Biomedicine & Pharmacotherapy 102 (2018) 633–638

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Effects of cholecalciferol cholesterol emulsion on renal fibrosis and aquaporin 2 and 4 in mice with unilateral ureteral obstruction

Ning Liu[a,1], Yalin Zhang[a,1], Han Su[a], Jinrong Wang[b], Zuwang Liu[a], Juan Kong[a,*]

[a] Department of Clinical Nutrition, Shengjing Hospital of China Medical University, Shenyang 110004, China
[b] Fushun City Center for Disease Control and Prevention, Fushun 113006, China

ARTICLE INFO

Keywords:
Cholecalciferol cholesterol emulsion
Renal fibrosis
Aquaporin

ABSTRACT

There was evidence that $1,25(OH)_2D_3$ regulated aquaporin (AQP) 2 and 4 expression while ameliorating renal fibrosis. This study investigated whether cholecalciferol cholesterol emulsion (CCE), a precursor of $1,25(OH)_2D_3$, has similar effects. The impact of CCE on renal fibrosis and AQP2 and AQP4 expression were studied in a unilateralureteral obstruction (UUO) mouse nephropathy model. CCE reduced both the extent of fibrosis and transforming growth factor (TGF)-β signaling compared with vehicle-treated UUO model controls. AQP2 protein expression was higher and AQP4 expression was lower in UUO-model mice treated with CCE than in vehicle-treated control mice. The results showed that CCE attenuated renal fibrosis by inhibiting TGF-β signaling, and regulated AQP2 and AQP4 expression in this UUO mouse model.

1. Introduction

Renal fibrosis involves progressive pathological changes that affect kidney physiology and result in gradual loss of function [1]. The extent of fibrosis reflects both damage progression and loss of kidney function [2–4]. Genomic factors, infection, and mechanical damage accelerate the progression of renal fibrosis [5], Renal fibrosis is characterized by increases in expression of various markers including transforming growth factor (TGF)-β, drosophila mothers against decapentaplegic protein (Smad2), Serpine1, alpha actin-2 (Acta2), alpha smooth muscle actin (α-SMA) and collagen type 1 (col1a1), which are involved in the pathogenesis of renal diseases [6–8].

Vitamin D (Vd) is a regulator of calcium homeostasis and plays a key role in bone metabolism [9,10], and $1,25(OH)_2D_3$, or calcitriol, the active metabolite of Vd, controls a range of physiological functions by binding to the Vitamin D receptor (VDR) [11–14]. Cholecalciferol cholesterol emulsion (CCE), a $1,25(OH)_2D_3$ precursor, is used to treat rickets, a condition caused by Vd deficiency in infants and children [15]. Previous studies found that Vd attenuated renal and hepatic fibrosis induced by the TGF-β signaling pathway [16–20].

Aquaporins (AQPs) are membrane proteins that act as channels to transport water and small nutrients through the cell membrane, and have been identified in microorganisms, plants, and animals [21,22]. Currently, 13 mammalian AQPs have been described in the lung, brain, kidney, and other organs [23–28]. Many studies have found an association of Vd with AQP expression. Renal ischemia/reperfusion injury has been associated with significant decreases of AQP1, AQP2 and AQP3 expression [29]. Iorio et al. [30] found that 14% of 70 AQP4 patients presenting with symptoms of neuromyelitisoptica(NMO), such as intractable nausea, vomiting, and hiccupping were seropositive for AQP4 antibodies, and neuroimaging has revealed high AQP4 expression in lesions characteristic of NMO [31].The severity of acute ischemic kidney injury was found to be increased in patients with Vd deficiency and downregulation of AQP2 expression [32]. In patients with transverse myelitis, Vd levels were lower in patients with recurrent than in those with monophasic disease, and 61% of those with recurrent disease were seropositive for AQP4 antibodies [33]. Vd clearly influences AQP2 and AQP4 expression, but the available data do not provide a clear picture of the relationship. In this study, the effects of CCE on renal fibrosis and AQP2, AQP4 expression were investigated in a unilateralureteral obstruction (UUO) mouse nephropathy model [6]. Renal tubulointerstitial fibrosis was induced without the use of exogenous toxins.

2. Materials and methods

2.1. Animals and treatments

Male ICR mice were obtained from the HuaFuKang Company (Beijing, China) and raised in the Animal Facility of Shengjing Hospital

* Corresponding author.
E-mail address: kongj1@sj-hospital.org (J. Kong).
[1] These authors contributed equally to this work and should be considered co-first authors.

https://doi.org/10.1016/j.biopha.2018.03.093
Received 29 December 2017; Received in revised form 15 March 2018; Accepted 15 March 2018

