European Journal of Pharmacology 744 (2014) 173–182

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Endocrine pharmacology

Combination therapy with spironolactone and candesartan protects against streptozotocin-induced diabetic nephropathy in rats

Amal Hofni [a,*], Mohamed A. El-Moselhy [a,b], Ashraf Taye [a], Mohamed M. Khalifa [a]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Minia University, 61511 Minia, Egypt
[b] Department of Pharmacology and Toxicology, Ibn Sina National College for Medical Studies, Jeddah, Saudi Arabia

ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form
5 October 2014
Accepted 8 October 2014
Available online 27 October 2014

Keywords:
Diabetic nephropathy
Candesartan
Spironolactone
Oxidative stress

ABSTRACT

Diabetic nephropathy is one of the most common causes of end-stage kidney disease. Aldosterone and angiotensin II appear to play a crucial role in the pathogenesis of this disease. The present study aimed to investigate effects of the combination therapy with spironolactone and candesartan on diabetic nephropathy and elucidate the underlying mechanism(s) involved.

Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (STZ) (55 mg/kg). The diabetic rats were orally treated with spironolactone (50 mg/kg/day) and/or candesartan (1 mg/kg/day) for 8 weeks.

Administration of STZ caused a marked elevation in the serum level of creatinine, urea and urinary albumin–creatinine ratio (ACR). This was associated with upregulated renal protein levels of nuclear factor-kappa B (NF-κB), transforming growth factor (TGF)-β, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) alongside increasing the renal superoxide anion (O_2^-) production, malondialdehyde (MDA) level and the systolic blood pressure. There was a marked decrease in nitric oxide (NO) bioavailability and antioxidant enzyme capacity. The combined therapy of spironolactone and candesartan significantly normalized the oxidative stress and fibrotic/inflammatory alterations. Additionally, the elevated blood pressure was attenuated by administration of candesartan alone or in combination. This was associated with improving the renal function parameters. The combined therapy exhibited more profound response compared to the monotherapy.

In conclusion, our results demonstrate that the combined therapy of spironolactone and candesartan can confer an additive benefit over the use of either drug alone against STZ-induced diabetic nephropathy, presumably via attenuating the inflammatory responses and oxidative status markers.

1. Introduction

Diabetic nephropathy, a common complication in patients with either type 1 or type 2 diabetes mellitus, has long been recognized to cause severe morbidity and mortality. Hyperglycemia-mediated metabolic abnormalities, hemodynamic abnormalities, and oxidative stress have all been implicated in the pathogenesis of diabetic nephropathy (Tan et al., 2007). Diabetes can influence numerous cell types in the kidney, including glomerular podocytes, mesangial and endothelial cells, tubular epithelia, interstitial fibroblasts, and vascular endothelia (Kanwar et al., 2008). Activation of renin–angiotensin–aldosterone system (RAAS) is thought to be the major mechanism underlying diabetic nephropathy (Kobori et al., 2012). Several reports have demonstrated that high glucose levels are the principal cause of renal damage in diabetes (Patinha et al., 2014; Xiao et al., 2014). Moreover, it has been reported that the activated RAAS/oxidative stress axis may play a central role in diabetic nephropathy pathogenesis (Tikellis et al., 2014). Many studies reported high glucose and angiotensin II stimulated collagen production by transforming growth factor (TGF)-β, a profibrotic cytokines (Jiao et al., 2011; Chou et al., 2013). In addition, cyclooxygenase-2 (COX-2) has been implicated in cardiovascular and renal pathophysiology, including the processes leading to the development of diabetic nephropathy (Cherney et al., 2008).

On the other hand, a crucial role of aldosterone in the pathogenesis of cardiovascular disease in humans has been established. Aldosterone is detrimental to patients with hypertension (Park and Schiffrin, 2002) and it can lead to progressive tissue damage in the heart, vasculature, and kidneys (Park and Schiffrin, 2002). Accumulating evidence suggests that the mineralocorticoid receptor antagonist, spironolactone, has been shown to prevent diabetic renal injury (Lian et al., 2012; Toyonaga et al., 2012). Moreover, the beneficial effects of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker in preventing diabetic nephropathy are widely accepted in current medical science (Abuissa and O'Keefe, 2008).

* Corresponding author. Fax: +20 86 2369075.
E-mail address: amal_arwa1@yahoo.com (A. Hofni).

http://dx.doi.org/10.1016/j.ejphar.2014.10.021
0014-2999/

Recently, it has been reported that simultaneous treatment with spironolactone and losartan may have protective effects against diabetic nephropathy in a type 2 diabetic rat (Lee et al., 2011). However, the putative role and the underling mechanism(s) by which the combination therapy with spironolactone and candesartan in diabetic nephropathy of type 1 diabetes remain to be fully defined. Therefore, the present study was conducted to examine the effect of the combined therapy of spironolactone and candesartan on the renal function in type 1 diabetic nephropathy. In addition, we attempted to elucidate the underlying mechanism(s) involved in this setting.

2. Materials and methods

2.1. Chemicals

Spironolactone was a gift from SEDICO (Egypt). Streptozotocin (STZ) was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA), Candesartan cilexetil (Takeda Chemical Industries, Osaka, Japan). Polyclonal rabbit/anti-rat primary antibodies against endothelial nitric oxide synthase (iNOS) were purchased from Thermo Fisher Scientific Inc./Lab Vision (Fremont, CA, USA). Polyclonal rabbit/anti-rat primary antibodies against transforming growth factor-beta (TGF-β) were purchased from Biorbyt (Cambridge, Cambrigshire, UK), mouse monoclonal anti NF-κB p65 (Santa Cruz, Biotechnology, Inc.) and anti-COX-2 (Biosciences, San Diego, CA). All chemicals were of highest grade commercially available.

