European Journal of Pharmacology 779 (2016) 147–156

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

# Direct renin inhibitor ameliorates insulin resistance by improving insulin signaling and oxidative stress in the skeletal muscle from post-infarct heart failure in mice

Arata Fukushima [a], Shintaro Kinugawa [a,*], Shingo Takada [a], Junichi Matsumoto [a], Takaaki Furihata [a], Wataru Mizushima [a], Masaya Tsuda [a], Takashi Yokota [a], Shouji Matsushima [a], Koichi Okita [b], Hiroyuki Tsutsui [a]

[a] Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[b] Graduate School of Lifelong Sport, Hokusho University, Ebetsu, Japan

ARTICLE INFO

*Article history:*
Received 6 November 2015
Received in revised form
10 March 2016
Accepted 11 March 2016
Available online 14 March 2016

*Keywords:*
Direct renin inhibitor
Insulin resistance
Heart failure
Skeletal muscle
Oxidative stress
(Pro)renin receptor

ABSTRACT

Insulin resistance can occur as a consequence of heart failure (HF). Activation of the renin-angiotensin system (RAS) may play a crucial role in this phenomenon. We thus investigated the effect of a direct renin inhibitor, aliskiren, on insulin resistance in HF after myocardial infarction (MI). MI and sham operation were performed in male C57BL/6 J mice. The mice were divided into 4 groups and treated with sham-operation (Sham, n=10), sham-operation and aliskiren (Sham+Aliskiren; 10 mg/kg/day, n=10), MI (n=11), or MI and aliskiren (MI+Aliskiren, n=11). After 4 weeks, MI mice showed left ventricular dilation and dysfunction, which were not affected by aliskiren. The percent decrease of blood glucose after insulin load was significantly smaller in MI than in Sham (14 ± 5% vs. 36 ± 2%), and was ameliorated in MI+Aliskiren (34 ± 5%) mice. Insulin-stimulated serine-phosphorylation of Akt and glucose transporter 4 translocation were decreased in the skeletal muscle of MI compared to Sham by 57% and 69%, and both changes were ameliorated in the MI+Aliskiren group (91% and 94%). Aliskiren administration in MI mice significantly inhibited plasma renin activity and angiotensin II (Ang II) levels. Moreover, (pro)renin receptor expression and local Ang II production were upregulated in skeletal muscle from MI and were attenuated in MI+Aliskiren mice, in tandem with a decrease in superoxide production and NAD(P)H oxidase activities. In conclusion, aliskiren ameliorated insulin resistance in HF by improving insulin signaling in the skeletal muscle, at least partly by inhibiting systemic and (pro)renin receptor-mediated local RAS activation, and subsequent NAD(P)H oxidase-induced oxidative stress.



## 1. Introduction

Insulin resistance is highly prevalent and an established risk factor for heart failure (HF), and it has been associated with reduced functional capacity and poor prognosis (Doehner et al., 2005; Ingelsson et al., 2005; Lopaschuk et al., 2010). Conversely, HF itself is known to trigger the occurrence of insulin resistance, accounting for a vicious cycle of functional exacerbation of these two conditions (AlZadjali et al., 2009; Witteles et al., 2004). Indeed, the peripheral effects of insulin resistance are likely to represent a major metabolic feature of the pathophysiology of HF, contributing to key clinical symptoms such as breathlessness and early muscle fatigue (Kinugawa et al., 2015; Okita et al., 2013; Wilson et al., 1993). Multiple mechanisms of insulin resistance have already been identified, including increased oxidative stress and hyperactivation of the renin-angiotensin system (RAS) (Of®-cers et al., 2002; Wei et al., 2006). We previously reported that insulin resistance was induced in experimental HF in mice (Ohta et al., 2011), and a later study showed that this induction was accompanied by increased local angiotensin II (Ang II) in the skeletal muscle and subsequent NAD(P)H oxidase-derived oxidative stress (Fukushima et al., 2014). In addition, the recent discovery of a (pro)renin receptor for renin and its precursor, prorenin, raises the possibility that these components of RAS may play significant pathophysiological roles in the insulin resistance of rats with high fructose diet-induced diabetes or in post-infarct HF mice (Fukushima et al., 2014; Nagai et al., 2009).

* Correspondence to: Department of Cardiovascular Medicine Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan.
*E-mail address:* tuckahoe@med.hokudai.ac.jp (S. Kinugawa).

http://dx.doi.org/10.1016/j.ejphar.2016.03.022
0014-2999/

Aliskiren is a potent direct renin inhibitor that blocks the first rate-limiting step in RAS, preventing the compensatory rise in plasma renin activity and other downstream components of this system from occurring during angiotensin converting enzyme (ACE) inhibitor or Ang II receptor blocker treatment (Gradman and Traub, 2007). Aliskiren has been shown to protect against the development of insulin resistance in an animal model of diabetes by improving skeletal muscle glucose transport as well as to improve insulin sensitivity in hypertensive patients with metabolic syndrome (Fogari et al., 2010; Iwai et al., 2010; Marchionne et al., 2012). In addition, it has been reported that aliskiren can inhibit the free form of mature renin and the (pro)renin receptor-bound forms of renin and prorenin, suggesting that these dual inhibitory effects play roles in both systemic RAS and (pro)renin receptor-mediated tissue RAS (Biswas et al., 2010). To date, clinical trials to investigate the effect of aliskiren on myocardial infarction (MI) and HF have failed to improve outcomes by administration of aliskiren in combination with an ACE inhibitor or Ang II receptor blocker (Gheorghiade et al., 2013; Solomon et al., 2011). However, it remains to be determined whether low-dose single treatment with aliskiren could ameliorate the insulin resistance associated with MI and HF.

