European Journal of Pharmacology 791 (2016) 244–253

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

Ramipril restores PPARβ/δ and PPARγ expressions and reduces cardiac NADPH oxidase but fails to restore cardiac function and accompanied myosin heavy chain ratio shift in severe anthracycline-induced cardiomyopathy in rat

Hana Cernecka [a,b], Gabriel Doka [a], Jasna Srankova [a], Lenka Pivackova [a], Eva Malikova [a], Kristina Galkova [a], Jan Kyselovic [a], Peter Krenek [a], Jan Klimas [a,*]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia
[b] Bayer Pharma AG, Wuppertal, Germany

ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form
31 August 2016
Accepted 31 August 2016
Available online 31 August 2016

Keywords:
Anthracycline
Chronic cardiomyopathy
Angiotensin-converting enzyme inhibitor
Peroxisome proliferator-activated receptors
Myosin heavy chain isoforms

ABSTRACT

We hypothesized that peroxisome proliferator-activated receptors (PPARs) might be involved in a complex protective action of ACE inhibitors (ACEi) in anthracyclines-induced cardiomyopathy. For purpose of study, we compared effects of ramipril on cardiac dysfunction, cardiac failure markers and PPAR isoforms in moderate and severe chronic daunorubicin-induced cardiomyopathy. Male Wistar rats were administered with a single intravenous injection of daunorubicin: 5 mg/kg (moderate cardiomyopathy), or 15 mg/kg (severe cardiomyopathy) or co-administered with daunorubicin and ramipril (1 mg/kg/d, orally) or vehicle for 8 weeks. Left ventricular function was measured invasively under anesthesia. Cardiac mRNA levels of heart failure markers (ANP, Myh6, Myh7, Myh7b) and PPARs (alpha, beta/delta and gama) were measured by qRT-PCR. Protein expression of NADPH subunit (gp91phox) was measured by Western blot. Moderate cardiomyopathy exhibited only minor cardiac dysfunction what was corrected by ramipril. In severe cardiomyopathy, hemodynamic dysfunction remained unaltered upon ramipril although it decreased the significantly up-regulated cardiac ANP mRNA expression. Simultaneously, while high-dose daunorubicin significantly decreased PPARbeta/delta and PPARgama mRNA, ramipril normalized these abnormalities. Similarly, ramipril reduced altered levels of oxidative stress-related gp91phox. On the other hand, ramipril was unable to correct both the significantly decreased relative abundance of Myh6 and increased Myh7 mRNA levels, respectively. In conclusion, ramipril had a protective effect on cardiac function exclusively in moderate chronic daunorubicin-induced cardiomyopathy. Although it normalized abnormal PPARs expression and exerted also additional protective effects also in severe cardiomyopathy, it was insufficient to influence impaired cardiac function probably because of a shift in myosin heavy chain isoform content.

1. Introduction

Anthracyclines are among the most effective anticancer treatments ever developed, but their clinical use is limited by their cumulative dose-related cardiotoxicity leading to cardiomyopathy (Hershman et al., 2008). There are several types of anthracyclines-induced cardiomyopathy in human, however, chronic forms that develop within the first year from anthracyclines exposure or with a delay of several years are largely irreversible (Menna et al., 2012). Chronic anthracycline cardiomyopathy is a serious clinical issue with well characterized functional and histopathological hallmarks. Anthracycline-damaged hearts typically display dilated cardiomyopathy characterized by a reduced ejection fraction, ventricular wall thinning and chamber dilatation. Free radical formation and oxidative stress have been implicated as central mechanisms underlying the cardiotoxic effects of anthracyclines (Zhou et al., 2001) but also other responsible mechanisms have been stressed (Simunek et al., 2009). Anthracyclines were shown to alter expression of heart-specific proteins including myocellular proteins (Boucek et al., 1999), calcium-regulating proteins (Kucerova et al., 2015) and sarcomeric proteins (Lencova-Popelova et al., 2014) suggesting a complex background of this specific cardiomyopathy.

* Correspondence to: Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Odbojarov 10, Bratislava 832 32, Slovakia.
E-mail address: klimas1@uniba.sk (J. Klimas).

http://dx.doi.org/10.1016/j.ejphar.2016.08.040
0014-2999/

Angiotensin-converting enzyme inhibitors (ACEi) are a class of drugs that have clearly shown positive therapeutic profiles for the treatment of heart failure caused by a number of cardiovascular diseases, including anthracyclines cardiomyopathy probably because they possess also free radical scavenger and antioxidant properties (Boucek et al., 2003), and recent clinical study has demonstrated that ACE inhibition significantly ameliorates development of heart failure in anthracyclines-induced cardiotoxicity (Cardinale et al., 2010). However, the exact mechanisms by which ACE inhibitors can prevent anthracyclines-induced cardiomyopathy remain unclear.

Aside from the central role of peroxisome proliferator-activated receptors (PPARs) in metabolism, recent studies have highlighted their role in processes accompanying most diseases of the cardiovascular system including myocardial infarction and cardiac failure. It has been shown that PPAR signalling pathway might be implicated in protective mechanism in anthracycline-induced cardiomyopathy (Shuai et al., 2011). Reduced expression of PPARα leads to development of cardiac hypertrophy and failure of fatty acid oxidation capacity (Stanley et al., 2005). A number of studies using transgenic approaches and pharmacological interventions have shown that PPARβ/δ plays a crucial role in cardiomyocyte growth and survival (van Bilsen and van Nieuwenhoven, 2010). Cardiomyocyte PPARγ maintains normal heart function and supresses cardiac growth and embryonic gene expression (Duan et al., 2005).

