Life Sciences 212 (2018) 70–79

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Effect of celastrol on the progression of polycystic kidney disease in a *Pkd1*-deficient mouse model

Ming-Yang Chang[a,*], Chun-Yih Hsieh[a], Chan-Yu Lin[a], Tai-Di Chen[b], Huang-Yu Yang[a], Kuan-Hsing Chen[a], Hsiang-Hao Hsu[a], Ya-Chung Tian[a], Yung-Chang Chen[a], Cheng-Chieh Hung[a], Chih-Wei Yang[a]

[a] Kidney Research Center, Department of Nephrology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan
[b] Department of Pathology, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Taoyuan, Taiwan

ARTICLE INFO

Keywords:
Inflammation
Polycystic kidney disease
Celastrol
Macrophage

ABSTRACT

Aims: Celastrol, a naturally occurring pentacyclic triterpene, has attracted considerable interest because it exhibits potent anti-inflammatory and anti-tumor properties. However, the effects of celastrol in autosomal dominant polycystic kidney disease (ADPKD) remain uninvestigated.

Main methods: We determined the effects of celastrol on ADPKD progression in a novel *Pkd1*-hypomorphic mouse model by intraperitoneal injection (postnatal day 35–63).

Key findings: Pkd1 miRNA transgenic (*Pkd1* miR TG) mice treated with 1 mg/kg/day of celastrol exhibited a lower renal cystic index (by 21.5%) than the vehicle-treated controls, but the fractional kidney weights and blood urea nitrogen levels were not significantly affected with celastrol treatment. At a high dose (2 mg/kg/day), celastrol caused marginal weight loss in the treated mice and had no significant effect on renal cystogenesis, thus indicating a potential toxic effect. We further identified that celastrol increased the phosphorylation level of adenosine monophosphate-activated protein kinase (AMPK) in the cystic kidneys. Moreover, celastrol reduced the renal mRNA expression levels of tumor necrosis factor-α, interleukin-1β, P2RX7, F4/80, CD68, transforming growth factor-β, collagen-1, and fibronectin, which were high in the *Pkd1* miR TG mice. Immunohistological analysis revealed that celastrol suppressed macrophage infiltration in the cystic kidneys; however, the renal fibrosis scores and proliferation indices remained high.

Significance: These results indicate that celastrol could be a potent anti-inflammatory agent and a natural AMPK enhancer. However, celastrol has only modest effects on renal cystogenesis and has a narrow therapeutic window. Further studies are needed to clarify whether celastrol has the potential for the treatment of ADPKD.

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a common disease, caused by mutations in *PKD1*, *PKD2,* and other genes, such as *GANAB* [32]. Renal function in patients with ADPKD may decline progressively because of the destruction of normal kidney structures caused by the growth of renal cysts [2]. ADPKD pathogenesis involves abnormal cell proliferation, fluid secretion, ciliary signaling, and extracellular matrix defects [31]. Other cellular abnormalities, such as inflammation and metabolic reprogramming, may contribute to cyst expansion and disease progression in ADPKD [15,45]. Inhibition of the cyclic adenosine monophosphate (AMP) pathway by using tolvaptan, a vasopressin V2 receptor antagonist, in patients with ADPKD reduced cyst growth and improved renal function [42]. However, whether a combination of treatments targeting metabolic reprograming, inflammation, and fibrosis are also beneficial in alleviating ADPKD remains unknown thus far [5].

Celastrol and triptolide represent two different classes of compounds that are isolated from roots of the Chinese herb thunder god vine [26,35]. Triptolide inhibits the early phase (up to postnatal day 8) of cyst growth through the suppression of cell proliferation and restoration of cytosolic Ca^{2+} release in *Pkd1* knockout mice [21–23]. In an observational cohort of patients with proteinuric ADPKD, treatment with triptolide was associated with a reduction in proteinuria and improvement in total kidney volume [7]. Contrary to those of triptolide, the effects of celastrol on the progression of ADPKD have not been reported.

Celastrol, a naturally occurring pentacyclic triterpene, has attracted

* Corresponding author at: Kidney Research Center, Department of Nephrology, Chang Gung Memorial Hospital, 5 Fu-Shing St., Kueishan, 333 Taoyuan, Taiwan.
E-mail address: mingyc@adm.cgmh.org.tw (M.-Y. Chang).

https://doi.org/10.1016/j.lfs.2018.09.047
Received 3 July 2018; Received in revised form 21 September 2018; Accepted 25 September 2018
Available online 27 September 2018
0024-3205/