2.2. Animals

Adult male Wistar rats (160–210 g, 10–12 week-old) were housed at room temperature with 12:12 h light/dark cycles and were given food and water ad libitum.

Experiments were conducted in accordance with the international ethical guidelines for animal care of the United States Naval Medical Research Centre, Unit No. 3, Abbaseya, Cairo, Egypt, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care international (AAALAC international). The adopted guidelines are in accordance with “Principles of Laboratory Animals Care” (NIH publication No. 85-23, revised 1985). The study protocol was approved by members of “The Research Ethics Committee” as well as by the Pharmacology &Toxicology Department, Faculty of Pharmacy, Minia University, Egypt.

2.3. Induction of diabetes

Streptozotocin was freshly dissolved in 10 mmol/l citrate buffer, pH 4.5, and was intraperitoneally injected at a single dose of 55 mg/kg for diabetes induction (Coskun et al., 2005). After 48 h, diabetic status was determined where rats with blood glucose levels $\geq$ 300 mg/dl were considered diabetics.

2.4. Experimental design

Rats were randomly divided into five groups of seven rats each, as follows:

1. Control group: normal non-diabetic rats, received the same volume of the vehicle (0.5% carboxymethylcellulose orally).
2. Diabetic group (STZ group): rats were treated with STZ as described above.
3. Diabetic group treated with the spironolactone (STZ+SPIRO): rats were orally treated with spironolactone at oral dose of 50 mg/kg (Pessoa et al., 2012).
4. Diabetic group treated with the candesartan (STZ+CAND): rats were orally treated with candesartan at oral dose of 1 mg/kg (Noda et al., 2001; Zoja et al., 2010).
5. Diabetic group treated with the combination of spironolactone and candesartan (STZ+SPIRO+CAND): rats were orally treated with spironolactone (50 mg/kg/day) and candesartan (1 mg/kg/day).

All drug treatments were maintained during the duration of diabetes (8 weeks). At the end of the experiment, animals were fasted for 12 h before urine samples were collected. The rats were killed; blood samples were collected and centrifuged to obtain clear sera. The longitudinal section of the left kidney was excised from each animal for histological and immunohistochemical examination. The renal cortex of the rest of kidneys was snap frozen in liquid nitrogen, stored at −80 °C, and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4) for western blotting and various biochemical analysis. Total protein concentration was also determined using a bicinchoninic acid (BCA) protein assay kit (Pierce Chemicals).

2.5. General and biochemical parameters

Body weights, the body weight/kidney weight ratio, and the level of blood glucose were determined at the end of the study. In addition, blood pressure was measured using tail-cuff plethysmography was determined.

Using commercially available kits, levels of serum and urinary creatinine (Diamond Diagnostics, Egypt), serum urea (Biodiagnostic, Egypt), microalbuminuria (BioSystems, Spain) as well as renal catalase (CAT) activity (Biodiagnostic, Egypt) were quantified according to the manufacturers' guidelines. Renal cortex lipid peroxidation was determined as thiobarbituric acid reacting substance and is expressed as equivalent of malondialdehyde, using 1,1,3,3-tetramethoxypropane as standard (Buege and Aust, 1978). Renal cortex nitric oxide bioavailability was measured as a total nitrite/nitrate, the stable degradation products of nitric oxide, by reduction of nitrate into nitrite using copperized cadmium, followed by color development with Griess reagent in acidic medium (Sastry et al., 2002).

2.6. Measurement of superoxide anion production

The kidney tissue was homogenized in a cold Krebs-HEPES buffer (10 mmol/l glucose, 0.02 mmol/l Ca-Tritriplex, 25 mmol/l $NaHCO_3$, 1.2 mmol/l KH2PO4, 120 mmol/l NaCl, 1.6 mmol/l $CaCl_2 \cdot 2H_2O$, 1.2 mmol/l $MgSO_4 \cdot 7H_2O$, and 5 mmol/l KCl, pH 7.4). Superoxide production was measured using lucigenin-derived chemisescence as previously described (Taye et al., 2010). Superoxide generation was measured using lucigenin-enhanced chemiluminescence (5 μmol/l) to minimize the redox cycling, incubated for 20 min. The reaction was started by addition of NADPH (100 μmol/l), and the relative light units (RLU) of chemiluminescences were measured over a period of 30 min using a luminescence spectrometer (Perkin-Elmer Ltd. UK). Results were expressed as counts per min and normalized to the protein content of each sample.

2.7. Western blot analysis

Protein expression of NF-κB and TGF-β was determined in kidney tissues using Western blot analysis. Frozen cortex of each kidney was homogenized in ice-cold lysis buffer containing: 20 mmol/l Tris · HCl, 140 mmol/l NaCl, 1 mmol/l EDTA, complete miniprotease inhibitor cocktail, 1% Triton X-100, 0.1% SDS, 1% sodium deoxycholate, 1 mmol/l NaF, and 1 mmol/l orthovanadate, pH 7.8. Following to protein concentration estimation using BCA, equal amounts of protein (20 μg/lane) were separated by Sodium Dodecyl Sulfate-Polyacrylamide Gel