Contents available at Sciverse ScienceDirect

Diabetes Research and Clinical Practice

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

International Diabetes Federation

Lumbrokinase attenuates diabetic nephropathy through regulating extracellular matrix degradation in Streptozotocin-induced diabetic rats

Huili Sun [a,1], *Na Ge* [a,1], *Mumin Shao* [b], *Xiaoyan Cheng* [a], *Yue Li* [c], *Shunmin Li* [a,*], *Jiangang Shen* [a,c,**]

[a] *Department of Nephrology, Shenzhen Affiliated Hospital, Guangzhou University of Traditional Chinese Medicine, Shenzhen, China*
[b] *Department of Pathology, Shenzhen Affiliated Hospital, Guangzhou University of Traditional Chinese Medicine, Shenzhen, China*
[c] *School of Chinese Medicine, The University of Hong Kong, 10 Sassoon Road, Hong Kong, China*

ARTICLE INFO

Article history:
Received 26 September 2012
Received in revised form
17 December 2012
Accepted 7 January 2013
Published on line 4 February 2013

Keywords:
Diabetic nephropathy
Lumbrokinase
Collagen IV
MMP-2
MMP-9

ABSTRACT

Objective: The present study was undertaken to investigate the therapeutic effect and underlying mechanisms of lumbrokinase on diabetic nephropathy.
Methods: Type I diabetes was induced in male Sprague-Dawley rats via intraperitoneal injection of Streptozotocin (STZ). Lumbrokinase was administered to the diabetic rats at a dose of 600,000 U/kg body weight by gavage. As a positive control, perindopril, an angiotensin-converting enzyme inhibitor (ACEI), was given to diabetic rats at a dose of 4 mg/kg body weight. Following 12 weeks treatment, we measured the creatinine clearance rate (Ccr), urinary albumin excretion (UAE) and kidney injury scores. In addition, the expression of collagen IV, MMP-2 and MMP-9 in renal tissue was evaluated.
Results: The diabetic rats developed proteinuria, glomerulosclerosis, tubulointerstitial fibrosis and a marked increase of renal cortical collagen IV. In contrast, MMP-2 and MMP-9 were significantly reduced in the renal cortex of diabetic rats. Interestingly, lumbrokinase treatment markedly reduced the proteinuria and improved the glomerulosclerosis and tubulointerstitial fibrosis in diabetic rats. The induction of collagen IV and the down-regulation of MMP-2 and MMP-9 was significantly attenuated by lumbrokinase. All these beneficial effects of lumbrokinase were comparable to the ACEI group.
Conclusion: Lumbrokinase treatment attenuated diabetic nephropathy in rats, possibly through increasing the activity of MMPs and the subsequent degradation of extracellular matrix.

1. Introduction

Diabetes mellitus is a metabolic disorder presenting with high blood glucose. About 41–60% of diabetic patients have diabetic nephropathy [1] which is characterized by a progressive mesangial expansion mainly due to the accumulation of the extracellular matrix (ECM) of collagen IV, laminin, fibronectin, proteoglycans, and other matrix proteins [2,3]. ECM is in

* *Corresponding author at*: Department of Nephrology, Shenzhen Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, 1 Fuhua Road, Futian District, Shenzhen 518033, Guangdong, China.
Tel.: +86 755 83002010; fax: +86 755 88359333.
** *Corresponding author at*: School of Chinese Medicine, The University of Hong Kong, 10 Sassoon Road, Hong Kong, China.
Tel.: +852 25890429; fax: +852 21684259.
E-mail addresses: shunminli@126.com (S. Li), shenjg@hkucc.hku.hk (J. Shen).
[1] Huili Sun and Na Ge contributed equally to this work.
0168-8227/$ – see front matter
http://dx.doi.org/10.1016/j.diabres.2013.01.012

dynamic flux with both synthetic and degradative components. Imbalance of synthetic and degradative components contributes to the progression of glomerular sclerosis in diabetic nephropathy.

Matrix metalloproteinases (MMPs) appear to be critical ECM-degrading enzymes. MMPs comprise a large family of Zn^{2+}-dependent enzymes which can degrade almost all extracellular matrix components [4–6]. Among MMPs, MMP-2 and MMP-9 have been implicated in the pathogenesis of diabetic nephropathy. Previous studies have shown the reduced gene expression and activities of MMP-2 and MMP-9 in the diabetic kidney [7]. High glucose decreased the degradation of mesangium matrix, which is substantially mediated by reduced MMPs activities [8]. As shown by streptozocin (STZ)-induced diabetic rats or glucose infused rats, exposure to hyperglycemia impaired nephrogenesis in fetuses [9]. Importantly, high glucose concentration in rat metanephric organ culture medium resulted in a dramatic reduction of MMP-2 and MMP-9 at both transcription and enzymatic activity levels [10]. In addition, the therapeutic effects of angiotensin-converting enzyme inhibitors (ACEIs) on diabetic nephropathy are also mediated by the modulation of matrix degradation [8]. Therefore, MMPs serve as potential therapeutic targets for drug development in treating diabetic nephropathy.

