2 transcription, promoted proteasome, and lysosome-mediated AQP2 degradation. Due to its multiple actions, steviol is a promising compound for further development in the treatment of PKD.

1. Introduction

Polycystic kidney disease (PKD) is an inherited disorder with the most common form caused by mutations in either the *PKD1* or *PKD2* genes [1]. The characteristics of PKD are abnormal renal cell proliferation and massive fluid secretion into the cyst lumen [2,3]. Increasing numbers of fluid-filled cysts occupy the renal parenchyma causing the decline in kidney function and patients eventually exhibit end-stage renal failure [4].

Accumulating evidence has shown that cyst enlargement in PKD is dependent on the activities of the cAMP activated-cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel [5] and water channel at the apical membrane of cyst-lining epithelial cells [3]. Chloride movement through CFTR creates an osmotic force within the cyst lumen to promote transport of sodium and water through tight junctions or transcellular leading to cyst enlargement [5]. It was reported that blockade of vasopressin 2 receptor (V2R) to decreased cAMP slowed renal cyst growth in the animal model of ADPKD [6]. Since vasopressin also increases AQP2 production and function, it is likely that this process may involve AQP2 as well. This notion was supported by the finding that AQP2 expression level was significantly increased with cyst size [7] Moreover, elevated vasopressin level was observed in cystic kidney of PKD mice [8]. Therefore, effective therapies targeting cyst enlargement in PKD would require inhibition of both CFTR chloride channel and AQP2 water channel in renal cyst-lining epithelial cells.

Previously, we reported that steviol retarded MDCK cyst enlargement through inhibition of CFTR [9]. It inhibited CFTR chloride

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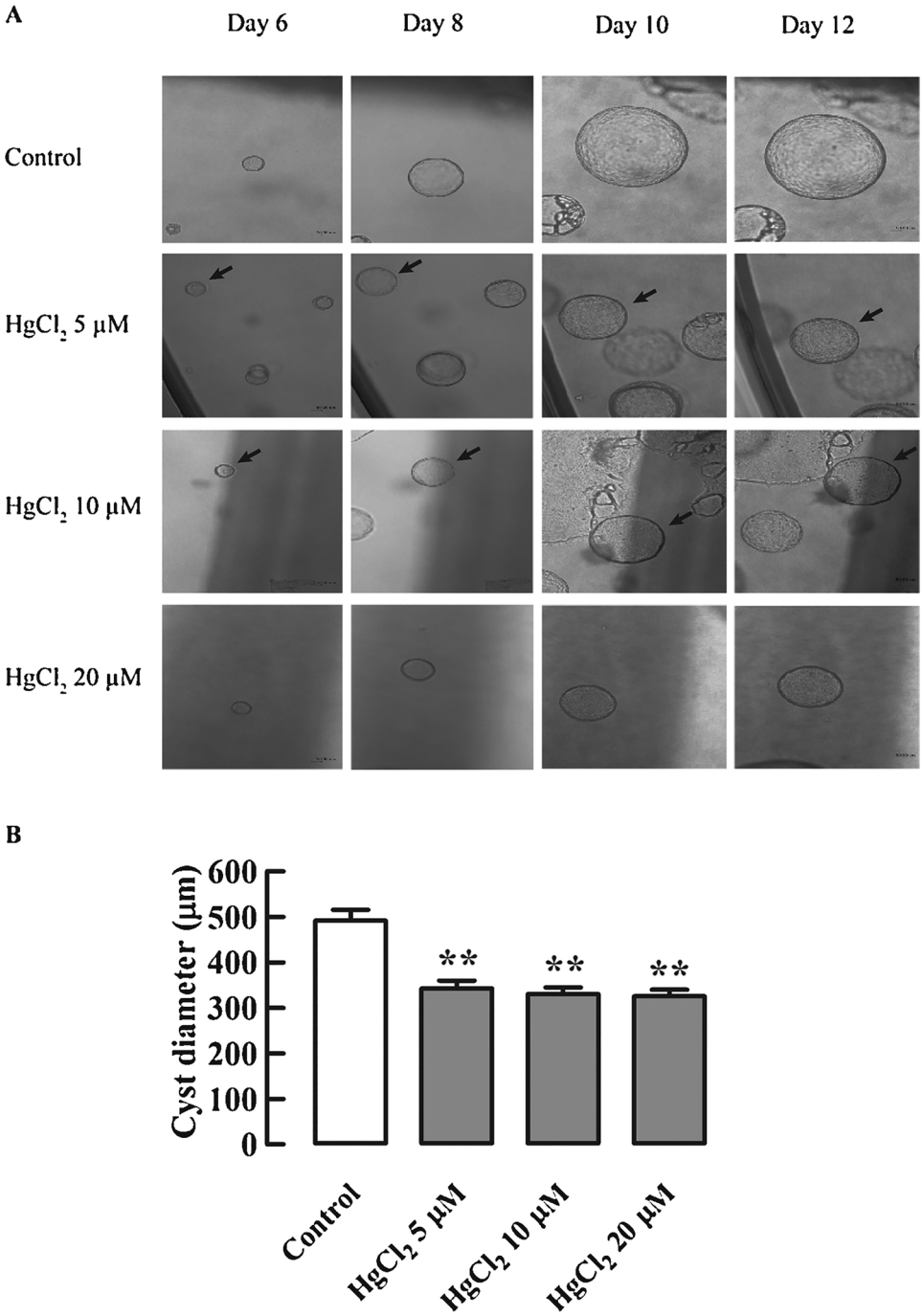

Fig. 1. Effect of mercury on cyst progression in the MDCK cyst growth model. (A) The representative light micrographs show MDCK cyst growth in 3D collagen gels after seeding of MDCK cells for 6 days. 10 μM forskolin without (1A, top) or with $HgCl_2$ (5, 10, 20 μM) was added to the culture media (1A). Bar = 100 μm, magnification = 10 × . (B) Inhibitory effect of mercury on MDCK cyst growth. The graph shows the outside cyst diameter at day 12 (mean ± SE, $n > 30$ cysts per condition, $^{**}P < 0.01$ compared with control).

channel activity and promoted the proteasome-mediated degradation of CFTR in both the MDCK cyst model and PKD mouse. However, the ability of steviol to slow growth of MDCK cysts was greater than that of $CFTR_{inh}$-172. This raises the possibility that the action of steviol may involve a mechanism in addition to an inhibition of CFTR activity. Interestingly, steviol and its parent compound, stevioside, were reported to induce diuresis in rat [10,11], which was likely to involve AQP2 function. Therefore, it is likely that steviol may have a direct effect on AQP2 expression which partly limiting cyst growth. In the present study, the effect and underlying mechanisms of steviol on AQP2 protein expression and cyst growth were investigated in both MDCK and *Pkd1*$^{-/-}$ cells.

2. Materials and methods

2.1. Cell culture

MDCK cells were kindly provided by Prof. David N. Sheppard (University of Bristal, Bristol, UK). Mouse renal cystic epithelial cells (PN24, *Pkd1*$^{-/-}$ cells) were kindly provided by Prof. Stefan Somlo (Yale University School of Medicine, CT, USA). MDCK cells were cultured in a 1:1 mixture of Dulbecco's modified Eagle medium (DMEM) and Ham's F-12 nutrient medium supplemented with 10% Fetal bovine serum (FBS), insulin (8.3×10^{-7} M), selenium X (6.8×10^{-9} M), transferrin (6.2×10^{-8} M) and supplemented with 100U/ml penicillin