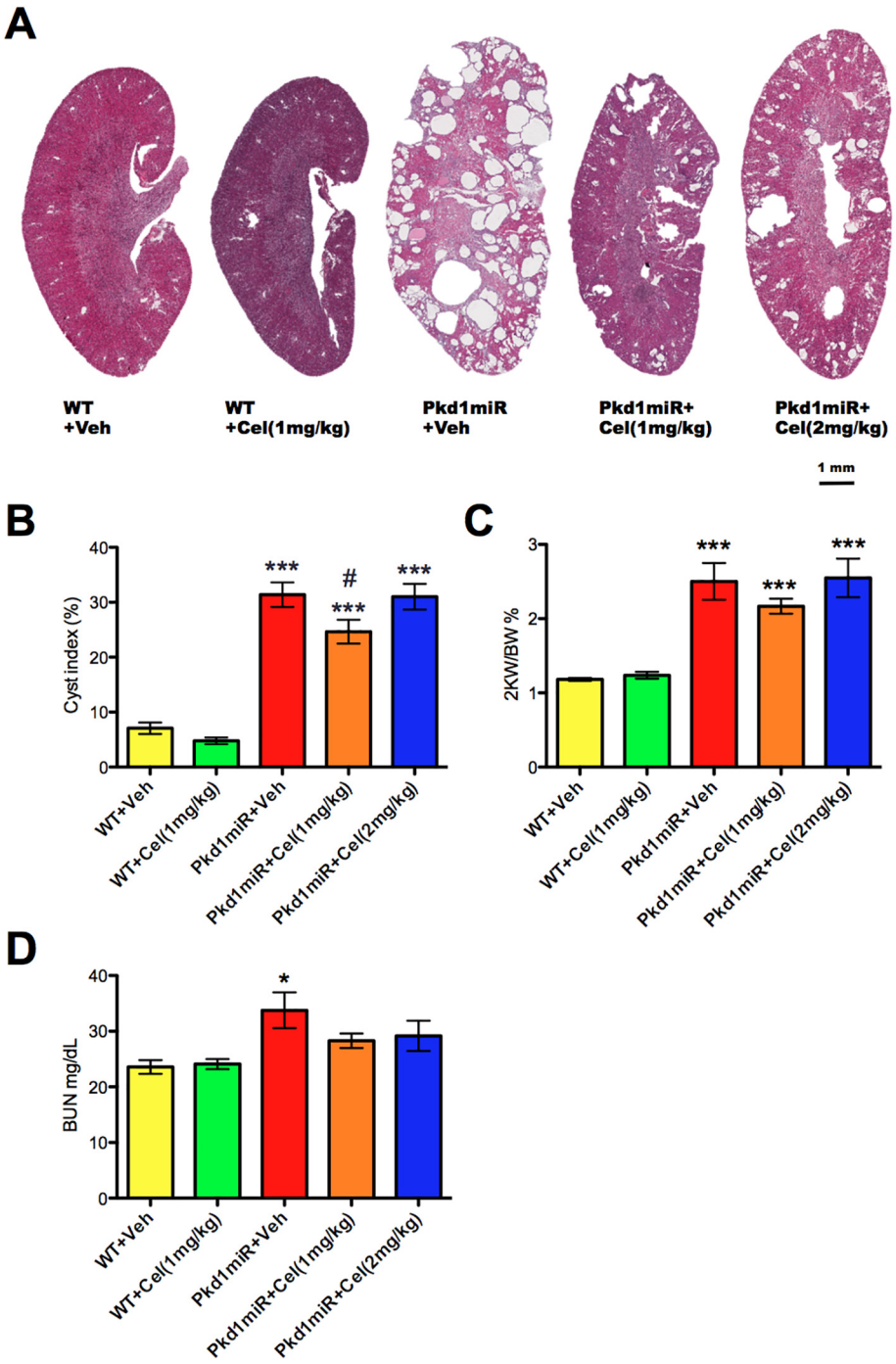

Fig. 1. Celastrol treatments partially ameliorated cystogenesis in the *Pkd1* miRNA transgenic mice. (A) Representative kidney sections (stained with hematoxylin and eosin) of the vehicle- and celastrol-treated *Pkd1* miRNA transgenic mice. Scale bar, 1 mm. (B) Cystic indices and (C) Ratios of the two-kidney weight to body weight in different experimental groups. (D) Serum levels of blood urea nitrogen in different experimental groups. The *Pkd1* miRNA transgenic mice were treated with celastrol (1 or 2 mg/kg/day) or vehicle, administered through intraperitoneal injection from day 35 for 4 weeks (n = 7–16 per group). A one-way analysis of variance, followed by a Newman–Keuls post hoc test, was applied. Data represent mean ± standard error of the mean. $^{*}P < 0.05$, $^{***}P < 0.05$ versus wild-type mice + vehicle; $^{\#}P < 0.05$ versus *Pkd1* miR Tg mice + vehicle. Cel, celastrol; Veh, vehicle; WT, wild-type.

considerable interest because it can be used for treating inflammation, cancers, obesity, and autoimmune diseases [3,12,17,20,36]. Small quantities of celastrol are extracted from *Tripterygium wilfordii* or other members of the Celastraceae (bittersweet) family [10]. Celastrol modulates proteasome activity [48], heat shock responses [38,50], and the nuclear factor kappa B (NF-κB) signaling pathway [18]. Furthermore, celastrol could reduce obesity by sensitizing leptin receptors in hyperleptinemic diet-induced obese mice [25], suggesting that celastrol plays a role in modulating metabolic processes. Therefore, we hypothesize that celastrol is a potential therapeutic agent for ADPKD.

In this study, we investigated the effects of celastrol treatment on renal cyst formation and disease progression in a *Pkd1*-deficient mouse model of ADPKD. We also determined the effects of celastrol on renal inflammation and fibrosis in the cystic kidneys of the *Pkd1* miR mice.

2. Materials and methods

2.1. Pkd1 miRNA mouse line

Pkd1 miRNA transgenic (*Pkd1* miR TG) mice with a C57BL/6 background were provided by Prof Si-Tse Jiang (National Rodent Model Resource Center, Taiwan) [43], and the wild-type littermates were used in the comparison study. All the mice were housed in the Chang Gung Memorial Hospital Animal Center (Taoyuan, Taiwan) under climate-controlled conditions with a 12-h light-dark cycle. They had free access to standard laboratory food and drinking water. The animal experiment protocols were approved by the Animal Care and Use Committee of Chang Gung Memorial Hospital. The study conformed to the Guide for the Care and Use of Laboratory Animals published by the National