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Original article

Long-term treatment with chaethomellic acid A reduces glomerulosclerosis and arteriolosclerosis in a rat model of chronic kidney disease

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ABSTRACT

The high prevalence of end-stage renal disease emphasizes the failure to provide therapies to effectively prevent and/or reverse renal fibrosis. Therefore, the aim of this study was to evaluate the effect of long-term treatment with chaethomellic acid A (CAA), which selectively blocks Ha-Ras farnesylation, on renal mass reduction-induced renal fibrosis. Male Wistar rats were sham-operated (SO) or subjected to 5/6 renal mass reduction (RMR). One week after surgery, rats were placed in four experimental groups: SO:SO rats without treatment (n = 13); SO + CAA: SO rats treated with CAA (n = 13); RMR:RMR rats without treatment (n = 14); and RMR + CAA:RMR rats treated with CAA (n = 13). CAA was intraperitoneally administered in a dose of 0.23 μg/kg three times a week for six months. Renal fibrosis was evaluated by two-dimensional ultrasonography and histopathological analysis. The kidneys of the RMR animals treated with CAA showed a significantly decrease in the medullary echogenicity ($p < 0.05$) compared with the RMR rats that received no treatment. Glomerulosclerosis and arteriolosclerosis scores were significantly lower ($p < 0.001$) in the RMR + CAA group when compared with the RMR group. There were no significant differences in interstitial fibrosis, interstitial inflammation and tubular dilatation scores between the RMR + CAA and RMR groups. These data suggest that CAA can be a potential future drug to attenuate the progression of chronic kidney disease.

1. Introduction

Chronic kidney disease (CKD) is an important challenge for healthcare systems worldwide [1]. The natural course of the CKD is to progress towards end-stage renal disease (ESRD) and death, unless dialysis or transplant is implemented [2]. Regardless of the initial cause, development of renal fibrosis is the hallmark of most progressive CKD [3]. Therefore, targeting the components of the fibrogenic pathways can be a therapeutic approach to inhibit or slow the progression of CKD to ESRD.

The Ras proteins – small monomeric GTPase of 21 kDa – are important mediators in the development of renal fibrosis [4–8] and thereby could be a potential therapeutic target against fibrotic nephropathies. These proteins are located in different plasma-membrane microdomains and subcellular compartments where activate several signalling pathways. The best characterized signalling pathways are: the Ras/Raf/MEK-ERK1/2, which is responsible for the induction of several cellular responses such as cell growth, differentiation and apoptosis; and the Ras/PI3 K/Akt, which is implicated in regulation of cell metabolism, cell motility and promotion of cell survival protecting cells from apoptosis [9–11]. Activation of these signalling pathways has been reported as mediators in renal fibrosis [4,7,8].

Abbreviations: CAA, chaethomellic acid A; CKD, chronic kidney disease; ECM, extracellular matrix proteins; EMT, epithelial-to-mesenchymal transition; ESRD, end-stage renal disease; IRI, renal ischemia-reperfusion injury; KO, knock-out; RMR, 5/6 renal mass reduction; SBP, systolic blood pressure; SO, sham-operated; TGF-β1, transforming growth factor-β1; UUO, unilateral ureteral obstruction; α-SMA, alpha-smooth muscle actin

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There are three closely related major isoforms of Ras proteins: Harvey (Ha)-, Kirsten (Ki)-, and neural (N)-Ras, which are expressed in mammalian cells and have different biological effects [9,12–14], namely in fibroblasts biology and fibrotic processes [6,15–17]. It has been reported that depletion of Ha-Ras isoform in cultured fibroblasts obtained from Ha-Ras knock-out (KO) mice up-regulates extracellular matrix proteins (ECM) synthesis and mediates proliferation and migration by modulating PI3 K/Akt and MEK/ERK activation [16,17]. In an in vivo study, fibrosis was lower in H-Ras KO than in wild type mice after unilateral ureteral obstruction (UUO) [4]. Therefore, inhibition of Ha-Ras isoform could be used as a potential therapeutic strategy to reduce fibrosis development.

Chaetomellic acid A (CAA) has been identified as a highly specific inhibitor of farnesyl transferase [18], which selectively blocks Ha-Ras farnesylation [19]. Sabbatini et al. [20] have demonstrated that pre-treatment with CAA of human renal proximal tubular cells or human umbilical vein endothelial cells significantly reduced apoptosis. The same researchers have also observed that in acute renal ischemia-reperfusion injury (IRI) model in rats, CAA administration preserves both renal function and histological damage [20,21]. Furthermore, in a rat model of excitotoxic lesion, CAA treatment increased the intracellular concentration of inactive Ha-Ras, leading to a marked decrease of superoxide anion production [19]. In another in vivo study, CAA administration reduced renal damage after UUO in mice [7]. However, up to now, all the studies performed on the effect of CAA in renal fibrosis have been performed in vitro or in rapid models of renal fibrosis such as UUO.

The 5/6 renal mass reduction (RMR) model has been widely used to study CKD. In this model, the development of renal fibrosis is characterized by the progressive development of glomerulosclerosis, tubulointerstitial fibrosis and vascular sclerosis, leading to ESRD [22,23]. Thus, in this study we aimed to evaluate the effect of long-term treatment with CAA on renal fibrosis in rats with RMR, a model similar to renal fibrosis observed in CKD [24].