Previously we showed that short-term ACE inhibition influences acute daunorubicin-induced cardiomyopathy and this might be related to PPARs (Cernecka et al., 2013). The purpose of this study was to determine the effect of ACE inhibitor ramipril on cardiac function and explore its protective molecular mechanism in chronic daunorubicin-induced cardiomyopathy in rat. Specifically, our objective was to determine the protective effects of ACE inhibitor ramipril in chronic cardiomyopathy induced by single dose of 5 mg/kg (moderate cardiomyopathy) or 15 mg/kg of daunorubicin (severe cardiomyopathy) and explore the potential involvement of PPAR isoforms in ramipril-mediated protection mechanism.

2. Materials and methods

2.1. Experimental animals

Male 10–12-week-old Wistar rats (Department of Toxicology and Laboratory Animal breeding, Dobra Voda, Slovak Republic) were used in the study. The rats were kept under standard conditions and received food and water *ad libitum*. All animal experiments were conducted in accordance with NIH Guide for the Care and Use of Laboratory Animals and approved by the Ethical Committee for Animal Experiments of the Faculty of Pharmacy, Comenius University, Bratislava and by the State Veterinary and Food Administration of Slovak Republic. Chronic daunorubicin cardiomyopathy was induced by a single intravenous injection of daunorubicin at two different doses: 5 mg/kg (D5) or 15 mg/kg (D15); Daunoblastina®, Pfizer, Sandwich, UK) under thiopental anesthesia (45 mg/kg, i.p.; Valeant Czech Pharma, Prague, Czech Republic). The control animals received vehicle: 0.9% NaCl solution (CON). The animals were assigned into the experimental groups receiving either oral ramipril (1 mg/kg, D5+R, D15+R) or vehicle (0.9% NaCl) via an intragastric probe for the duration of 8 weeks.

2.2. Functional measurements

Standard 12-lead electrocardiography (ECG) was performed in rats anaesthetized with tribromoethanol (375 mg/kg, i.p.) as previously reported (Klimas et al., 2012; Krenek et al., 2009). Duration of QT, as a measure of cardiac repolarization, was determined and corrected to rat cardiac cycle length as QTc (in milliseconds)=$QT/(RR/150)^{1/2}$ (Kmecova and Klimas, 2010). Following ECG measurements, arterial systolic and diastolic blood pressure (sBP and dBP, respectively) were measured invasively in *a. carotis*. Left ventricular function was measured *in situ* via catheterization of the left ventricle (Spel Advanced HaemoSys; Experimetria Ltd.) as described (Klimas et al., 2010). The functional response of left ventricle to beta-adrenergic stimulation was measured upon infusion of increasing doses of dobutamine into *v. jugularis*. Next, systolic blood pressure and hemodynamic parameters (+dP/dt and -dP/dt) were analysed in the left ventricle.

2.3. RNA isolation and RT-PCR

Total RNA was isolated from left ventricles using Tri-Reagent (Ambion, USA). Isolated RNA was verified to be intact by using agarose gel electrophoresis. Reverse transcription was performed using (High capacity cDNA Reverse Transcription Kit with RNAse inhibitor®; Applied Biosystems), 1 μg RNA was used per reaction. Real-time PCR was performed using SYBR Green detection (Power SYBR Green PCR Master Mix kit; Applied Biosystems) on ABI Prism 7300 Real-Time PCR System (Applied Biosystems, USA). Expressions of cardiac genes were evaluated using gene-specific primers. β-actin or β-2-microglobulin served as housekeeping genes and we used the delta-delta Ct method (Livak and Schmittgen, 2001). The primers sequences used are listed in Table 1.

2.4. SDS and Western blotting

Tissue samples from left ventricles were snap-frozen in liquid nitrogen and homogenised in buffer containing 10 mM EDTA-Na, 10% sodium dodecylsulphate and 1 mM phenylmethylsulfonyl fluoride, as described previously (Kucerova et al., 2015). Each sample was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis on a 4–12% gel under reducing conditions and transferred to polyvinylidene fluoride (PDVF) transfer membrane (Immobilon-P, Millipore Corp., Billerica, MA, USA). After blocking, membranes were incubated with antibodies against the following proteins: eNOS, iNOS, hsp90, cav-3, cav-1 and gp91phox (BD Transduction Laboratories, Franklin Lakes, NJ, USA). β-actin (Sigma-Aldrich, Saint Louis, MO, USA) was used as a loading control. Immunoreactive proteins were detected by chemiluminescent detection (ECL Plus, Amersham, Buckinghamshire, UK).

2.5. Statistical analysis

All data shown are expressed as average ± standard error of mean (S.E.M.). Real-time PCR data were analysed by the published method (Livak and Schmittgen, 2001). Kruskal–Wallis test was used to determine statistical significance. Values $P < 0.05$ were considered significant. The data were analysed by GraphPad Prism 4 for Windows (GraphPad Software, Inc., version 4.00).

3. Results

3.1. Ramipril restored clinical features only in moderate daunorubicin cardiomyopathy

We observed pronounced differences between the two doses of daunorubicin. While the lower dose of daunorubicin induced only a moderate cardiomyopathy, the higher dose was associated with a severe cardiomyopathy and consequent clinical manifestations. Only 65% of D15 group survived for 8 weeks until hemodynamic