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XLF-III-43, a novel coumarin–aspirin compound, prevents diabetic nephropathy in rats via inhibiting advanced glycation end products

Hongyan Li, Xuguang Zheng, Hongbo Wang, Yi Zhang, Hongqi Xin, Xiaoguang Chen *

Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Xian Nong Tan Street, Beijing 100050, China

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ABSTRACT

Advanced glycation end products (AGE) have been implicated in the pathogenesis of diabetic complications. The purpose of this study was to examine the novel coumarin–aspirin compound XLF-III-43 in the inhibition of AGE formation in diabetic nephropathy. *In vitro* analysis showed XLF-III-43 in a dose-dependent manner decreased glucose induced formation of glycation adducts on albumin and inhibited AGE–lysozyme crosslinking. The streptozotocin-induced diabetic rats were used to investigate the beneficial effects of XLF-III-43 treatment on diabetic nephropathy. Administration of XLF-III-43 significantly decreased ($P<0.05$) blood urea nitrogen and urinary albumin excretion. Moreover, XLF-III-43 ameliorated kidney hypertrophy, mesangial expansion and glomerulosclerosis in diabetic rats relative to untreated model group. These data correlated with decreased both AGE and downstream markers of AGE stress (TGF-β1, CTGF, fibronectin and collagen IV fibrolysis) in kidneys of diabetic rats. These data support further development of XLF-III-43 for prevention of nephropathy via inhibition of AGE formation consequent to chronic hyperglycemia.

1. Introduction

One of the most common and serious diabetic complications is the development of nephropathy and end-stage renal disease (Barnett, 2006). Advanced glycation end products (AGE) are heterogeneous products formed by the non-enzymatic reactions between reducing sugars and free amino groups of proteins, lips and nucleic acids (Bucala et al., 1984; Bucala, 1997). AGE have been implicated as one of the major factors in the pathogenesis of numerous pathologies (aging, atherosclerosis, rheumatoid arthritis, Alzheimer's) notably including diabetic complications (Ramasamy et al., 2005; Ahmed and Thornalley, 2007; Kanwar et al., 2008). AGE can interfere with protein function and promote formation of aggregates. In kidney, AGE can become trapped in glomerular basement membranes and covalently crosslink to collagen resulting in membrane thickening and distortion (Gross et al., 2005). In addition to these direct effects, AGE can bind mesangial cell surface receptors stimulating formation of transforming growth factor β1 (TGF-β1) and connective tissue growth factor (CTGF), which in turn mediates mesangial expansion and glomerulosclerosis (Bohlender et al., 2005; Schena and Gesualdo, 2005). Persistent hyperglycemia accelerates AGE formation and crosslinked deposits resulting to pathological injury in kidneys (Peppa et al., 2003; Ahmed, 2005; Ramasamy et al., 2005).

Beyond good glucose control, the design and discovery of inhibitors that either prevent the formation of AGE or promote the degradation of existing AGE offer a promising therapeutic approach for diabetes related complications (Reddy and Beyaz, 2006; Peyroux and Sternberg, 2006; Desai and Wu, 2007). AGE-inhibitors include aminoguanidine (AG, Pimagedine) (Youssef et al., 1999; Thornalley, 2003; Bolton et al., 2004), pyridoxamine (Voziyan and Hudson, 2005; Williams, 2004), aspirin (Jafarmejad et al., 2008), OPB9195 (Miyata et al., 2000; Nakamura et al., 1997) and LR compounds (Rahbar, 2007; Figarola et al., 2005a,b). Compounds that break AGE crosslink AGE include phenacylthiazolium bromide (PTB) (Vasan et al., 1996; Yang et al., 2003) and ALT-711 (Alagebrium) (Cooper et al., 2000; Peppa et al., 2006). Some of the agents targeting AGE have been approved for clinical trials (Williams, 2004; Goh et al., 2008).