2. Materials and methods

2.1. Animals and experimental conditions

Sixty male Wistar rats (weighing approximately 135 g) were acquired from Harlan-Interfauna (Barcelona, Spain). Rats were housed in standard cages (Tecniplast, Buguggiate, Italy) with corncob for bedding (Mucedola, Milan, Italy) in a controlled room: 12/12 h' light-dark cycle, temperature (23 ± 2 °C) and humidity (50 ± 10%); animals were fed with a standard rat chow (Mucedola®, Milan, Italy) and water *ad libitum*. All experimental procedures followed the European (European Directive 2010/63/EU) and National (Decree-Law 113/2013) legislation on the protection of the animals used for scientific purposes.

2.2. Experimental design

After seven weeks of acclimatization, rats (weighing 359 to 402 g) were sham-operated (SO) or submitted to RMR. All surgical procedures were carried out under general anaesthesia (ketamine/xylazine, 70/10 mg/kg; intraperitoneally) and aseptic conditions. The animals assigned to the RMR groups (n = 34) were subjected to 5/6 RMR by surgical resection through a midline laparotomy, as described previously [25]. The right kidney was exposed, decapsulated and removed. Then, the left kidney was exposed, decapsulated and both the upper and lower poles (two thirds of the left kidney) were resected. Excised kidney and poles were weighed immediately after removal. The sham-operated group rats (n = 26) underwent the same abdominal incision and manipulation of the right and the left kidneys without removal of renal mass. Special care was taken to prevent damage to the adrenal glands during the surgeries. The percentage of renal tissue removed was calculated based on the removed tissue, assuming that the right and left kidneys had equal weights. Two days after renal mass reduction, serum creatinine was measured (Daytona® Rx, Randox). Rats weights were recorded weekly. Animals were daily observed to assess their general health and mortality.

One week after surgery surviving animals (n = 53) were distributed into four groups: SO, SO rats receiving no treatment (n = 13); SO + CAA, SO rats receiving CAA treatment (n = 13); RMR, RMR rats receiving no treatment (n = 14); RMR + CAA, RMR rats receiving CAA treatment (n = 13). Rats from SO groups were distributed randomly and the animals from RMR groups were distributed according to the serum creatinine concentrations and the percentage of the removed renal tissue to ensure equal reduction in renal mass. CAA was intraperitoneally administered (0.23 μg/Kg; Santa Cruz Biotechnology, California, USA) [21] three times a week for six months.

2.3. Ultrasonographic evaluation

Six months after the surgical procedure, in the left kidney of each animal was evaluated the mean cortical and medullary echogenicity by ultrasonography using two-dimensional ultrasonography (B mode) as reported previously by Nogueira et al. [26].

2.4. Renal function assessment

Blood and urine samples were collected at the sixth month as we have previously described [27]. Plasma creatinine, urinary creatinine and proteinuria were measured using a chemistry analyser (Daytona® Rx, Randox) as per manufacturers' instructions. Creatinine clearance was calculated according to standard formula [Uc x V/Pc, where Uc = urine creatinine (mg/dl), V = urine volume (ml/min/100 g body weight) and Pc = plasma creatinine (mg/dl)].

2.5. Animals' sacrifice

Six months after the surgery, surviving animals were anesthetized with isoflurane. Systolic blood pressure (SBP) was measured through femoral artery catheterization as we have previously described [27]. After that, the rats were sacrificed using an overdose of anaesthesia followed by exsanguination by cardiac puncture as indicated by the Federation of European Laboratory Animal Science Associations [28]. A complete necropsy was performed, either the remnant kidney from RMR rats or both kidneys from SO rats were removed, weighed and examined macroscopically. Relative left kidney weights were calculated as the ratio of the left kidney weight to the rats' total body weight [29].

2.6. Renal fibrosis evaluation

Samples were fixed in neutral buffered formalin 10%, embedded in paraffin wax, by routine methods, and 2 μm thick sections, including renal cortex and medulla, were stained for routine histopathological diagnosis with Haematoxylin and Eosin (HE), Masson's trichrome and Reticulin special stains. Renal fibrosis was evaluate under light microscopy by two different researchers blindly and scored as previously reported by Asaba *et al.* [30]: glomerulosclerosis (0: normal; 1: matrix expansion or sclerosis less than 25%; 2: 26–50%; 3: 51–75%; and 4: more than 75%); interstitial fibrosis (0: normal; 1: mild fibrosis around vasculature; 2: mild fibrosis around tubules; 3: moderate fibrosis with tubular casts or tubular damage; and 4: severe fibrosis with cell infiltration) and arteriolosclerosis (0: normal; 1: medial thickening; 2: segmental hyalinosis; 3: global hyalinosis; and 4: luminal occlusion with thrombus or infiltrating cells). The interstitial inflammation (presence of aggregates of lymphocytes and neutrophils in the interstitium) and the tubular dilatation (significant increase in luminal diameter, more than two folds, associated with flattening of the epithelial lining) were assessed according to Moubarak et al. [31] (0: not present; 1: minimal damage with rare and small foci; 2: mild damage