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Comparison of the effects of levocetirizine and losartan on diabetic nephropathy and vascular dysfunction in streptozotocin-induced diabetic rats

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ABSTRACT

This work was designed to investigate the effects of levocetirizine, a histamine H_1 receptor antagonist, on diabetes-induced nephropathy and vascular disorder, in comparison to an angiotensin II receptor antagonist, losartan. Diabetes was induced in male Sprague Dawley rats by a single intraperitoneal injection of streptozotocin (50 mg/kg). Diabetic rats were divided into three groups; diabetic, diabetic-levocetirizine (0.5 mg/kg/day) and diabetic-losartan (25 mg/kg/day). Treatments were started two weeks following diabetes induction and continued for additional eight weeks. At the end of the experiment, urine was collected and serum was separated for biochemical measurements. Tissue homogenates of kidney and aorta were prepared for measuring oxidative stress, nitric oxide (NO), transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α). Moreover, histological analyses were conducted and aortic vascular reactivity was investigated.

Levocetirizine improved renal function in diabetic rats (evidenced by mitigation of diabetes-induced changes in kidney to body weight ratio, serum albumin, urinary proteins and creatinine clearance). Moreover, levocetirizine attenuated the elevated renal levels of TNF-α and TGF-β1, ameliorated renal oxidative stress and restored NO bioavailability in diabetic kidney. These effects were comparable to or surpassed those produced by losartan. Moreover, levocetirizine, similar to losartan, reduced the enhanced responsiveness of diabetic aorta to phenylephrine. Histological evaluation of renal and aortic tissues further confirmed the beneficial effects of levocetirizine on diabetic nephropathy and revealed a greater attenuation of diabetes-induced vascular hypertrophy by levocetirizine than by losartan. In conclusion, levocetirizine may offer comparable renoprotective effect to, and possibly superior vasculoprotective effects than, losartan in streptozotocin-diabetic rats.

1. Introduction

Diabetes mellitus has become a major threat to human health, affecting over 300 million people across the world (Danaei et al., 2011). Diabetic patients often develop macrovascular and microvascular complications, which represent a major cause of morbidity and premature mortality (Bate and Jerums, 2003). Chronic hyperglycemia has been proposed as a central underlying mechanism in the development and progression of diabetic vascular complications (Forbes and Cooper, 2013; Wu et al., 2006). Conditions leading to diabetic vascular dysfunction also include effects of oxidative stress in the diabetic milieu (Ceriello, 2006) and reduced vascular nitric oxide (NO) bioavailability (Satoh et al., 2005), which collectively contribute to endothelial dysfunction and impairment of flow-mediated vasodilation (Liu et al., 2014).

Nephropathy is one of the microvascular diabetic complications and is an important cause of end stage renal failure (Kuhad and Chopra, 2009). Activation of the local renin-angiotensin-aldosterone system (RAAS) seems to be a key player in the development of long-term diabetic renal complications (Giacchetti et al., 2005; Ruggenenti et al., 2010). Therapeutic interventions which inhibit RAAS, including angiotensin converting enzyme inhibitors (Lewis et al., 1993) and angiotensin II receptor antagonists such as losartan (Arozal et al., 2009; Perico et al., 2004; Yavuz et al., 2003), are currently considered the first line renoprotective therapies for diabetic nephropathy. However, disappointing results from some clinical trials using RAAS blockade to protect against diabetic nephropathy have also been reported (Bilous et al., 2009; Mauer et al., 2009; UK Prospective Diabetes Study Group, 1998). Moreover, angiotensin II receptor antagonists provide imperfect renoprotection when administered in the advanced phase of diabetes (Benigni et al., 2003), which emphasizes the need of

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alternative therapies.

Histamine H_1-receptor antagonists provide a highly successful approach for controlling allergic and inflammatory conditions (Simons and Simons, 2008). The anti-inflammatory effects of H_1-antihistamines are thought to be mediated via H_1-receptor-dependent (Bakker et al., 2001; Roumestan et al., 2008) and independent (Abdelaziz et al., 1998; Okamoto et al., 2009; Roumestan et al., 2008) mechanisms. We have recently reported that levocetirizine, a non-sedating antihistamine, has insulin-sensitizing effects in fructose-fed rats, which could be explained on the basis of its antioxidant and anti-inflammatory effects (Shawky et al., 2014). Several studies showed that anti-inflammatory and antioxidant agents attenuated the development and progression of diabetic nephropathy in rats (Alderson et al., 2004; Han et al., 2004; Ko et al., 2008; Sharma et al., 2006). Therefore, this work was aimed to investigate the effects of levocetirizine on diabetic nephropathy and vascular dysfunction, in comparison to losartan, using streptozotocin (STZ)-induced diabetic model in rats. The potential mechanisms involved in levocetirizine-mediated effects were also explored.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats weighing 190–210 g were used in this study. The animals were kept at constant environmental and nutritional conditions at room temperature with a 12 h/12 h light/dark cycle and allowed free access to standard laboratory food and water throughout the experiment. Animals care and handling were conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23, revised 1985) and had been approved by the committee on animals' experimentation of Mansoura University.

