European Journal of Pharmacology 718 (2013) 160–166

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

Protective effects of aliskiren on ischemia–reperfusion-induced renal injury in rats

Zhen Wang [a,b,1], Yukai Liu [a,b,1], Yu Han [a,b,1], Weiwei Guan [a,b], Xun Kou [a,b], Jinjuan Fu [a,b], Di Yang [a,b], Hongmei Ren [a,b], Duofen He [a,b], Lin Zhou [a,b,*], Chunyu Zeng [a,b,*]

[a] Department of Cardiology, Daping Hospital, The Third Military Medical University, Chongqing 400042, China
[b] Chongqing Institute of Cardiology, Chongqing, China

ARTICLE INFO

Article history:
Received 17 April 2013
Received in revised form
24 July 2013
Accepted 27 August 2013
Available online 13 September 2013

Keywords:
Aliskiren
Renal ischemia–reperfusion
Angiotensin II
Oxidative stress
Apoptosis

ABSTRACT

The protective effect of aliskiren on ischemia–reperfusion (I/R) injury in the heart and brain has been reported. Whether or not this protective effect extends into the alleviation of renal I/R injury is not known. Therefore, we investigated the protective effect of aliskiren in the kidney in this study. Sprague-Dawley rats were randomly divided into four groups: sham control group; sham control with aliskiren pretreatment; I/R group and I/R with aliskiren pretreatment. Aliskiren (3 mg/kg) or vehicle was administrated intravenously via vena cava. Blood samples and the left kidneys were then collected to check for renal function, angiotensin II (Ang II), apoptosis and oxidative stress levels. Compared with the sham rats, serum creatinine (SCR) and blood urea nitrogen (BUN) were significantly increased in the I/R rats, accompanied by histopathological damage to the kidney, which included tubular cell swelling, desquamation, and cast formation. There were also more apoptotic cells and leukocyte infiltration in the I/R rats than in the sham rats. Pretreatment with aliskiren ameliorated I/R induced renal injury, i.e. reduced SCR and BUN levels, ameliorated renal histopathological changes, and decreased the apoptosis of cells and leukocyte infiltration in kidney. I/R injury also decreased superoxide dismutase (SOD) and glutathione (GSH-reduced form) levels, which were blocked with the aliskiren pretreatment. Aliskiren pretreatment exerts a protective effect on ischemia/reperfusion injury in the kidney, via amelioration of oxidative stress, and reduction in leukocyte infiltration and cellular apoptosis.

1. Introduction

Renal ischemia–reperfusion (I/R) injury is involved in many diseases, including shock, nephrectomy, renal arterial revascularization and renal transplantation, and may worsen renal function or even cause renal failure (Bergler et al., 2012; Landry et al., 2010; Matin and Novick, 2001). Ameliorating renal I/R injury is a very important condition to consider in the protection of renal function, especially in patients with kidney insufficiency.

Although the mechanisms underlying I/R injury are complex, renin–angiotensin–aldosterone system (RAAS) may play an important role in the progression of I/R injury. Previous studies have proven that both angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), the classic RAAS inhibitors, have beneficial effects on I/R injury in the kidney (Barrilli et al., 2004; Lukitsch et al., 2012; Pazoki-Toroudi et al., 2003). Direct renin inhibitors (DRIs) are a new type of RAAS inhibitors that blocks the RAAS at its first rate-limiting step, by blocking the conversion of angiotensinogen to angiotensin I. This inhibits plasma renin activity (PRA) and reduces the production of angiotensin II (Ang II) and aldosterone. Aliskiren is the first of its class of orally-taken DRIs. It has been demonstrated to be effective in BP reduction and end-organ protection such as cardiovascular and renal protection in preclinical and clinical experiments through its effects on RAAS inhibition. As aliskiren can directly decrease plasma renin activity, it may be more effective on RAAS blockade and reduction of Ang II level as compared to ACEI or ARB (Morganti and Lonati, 2011). Previous studies show the protective effect of aliskiren on I/R injury in the heart and brain (Schmerbach et al., 2010; Shi et al., 2011). Whether or not there is also a protective effect on renal I/R injury is not known. The present study will evaluate the effect of aliskiren on renal I/R injury and investigate its protective mechanisms in I/R rats.

2. Materials and methods

2.1. Animals and surgical procedures

Adult male Sprague-Dawley (SD) rats, weighing 250–260 g, were obtained from the Laboratory Animal Center of Daping Hospital. All

* Corresponding authors at: The Third Military Medical University, Department of Cardiology, Daping Hospital, Chongqing 400042, China. Tel.: +86 23 68757808.
E-mail addresses: zhoulin@mail.tmmu.com.cn (L. Zhou), cyzeng1@hotmail.com (C. Zeng).
[1] Co-first author.

0014-2999/$ - see front matter Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.08.038

procedures were approved by the Experimental Animals Committee of Daping Hospital. The rats were randomly divided into four groups: sham control group; sham control with aliskiren pretreatment; I/R group and I/R with aliskiren pretreatment. Aliskiren was dissolved in injectable sterile saline solution before being used.

Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg), and placed on a heating pad to maintain a constant body temperature of 37 °C during the surgery. A laparotomy was done and a right kidney nephrectomy was performed. Aliskiren (3 mg/kg) or vehicle (distilled saline) was administered by intravenous injection. Fifteen minutes later, the left renal pedicle was occluded using a small microvascular clamp and renal ischemia was induced for 45 min. For the sham control group, only the laparotomy and the right kidney nephrectomy were performed. The incisions were closed with 4-0 silk sutures. The animals were then allowed to recover with free access to food and water. Blood was collected and the left kidney was harvested for analysis 24 h after reperfusion (Zheng et al., 2013).

