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Sodium valproate ameliorates diabetes-induced fibrosis and renal damage by the inhibition of histone deacetylases in diabetic rat

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ABSTRACT

Recent reports emphasize the contribution of histone deacetylases (HDACs) in the pathogenesis of diabetic renal injury and fibrosis. Valproic acid (VPA) is a first-line drug used for the treatment of epilepsy and migraine as well as established as a HDAC inhibitor. The present study was aimed to evaluate the anti-fibrotic and renoprotective effects of VPA in diabetic nephropathy (DN). Diabetes was induced by single injection of STZ (50 mg/kg), whereas VPA at the doses of 150 and 300 mg/kg/day was administered for 8 consecutive weeks by oral route in Sprague Dawley rats. The renal injuries and fibrosis were assessed by histology, fibrosis specific staining and fibroblast activation by a transmission electron microscope, while expression of proteins of interest was evaluated by western blotting and immunohistochemistry. VPA treatment ameliorated the histological alterations as well as fibrosis, and decreased the expression of TGF-β1, CTGF, α-SMA, fibronectin, collagen I, COX-2, ICAM-1 and HDAC4/5/7. Further, VPA treatment significantly increased histone H3 acetylation and MMP-2 expression. The present study clearly established that VPA treatment ameliorates the renal injury and fibrosis in diabetic kidney by preventing the myofibroblast activation and fibrogenesis by HDAC inhibition and associated mechanisms, thereby improving the profibrotic and anti-fibrotic protein balance.

1. Introduction

Diabetic nephropathy (DN) is characterized by the thickening of glomerular basement membranes and increased extracellular matrix (ECM) in the glomerular and tubulo-interstitial region, which finally leads to end-stage renal disease (Ban and Twigg, 2008; Riser et al., 2010). Several mechanisms have been reported to play significant role in the pathogenesis of DN, but transforming growth factor-β1 (TGF-β1)-induced fibrogenesis takes a central position (Noh et al., 2009; Van Beneden et al., 2013). Moreover, connective tissue growth factor (CTGF) in association with TGF-β1, promotes the development of fibrosis in a variety of experimental models (Kliem et al., 1996; Noh et al., 2009). Generally the myofibroblasts are originated from tissue-specific fibroblasts and/or pericytes during tissue injury and repair (Hinz et al., 2007). In the kidney, the myofibroblasts are originated from the differentiation of resident fibroblasts or transformation of epithelial cells, term as epithelial–mesenchymal transition (EMT) (Cook, 2010; Hinz et al., 2007). Recent reports highlighted that EMT plays a pivotal role in renal fibrotic disease including DN (Fragiadaki and Mason, 2011). It has also been reported that myofibroblasts play an important role in adriamycin-induced nephropathy as well as in ischemia/reperfusion injury (Broekema et al., 2007; Li et al., 2006). The increased myofibroblast population leads to structural abnormalities and reduces the organ function, thereby further accelerates progression of fibrosis. Activated fibroblasts result a series of changes in the morphology and gene expression profile such as increased proliferation, motility, α-SMA expression as well as ECM production and decreased ECM degrading enzymes like matrix metalloproteinase (MMPs) (Aher et al., 2015; Li et al., 2006).

Recently, several studies highlighted the importance of epigenetic mechanisms in the pathogenesis of diabetic complications including DN (Gilbert et al., 2011; Kato and Natarajan, 2014; Reddy et al., in press). Histone deacetylases (HDACs) are involved in several molecular signaling relevant to the pathogenesis of DN (Lee et al., 2007; Villeneuve and Natarajan, 2010). HDAC inhibitors including VPA have been acknowledged as potential anti-fibrotic molecules in various fibrotic disorders in multiple organs (Mannaerts et al., 2010; Van Beneden et al., 2013). Further, knockdown of HDAC1 in renal interstitial fibroblasts and tubular epithelial cells confirmed the contribution of HDACs in myofibroblast activation, proliferation and chemokine production (Liu et al., 2013). HDAC inhibitors can prevent the TGF-β1-mediated fibroblast activation and subsequently reduced the ECM deposition and fibrosis (Gilbert et al., 2011; Khan and Jena, 2014a; Noh et al., 2009). Further,

Abbreviations: α-SMA, Alpha smooth muscle actin; COX-2, Cyclooxygenase 2; CTGF, Connective tissue growth factor; DAB, 3,3′-Diaminobenzidine; ECM, Extracellular matrix; HDACs, Histone deacetylases; ICAM-1, Intercellular adhesion molecule 1; MMP-2, Matrix metalloproteinase-2; STZ, Streptozotocin; TGF-β1, Transforming growth factor-beta 1; VPA, Valproic acid.