XLF-III-43 is a novel compound that combines a coumarin moiety with aspirin ($C_{18}H_{12}N_2O_9$; chemical structure shown in Fig. 1). XLF-III-43 shows low toxicity with an oral maximum tolerance dose (MTD) of 5 g/kg. The LD_{50} by i.p. injection is 453.5 mg/kg in rats. In the present study, the potential role of XLF-III-43 on direct inhibition AGE formation and glycation induced protein modifications was examined. The potential translation of XLF-III-43 in treatment of diabetic nephropathy was determined using the streptozotocin-induced diabetic rats.

* Corresponding author. Tel./fax: +86 10 63165207.
E-mail addresses: lhy2116@gmail.com (H. Li), zhengxg@gmail.com (X. Zheng), Hongbowang1980@gmail.com (H. Wang), pkucat@imm.ac.cn (Y. Zhang), Xinhongqi@gmail.com (H. Xin), chxg@imm.ac.cn, lhy2116@yahool.com.cn (X. Chen).

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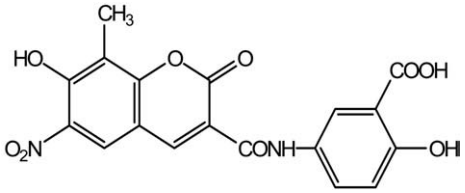

Fig. 1. Chemical structure of XLF-III-43 ($C_{18}H_{12}N_2O_9$, MW 400.32).

2. Material and methods

2.1. Material

XLF-III-43 was synthesized by the Department of Medical Chemistry, Institute of Materia Medica (Beijing, China). Purity was determined by HPLC and found to be >99%. All reagents were products of Beijing Chemical Plant (Beijing, China) unless specifically noted.

2.2. In vitro assays of AGE formation

AGE was synthesized by triplicate incubation BSA (50 g/l, fraction V, fatty acid free, low endotoxin, Sigma, MO, USA) with 0.5 M D-glucose in sterile phosphate buffer (PB, 0.2 M, pH 7.4) containing 0.02% sodium azide at 37 °C for 12 weeks according to the previous methods (Vinson and Howard, 1996). BSA-control samples were prepared under the same conditions without the addition of sugar. XLF-III-43 dissolved in DMSO was diluted to 0.008, 0.04 and 0.2 mM with PB and the final 2% DMSO was set up as solvent control. Additionally, 0.2 and 1 mM aminoguanidine (AG, Sigma, MO, USA), a putative anti-glycation agent, was used as a positive control.

After desired incubation, the samples were dialyzed in PBS (pH 7.4) with dialysis membrane of 14 kDa molecular weight cut-off (Union Carbide, Ca, USA). AGE specific fluorescence was detected at excitation 370 nm and emission 440 nm using the fluorophotometer (SPECTRA MAX GEMINI XS, USA) and expressed as relative fluorescence units (RFU) per mg of BSA. Simultaneously, ELISA was used to detect N-carboxymethyl lysine AGE (CML-AGE), the most common AGE (Reddy et al., 1995), according to the previous method (Ikeda et al., 1996). Briefly, each well of a 96-well plate was coated with dialysis samples (0.1 ml, 2 μg/ml) or CML-AGE standards (0.1 ml, 0–0.2 μg/ml, MBL, MA, USA). Then, the plate was added with anti-CML-AGE antibodies (0.1 ml, 50 ng/ml, MBL, MA, USA), horseradish peroxidase (HRP)-conjugated antibody, and HRP was visualized with 1 M TMB (tetramethylbenzidine, Sigma, MO, USA) step-by-step. The concentration of CML-AGE was expressed as μg per mg of BSA.

2.3. In vitro assays of AGE crosslink

According to Rahbar's (2007) methods, the effect of XLF-III-43 on glucose mediated protein crosslink was analyzed by incubation of 5 g/l lysozyme with 0.1 M D-glucose with and without 0.008, 0.04 and 0.2 mM XLF-III-43 at 37 °C. Concurrently, 1 and 10 mM AG were run as positive control and the final 2% DMSO was served as solvent control. After 1 day and 3 days incubation, equal aliquots (40 μg protein) from the reaction mixture were conducted by SDS-PAGE and the coomassie stained bands were analyzed using Image Pro. The assays were repeated 3 times.