2.2. Organ and tissue samples

Blood samples were obtained and centrifuged ($3000 \times g$ for 10 min) to extract the serum, which was then stored at −80 °C until analyzed. The rat kidneys were divided into two longitudinal sections. The first section was used to detect oxidative stress and apoptosis molecules. It was weighed, and part of the renal cortex was homogenized in ice-cold lysis-buffer. The tissue homogenate was sonicated and kept on ice for 1 h. The lysate was then centrifuged for 30 min at 12,000 rpm. The supernatant was collected and similarly stored at −80 °C for further analysis (Chen et al., 2008). The concentration of the protein was quantified by using a protein assay kit (Bio-Rad Laboratories, Hercules, California, USA) with bovine serum albumin used as a standard (Altintas et al., 2012; Zeng et al., 2009). The remainder of the kidney was stored at −80 °C for further studies. The other half of the kidney section was fixed in 4% paraformaldehyde for hematoxylin–eosin (HE) and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining.

2.3. Assessment of renal function

The renal function of the experimental rats was assayed for serum creatinine (SCR) and blood urea nitrogen (BUN) levels. Serum samples were analyzed by using an automated Beckman Analyzer (Beckman Instruments GmbH, Munich, Germany) (Efrati et al., 2012; Lai et al., 2009).

2.4. Serum Ang II assessment

Rat serum Ang II level was measured to determine the activation of RAAS by radioimmunoassay (Braithwaite et al., 2012). Serum samples were tested following the recommendations of the manufacturer of the commercial kits (Angiotensin II Radioimmunoassay Kit, North Institute of Biotechnology, Beijing, China).

2.5. Caspase-3 and caspase-9 activity assay

The activity of caspase-3 and caspase-9 in the renal cortex were detected using commercial assay kits for caspase activity (Beyotime Institute of Biotechnology, Haimen, China). Caspase-3 and -9 activities were measured through the cleavage of substrates specific for caspase-3 (Ac-DEVD-pNA) or caspase-9 (Ac-LEHD-pNA) and the release of the chromophore, *p*-nitroaniline (pNA) (Zhu et al., 2010).

2.6. MDA activity assay

Renal cortical samples from the four rat groups were used for the malondial-dehyde (MDA) assay. The concentration of MDA was measured by the thiobarbituric acid (TBA) method. The amount of lipid peroxides (LPO), the by-product of MDA, was assayed to determine MDA concentrations. Absorbance was measured at 532 nm by using Model 680 spectrophotometer (Bio-Rad, California, USA) (Assay kit was purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China) (Qiao et al., 2013).

2.7. SOD activity assay

Total SOD activity was measured using the nitroblue tetrazolium (NBT) method (Assay kit from Beyotime Institute of Biotechnology, China). Briefly, the SOD activity in renal homogenates was measured by the inhibition of NBT reduction caused by the xanthine-XO system as the superoxide generator. Activity was assessed during the ethanol phase of the lysate after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount of enzyme that can cause a 50% inhibition in the NBT reduction rate. SOD activity was expressed as units/mg protein (Sun et al., 2010).

2.8. GSH activity assay

Reduced glutathione (GSH) activity was measured by using a commercial assay kit (Beyotime Institute of Biotechnology, China). Briefly, total glutathione (T-GSH) was assayed using the 5, 5-dithio-*bis* (2-nitrobenzoic) acid (DTNB)-GSSG reductase recycling. GSSG was determined by the same method in the presence of vinyl pyridine. The absorbance at 412 nm was measured by a Model 680 spectrophotometer (Bio-Rad, California, USA). GSH levels were evaluated by subtracting GSSG from T-GSH. The results were expressed in μmol GSH/g tissue (Huang et al., 2010).

2.9. MPO activity assay

Myeloperoxidase (MPO) activity was determined in kidney homogenates to assess leukocyte infiltration (Assay kit from Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Kidney samples stored at −80 °C were weighed and homogenized using a Tekmar tissue grinder in ice-cold 50 mM potassium phosphate buffer (pH 7.4) with 10 times volume as renal samples. The homogenate was centrifuged at 15,000*g* for 15 min at 4 °C, and the resultant supernatant fluid was discarded. The pellet was washed twice, resuspended in ice-cold 50 mM potassium phosphate buffer with 0.5% hexadecyltrimethylammonium bromide, and sonicated. The suspension was subjected to three freeze-thaw cycles, sonicated for 10 s, and centrifuged at $15{,}000 \times g$ for 15 min at 4 °C. The supernatant fluid was added to an equal volume of solution consisting of *o*-dianisidine (10 mg/ml), 0.3% H_2O_2, and 45 mM potassium phosphate, pH 6.0. Absorbance was measured at 460 nm over a period of 5 min. The results were expressed in U/g wet tissue (Chen et al., 2008; Wang et al., 2013).

2.10. Histological analysis

The fixed kidneys were dehydrated in increasing concentrations of ethanol, cleared in xylene, and then embedded in paraffin. The samples were cut into 4 μm thick sections then stained with haematoxylin and eosin. Each prepared sample section was examined by a pathologist in a blinded study. Morphological changes to the tubules were scored to assess the degree of renal damage. These changes included tubular epithelial cell swelling, vacuolization, cast