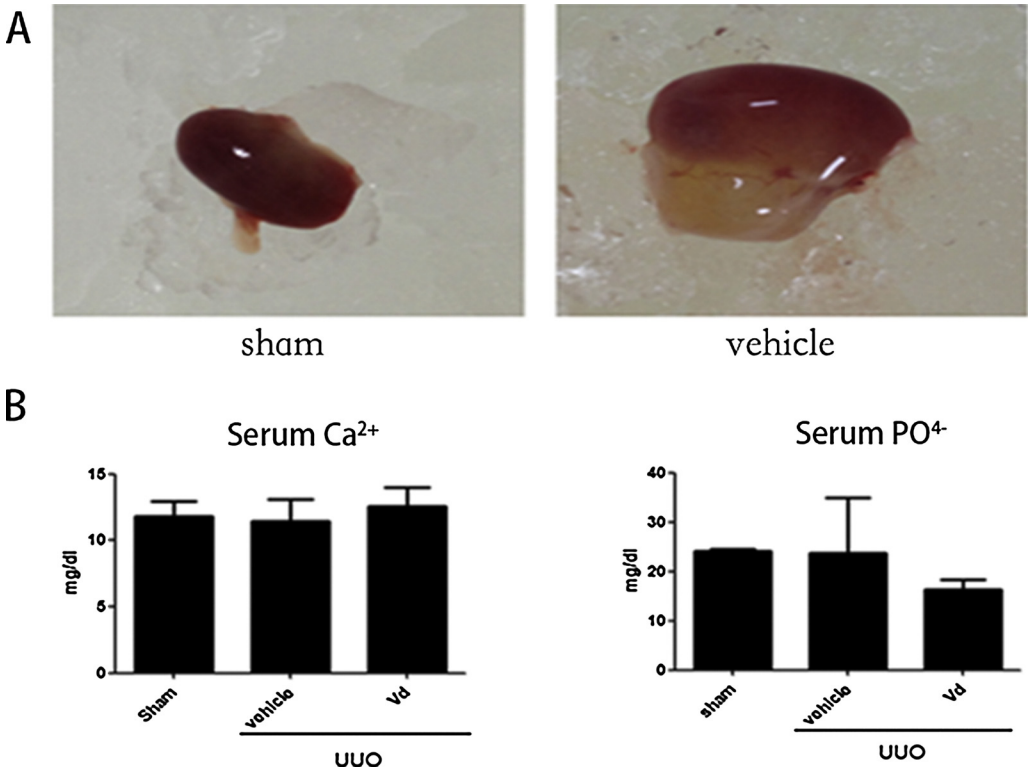

Fig. 1. Establishment and treatment of the UUO model. (A) The renal histology in UUO model mice was characterized by significant edema compared with sham group. (B) Serum Ca^{2+} and PO^{4-} concentrations in the Vd, sham, and vehicle groups were similar. Values were means ± SEMs. $p > 0.05$ ($n = 6$ in each group).

of China Medical University (Shenyang, China). CCE and calcitriol were provided by the Shengjing Hospital of China Medical University. All experimental protocols were approved by the Animal Research Committee of Shengjing Hospital of China Medical University (Approval 2017PS311K). All experimental procedures followed the committee guidelines, and every effort was made to minimize discomfort. The UUO mouse model was developed by surgical ligation of the right ureter. The mice were randomly assigned to a Vd group given CCE (10 μL in 100 mL of water) for 14 days after ligation, a vehicle group with ureter ligation but no CCE, and a sham group with surgery but no ureter ligation. Drinking water containing CCE was protected from sunlight. Food and water were provided adlibitum. On day 14 after surgery, the right kidney was photographed and renal tissues and blood were harvested. Serum was separated for assay of ionic calcium (Ca^{2+}) and phosphorus (PO^{4-}) by commercially available kits (C004-2 and C006-3, Nanjing jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions. Renal tissues were cut into small pieces for culture in Dulbecco's modified Eagle medium (Biological Industries, Beit haemek, Israel) with 10% fetal bovine serum (Biological Industries), 100 U/mL penicillin, 100 mg/mL streptomycin, and incubated at 37 °C in a humidified incubator with 5% CO_2. Cultures were treated with recombinant mouse TGF-β1 protein (7666-MB, R&D Systems, Minneapolis, MN, USA)in accordance with the manufacturer's instructions, TGF-β + 0.01 nM calcitriol, TGF-β + 0.1 nM calcitriol, or TGF-β + 1 nM calcitriol. Control cultures received no experimental treatment. After 8 h of incubation, renal tissues were harvested for western blotting.

2.2. Periodic acid Schiff (PAS) and Masson trichrome staining

Renal tissues were fixed in paraformaldehyde, embedded in paraffin, sectioned at 5 μm, and stained with PAS (G1340, Solarbio, Beijing, China) and Masson trichrome (G1281, Solarbio) for evaluation of fibrosis, lesion localization, and the extent of extracellular matrix deposition.

2.3. Immunohistochemistry (IHC)

Renal tissue sections were dewaxed, antigen retrieval was performed with 0.01 M citrate buffer, and the sections were incubated with 3% H_2O_2 and washed with phosphate buffered saline (PBS). After incubating with TGF-β (A2124, ABclonal, Wuhan, China), Smad2 (A7699, ABclonal), VDR (sc-1008, Santa Cruz Biotechnology, CA, USA), AQP2 (sc-28629, Santa Cruz Biotechnology), and AQP4 (sc-20812, Santa Cruz Biotechnology) primary antibodies, secondary antibodies, and washing with PBS, the tissues were stained with 3,3′- diaminobenzidine (DAB; 40412a, MXB, Fuzhou, China). The tissues were then prepared for evaluation by light microscopy.

2.4. Western blotting

Proteins were extracted from fresh or frozen renal tissues with lysis buffer. The protein concentration was measured by Bradford protein assay (PC0010, Solarbio). The proteins were separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoridemembranes (Millipore, MA, USA). After incubating with TGF-β, Smad2, VDR, AQP2, AQP4, α-SMA (A2547; Sigma–Aldrich, St. Louis, MO, USA), Fn (sc-56250, Santa Cruz Biotechnology), PAI-1 (sc-5297, Santa Cruz Biotechnology), β-actin (sc-47778, Santa Cruz Biotechnology), and GAPDH (60004-1-Ig, Proteintech, Wuhan, China) primary antibodies and secondary antibodies, the band densities were measured with Image Laboratory software (Bio-Rad, Hercules, CA, USA).

2.5. Quantitative real-time PCR (qPCR)

Total RNA extracted from renal tissue was reversely transcribed into cDNA using a Prime Script RT reagent kit (RR047A; TaKaRa, Kusatsu,