In the present study, we examined the effects of aliskiren on the insulin resistance and the insulin signaling in the skeletal muscle from post-infarct HF mice, mainly focusing on its effects on the (pro)renin receptor-mediated tissue RAS and oxidative stress in the skeletal muscle.

## 2. Materials and methods

All procedures and animal care were approved by our institutional animal research committee and conformed to the Guidelines for the Care and Use of Laboratory Animals of the Hokkaido University Graduate School of Medicine.

### 2.1. Experimental animals

Male C57BL/6J mice, 8–10 weeks old and 20–21 g body weight (BW), were maintained on a normal diet (CE-2; CLEA Japan, Tokyo) containing 4.2% fat and 54.6% carbohydrate. MI was established by ligating the left coronary artery as described previously (Fukushima et al., 2014; Kinugawa et al., 2000). Sham operation without ligation of the coronary artery was also performed. Each group of mice was then randomly divided into 2 groups, a group with and a group without aliskiren (10 mg/kg BW/day; Novartis Pharmaceuticals, Basel, Switzerland) administered subcutaneously for 4 weeks using an osmotic minipump (model 2004; Alzet, Palo Alto, CA). The non-depressor concentration of aliskiren was chosen on the basis of our preliminary data of blood pressure measurement by using the indirect tail-cuff method (MK-1030; Muromachi Kikai Co., Ltd., Tokyo, Japan) (Supplementary material). Experiments were performed at 4 weeks after operation in the following 4 groups: Sham (n=10), Sham+Aliskiren (n=10), MI (n=11), and MI+Aliskiren (n=11).

### 2.2. Echocardiographic and Hemodynamic measurements

Echocardiographic and hemodynamic measurements were performed under light anesthesia with tribromoethanol/amylene hydrate (avertin; 2.5% wt/vol, 8 μl/g BW ip), which has short duration of action and modest cardiodepressive effects and spontaneous respiration, as described previously (Fukushima et al., 2014; Ohta et al., 2011). Standard echocardiographic short- and long-axis views were obtained at the levels of the papillary muscles. Left ventricular function, ventricular size and wall thickness were measured from M-mode frames at a paper speed of 50 mm/s. To perform hemodynamic measurements, a 1.4 Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted into the right carotid artery and then advanced into the left ventricle (LV) to measure LV pressures.

### 2.3. Tissue preparation and organ histology

Heart, lung, and hindlimb skeletal muscle including the quadriceps, gastrocnemius, and soleus were excised 3 min after intraperitoneal injection of saline, with or without human regular insulin (1.0 U/kg BW) and weighed under deep anesthesia with avertin (2.5% wt/vol, 10 μl/g BW, ip). To determine the infarct size, myocyte cross-sectional area, and total collagen volume in cardiac tissue, ventricular tissue was fixed in 6% formaldehyde, cut into three transverse sections–the apex, middle ring, and base–and stained with hematoxylin-eosin or Masson's trichrome as described previously (Matsushima et al., 2009; Sobirin et al., 2012).

### 2.4. Plasma biochemical measurement

After the animals were fasted for 8 h, blood samples were collected from the inferior vena cava, the blood glucose level was determined using a glucometer (Glutest Ace R; Sanwa Kagaku Kenkyusho, Nagoya, Japan) and the plasma insulin was measured by an ELISA kit (Morinaga Institute, Kanagawa, Japan). The homeostasis model assessment index (HOMA-IR) was calculated using the formula of fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5. Total cholesterol, triglyceride, and nonesterified fatty acid (NEFA) were measured by a commercial ELISA kit (Wako Pure Chemical Industries, Osaka, Japan). The plasma renin activity level was determined using a SensoLyte 520 Renin Assay Kit (AnaSpec Inc., San Jose, CA). The plasma angiotensin (Ang) II level was measured by using an enzyme immunoassay (EIA) kit (Phoenix Pharmaceuticals Inc., Burlingame, CA) as previously described (Fukushima et al., 2014).

### 2.5. Intraperitoneal insulin tolerance test

For the insulin tolerance test, mice were injected intraperitoneally with human regular insulin (0.5 U/kg BW) and blood samples were collected before and 15, 30, 45, 60, 90, and 120 min after the injection. Blood glucose levels were determined using a glucometer (Glutest Ace R; Sanwa Kagaku Kenkyusho, Nagoya, Japan) (Takada et al., 2014). Data are shown as a percent change in blood glucose levels after insulin load.

### 2.6. Western blot analysis

Forty milligrams of frozen quadriceps skeletal muscle tissue was homogenized for 30 s with a Polytron homogenizer in a homogenization buffer containing 20 mM $NaHCO_3$, pH 7.0, 0.25 M sucrose, 5 mM $NaN_3$, 1 mM leupeptin, 1 mM aprotinin, and 1 mM pepstatin) at 4 °C. Twenty μg of denatured proteins was subjected to 8–12% SDS-PAGE on a polyvinylidene difluoride (PVDF) membrane as previously described (Takada et al., 2013). After blocking in 5% fat-free milk for 1 h, the membranes were probed with the following antibodies: ATP6P2/(pro)renin receptor (Abcam Inc., Cambridge, MA), Akt, phosphoserine Akt (Ser473), and glucose transporter 4 (GLUT4) (Cell Signaling Technology, Beverly, MA). The membranes were then incubated with the appropriate secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. These bands were visualized by enhanced chemiluminescence and quantified with Image J software (NIH, Bethesda, MD). The resulting values were expressed as the ratio of target band intensity to total protein or internal control intensity. GAPDH (Cell