Lumbrokinase, an extract of lumbricus rubellus, was identified in recent decades [11]. Lumbricus rubellus, as a Traditional Chinese Medicine, has been used for thousands of years in China. Lumbrokinase consists of a group of bioactive protelytic enzymes. Previous studies demonstrated many beneficial properties of lumbrokinase, including anti-inflammatory, anti-oxidative stress, anti-fibrotic, anti-microbial and anti-cancer effects [12–15]. Lumbrokinase is easily absorbed in the intestinal tract without destruction of its activity [16] and capsules of lumbrokinase are widely used in China, Japan, Korea, Canada and United States [17–20]. Similar to tissue plasminogen activator (t-PA), lumbrokinase dissolves fibrin clot by converting plasminogen to plasmin with the advantage of a relative broad optimal pH range and good heat stability [16,21–23]. Lumbrokinase was reported to protect ischemic brains through inhibition of intercellular adhesion molecule-1 (ICAM-1) and the activation of Janus Kinase1/Signal transducers and activators of transcription1 (JAK1/STAT1) in experimental cerebral ischemia-reperfusion model [24]. However, the effect of lumbrokinase on diabetic nephropathy is poorly understood.

In the present study, a diabetic rat model was established using STZ injection. Therapeutic effect of lumbrokinase on diabetic nephropathy was evaluated by determination of renal function, ECM deposition and the expression of MMP-2 and MMP-9 in renal tissues. The findings from the present study revealed an important role of lumbrokinase in amelioration of diabetic nephropathy. The regulation of collagen IV, MMP-2 and MMP-9 in kidney by lumbrokinase may be involved in the potential mechanisms of renal protection in diabetes.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats, weighting 200–220 g, were purchased from the Laboratory Animal Center of Guangzhou Medicine. All experiments were conducted in accordance with the NIH statements of ''Principles of laboratory animal care''. Rats were treated according to the guidelines of the Institutional Animal Care and Use Committees of the Guangzhou University of Traditional Chinese Medicine and University of Hong Kong. Animals were housed at constant room temperature (21 °C) under a controlled 12 h light to 12 h dark cycle and had free access to water and standard laboratory diet.

2.2. Streptozotocin-induced diabetes mode and drug treatment

Experimental diabetes was induced by a single intraperitoneal injection of 60 mg/kg body weight Streptozotocin (STZ, Sigma Aldrich, St. Louis, MI, USA) in 0.01 mol/l citrate buffer (pH 4.2) after a 16 h overnight fasting. Induction of the diabetes was confirmed by measuring the blood glucose level after 3 days STZ administration. The rats with fasting blood glucose concentration >16.7 mmol/l were classified as successful diabetes model and used in the study [25,26]. The rats were randomly allocated into the following experimental groups: Normal control rats (non-diabetic group, $n = 12$), STZ-induced diabetic rats (diabetic group, $n = 12$); lumbrokinase-treated diabetic rats (diabetic + lumbrokinase group, $n = 12$); ACE inhibitor perindopril-treated diabetic rats (diabetic + ACEI group, $n = 12$). Lumbrokinase (Lum, Bokang Pharmaceutical Company, Zhuhai, China) were administered to the rats at a dose of 600,000 U/kg body weight by gavage for 12 weeks. According to previous studies, the suitable concentration of lumbrokinase was determined as 600,000 U/kg body weight in this experiment. As a positive control, perindopril (PER, an ACE inhibitor, Servier Laboratories, Neuilly, France) was administered to the rats at a dose of 4 mg/kg body weight by gavage for 12 weeks.

The purification of lumbrokinase from lumbricus rubellus included 5 steps: 1, hydrolysis and autolysis; 2, centrifugal separation; 3, membrane separation and particles obtainment; 4, ultrafiltration and 5, lyophilization. One kilogram of the lumbrokinase was obtained from the 100 kg of fresh Lumbricus rubellus. The specific activity of lumbrokinase had 20,000 U pers 1 mg protein.

2.3. Physiological and metabolic parameters

Body weight was measured every week. The kidney index was 1000 × kidney weight/body weight. The value of serum creatinine (Cr) and urinary creatinine were determined by the automatic biochemistry analyzer (Olympus 2000, Tokyo, Japan). Ccr was calculated as urinary creatinine × urine volume/serum creatinine, and was expressed as microliters per minute per gram. Systolic blood pressure (SBP) was measured by tail plethysmography in conscious, preheated rats at the 12th week as described (IITC Life Science, Woodland Hills, CA, USA) [26]. In brief, rats were restrained and warmed with a heat lamp before measurement, then placed in a holder with the tail exposed, allowing access to the tail-cuff. An integrated sensor-cuff occluder operated to stop tail pulsation on inflation and to detect the return of tail pulsations passing through the occluder cuff on each deflation cycle with the