2.2. Drugs and chemicals

STZ and acetylcholine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Commercially available human insulin-zinc suspension (Mixtard®, Novo Nordisk, Bagsvaerd, Denmark), losartan tablets (Al-Ameriya Pharmaceutical Industries Co., Alexandria, Egypt) and levocetirizine tablets (Marcyrl Pharmaceutical Industries Co., Cairo, Egypt) were obtained from private retail pharmacies. All other chemicals used in this study were of fine analytical grade.

2.3. Experiment protocol

Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg) in sterile saline, as previously reported (Saito et al., 2006). Two days later, the diabetic state was confirmed by measurement of blood glucose level using GlucoDr.® glucose-measuring device. Animals with serum glucose level more than 250 mg/dl were used in this study. Diabetic glycemia was controlled throughout the study by subcutaneous injection of insulin (4 IU/kg) twice a week to maintain body weight (Alderson et al., 2004). Two weeks after diabetes induction, diabetic rats were randomly divided into three groups of eight animals, which received either 0.5% carboxymethyl cellulose (CMC, 3 ml/kg/day, orally) [diabetic control], losartan (25 mg/kg/day in 0.5% CMC, orally) [Losartan- diabetic] or levocetirizine (0.5 mg/kg/day in 0.5% CMC, orally) [Levocetirizine-diabetic] for eight weeks. A group of 10 age-matched normal rats served as non-diabetic control and was given 0.5% CMC (3 ml/kg/day, orally).

Selection of doses of losartan and levocetirizine in this study was based on previously reported doses in animal studies (Arozal et al., 2009; Shawky et al., 2014; Sleem et al., 2014), with slight modification. The dose of levocetirizine used in the present study is close to human therapeutic dose (10 mg/kg/day). At this dose, levocetirizine should be devoid of central antihistamine effects due to slow transport across blood-brain barrier (Gupta et al., 2007).

At the end of the experiment, rats were placed in metabolic cages for 24 h to collect urine for determination of urine output and the excretion rates of protein, creatinine and urea. Blood was collected via retro-orbital puncture under light ether anaesthesia into a non-heparinized tube from which serum was separated by centrifugation for 10 min at 1000*g*. A sample of fresh whole blood was obtained for glycated hemoglobin A_{1c} (HbA_{1c}) measurement. Moreover, kidney and aorta were harvested and processed for homogenate preparation and histological evaluation. Isolated aortas were also used for determination of vascular reactivity to phenylephrine.

2.4. Biochemical measurements

An assay kit (Biosystems, Spain) was used for evaluation of HbA_{1c} level in blood. Commercially available kits from Biodiagnostic (Giza, Egypt) were used for accurate determination of urinary protein, serum albumin and urinary and serum creatinine and urea according to the manufacturer's instructions (Daughaday et al., 1952; Fawcett and Scott, 1960). Creatinine and urea clearance were calculated from serum and urine levels of creatinine and urea, respectively, as previously reported (Petersen et al., 1999; Sharma et al., 2006).

2.5. Preparation of tissue homogenates of kidney and aorta

10% w/v renal and aortic tissue homogenates were made in 0.9% NaCl, pH 7.4 using a hand-held homogenizer (Omni international, USA) and centrifuged for 15 min at 1000*g*, 4 °C to obtain supernatants, which were used for measuring the following parameters:

2.5.1. Determination of superoxide dismutase (SOD) activity

The enzymatic activity of SOD was determined via assessing the SOD-mediated inhibition of pyrogallol autooxidation (Marklund, 1985). One unit (U) of SOD activity is defined as the amount of the enzyme causing 50% inhibition of auto-oxidation of pyrogallol. SOD activity was expressed as U/g wet tissue.

2.5.2. Determination of malondialdehyde (MDA) levels

Lipid peroxidation was determined by measuring homogenate MDA concentration, based on its reaction with thiobarbituric acid in acidic medium (pH 3.5) at 95 °C for 30 min to form thiobarbituric acid reactive substances (TBARS). The absorbance was read at 532 nm using a colorimeter WPA colourwave (Model CO 7500, Cambridge, England) and results were expressed as MDA equivalents using 1, 1, 3, 3 tetramethoxypropane as a standard (Ohkawa et al., 1979).

2.5.3. Determination of reduced glutathione (GSH) levels

The tissue levels of acid-soluble thiols, mainly GSH, were assayed according to the method of Ellman (1959), which is based on the reduction of Ellman's reagent (5, 5′ dithiobis (2-nitrobenzoic acid), DTNB) by GSH. The absorbance was measured at 405 nm and GSH concentrations were expressed as μmol/g tissue.

2.5.4. Measurement of total nitrite/nitrate (NOx)

Tissue levels of total nitrite and nitrate, the stable metabolites of NO, were determined using a commercial assay kit (R and D