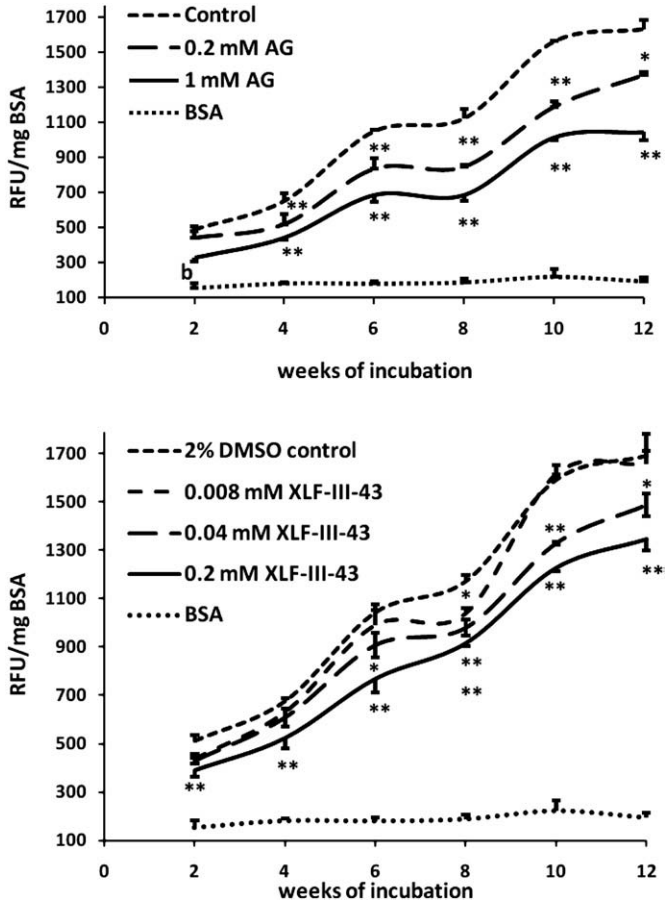

Fig. 2. XLF-III-43 inhibits AGE fluorescence argumentation (50 g/l BSA was incubated with 0.5 M glucose for 12 weeks. AG: aminioguanidine run as positive control ($n=3$; *$P<0.05$; **$P<0.01$).

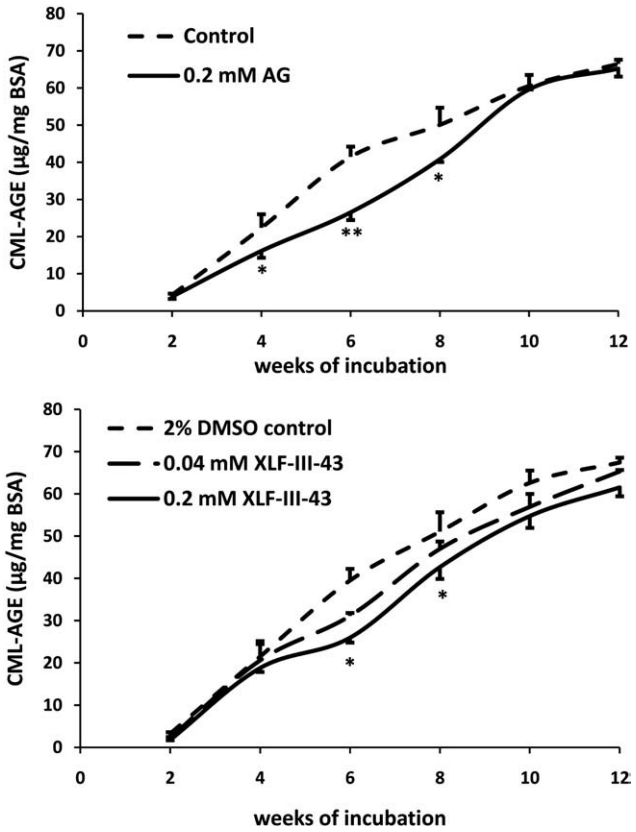

Fig. 3. XLF-III-43 inhibits CML-AGE formation (50 g/l BSA was incubated with 0.5 M glucose for 12 weeks. CML-AGE was measured by ELISA. AG: aminioguanidine run as positive control ($n=3$; *$P<0.05$; **$P<0.01$).