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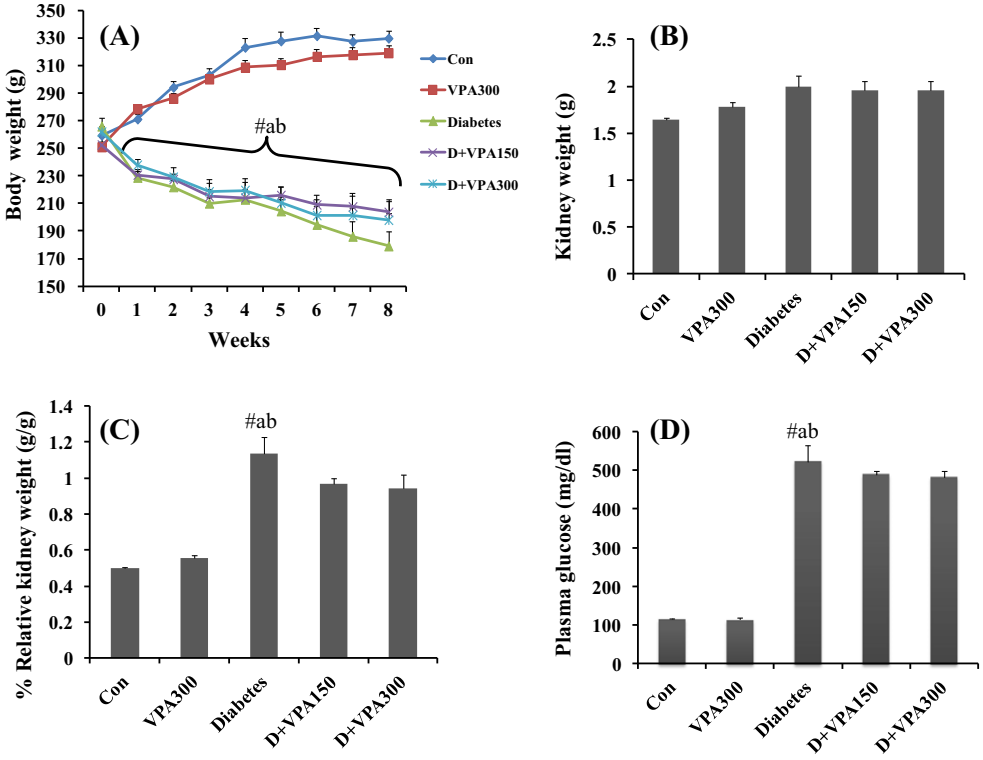

Fig. 1. Effect of the diabetes and VPA treatment on the (A) body weight and (B & C) absolute as well as relative kidney weight and (D) plasma glucose. All the values are expressed as mean ± SEM, (n = 6–8), #P < 0.001, 'a' vs. control 'b' vs. VPA control and 'c' vs. diabetic control.

HDAC inhibitors can also modulate the function of renal endothelial and epithelial cells and regulate the expression of CTGF and collagen I as well as other profibrotic molecules (Komorowsky et al., 2009). Recent study shows the expressions of different HDACs in diabetic kidney of patients and STZ treated rats, which proves that HDAC4 is the major player in the pathogenesis of DN (Wang et al., 2014). Together, above reports highlighted the role of HDACs and their inhibitors in the renal injury and fibrosis in DN and various other pathological conditions.

Valproic acid (VPA) is a short-chain fatty acid and first-line drug used for the treatment of epilepsy, migraine and other psychiatric disorders. Presently, VPA has been proven as a HDAC inhibitor and subdued the class I and II HDACs (Chateauvieux et al., 2010; Gottlicher et al., 2001). VPA exerts anti-inflammatory and anti-oxidant activity thereby protects the multiple organ damage in several pathological conditions (Khan and Jena, in press; Shang et al., 2010; Zhang et al., 2008). Further, VPA has been reported to reduce the glomerulosclerosis and proteinuria as well as fibrosis in adriamycin-induced nephropathy in mouse as well as DN (Khan et al., in press; Van Beneden et al., 2011). Additionally, VPA prevents the hepatic fibroblast activation in in vitro and in vivo experiments as well as prevents penile fibrosis and erectile dysfunction in cavernous nerve-injured rat (Aher et al., 2015; Hannan et al., 2014; Watanabe et al., 2011). Therefore, we hypothesized that VPA can exert protective effects on TGF-β1-mediated fibrogenesis, myofibroblast activation and renal fibrosis in the kidney of diabetic rat. The present results clearly demonstrated that VPA treatment significantly ameliorates the fibrosis by preventing the diabetes-associated fibrogenesis and activation of myofibroblast in the kidney through HDAC inhibition.

2. Methods

2.1. Animals

Animal experiment protocol was approved by the Institutional Animal Ethics Committee (IAEC) and experiment was performed on male Sprague–Dawley rat (250–280 g) procured from the Central Animal Facility of the institute in accordance with the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines. Animals were kept under controlled environment at room temperature (22 ± 2 °C) with humidity (50 ± 10%) and an automatically controlled 12 h light and dark cycle. Feed and water were provided ad libitum. Animals were acclimatized for one week prior to commencement of actual experiment.

2.2. Chemicals

Until mentioned otherwise, all the chemical and reagents including sodium valproate (CAS no. 1069-66-5) were purchased from Sigma-Aldrich chemicals, Saint Louis, MO, USA, while primary and secondary antibodies were purchased from Santa Cruz Biotechnology, CA, USA.

2.3. Experimental design and animal treatment

Animals were randomized into five groups; group-1: control (Con, n = 6), received saline; group-2: VPA control (VPA300, n = 6), received VPA 300 mg/kg/day for 8 weeks; group-3: diabetic control (D, n = 8), received single injection of streptozotocin (STZ, 50 mg/kg); groups-4 and 5: VPA treated diabetic animals (D + VPA150 & D + VPA300, n = 8), received VPA at the doses of 150 and 300 mg/kg/day for 8 consecutive weeks. The doses of VPA were selected on the basis of previous studies (Ahmad et al., 2013; Khan et al., 2011; Khan and Jena, in press, 2013). VPA was dissolved in distilled water and administrated by oral route according to body weight.

2.4. Induction of diabetes

Experimental diabetes was induced by a single injection of STZ (50 mg/kg) dissolved in ice-cold 10 mM citrate buffer (pH 4.4) and administered by *i.p.* route immediately. Age-matched control rat received an equivalent volume of vehicle. After 48 h of STZ injection, animals were kept for 6–8 h fasting and plasma glucose was measured using