Vascular Pharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Vascular Pharmacology

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

Exaggerated hypertensive response to combretastatin A-4 phosphate in hypertensive rats: Effective pharmacological inhibition by diltiazem

Qingen Ke [a,1], Mohammed A. Samad [a,1], Soochan Bae [a], David J. Chaplin [b], Peter M. Kang [a,*]

[a] Cardiovascular Institute, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA
[b] OXiGENE, Inc., South San Francisco, CA, USA

ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form 8 May 2015
Accepted 9 May 2015
Available online xxxx

Keywords:
Anticancer agents
Blood pressure
Calcium channel blockers
Cardiac toxicity

ABSTRACT

Combretastatin A-4 phosphate (CA4P), a tubulin depolymerizing agent, shows promise in anti-cancer therapy and is associated with dose-dependent transient hypertension. The cardiac consequence of this hypertensive effect is unknown. This study was conducted to examine the cardiotoxic effect of CA4P on a rat model of hypertension. Hypertensive rats were created by feeding a 6% high salt (HS) diet to Dahl salt sensitive (DSS) rats for 2.5 weeks. Cardiac toxicity was measured using serum troponin I levels 24 h after CA4P administration. In rats fed HS diet, there was a significant increase in mean arterial blood pressure (MAP) from baseline, which was further increased by 80% following CA4P administration with peak systolic blood pressure (BP) of 247 mm Hg. Treatment with the calcium channel blockers, diltiazem and nicardipine, completely inhibited the hypertensive effects of CA4P. Nitroglycerin or enalapril, however, failed to completely block the hypertensive effects of CA4P. CA4P injection also significantly increased the cardiac troponin I level in hypertensive rats though pretreatment with diltiazem effectively blocked troponin I increase after CA4P administration. Based on these findings, an exaggerated hypertensive response to CA4P is associated with myocardial damage in hypertensive rats. Calcium channel blockers effectively blocked both CA4P induced hypertension and cardiac damage.

1. Introduction

Combretastatins are a class of tubulin depolymerizing agents isolated from the South African willow tree *Combretum caffrum* [1–3]. Combretastatin-A4 phosphate (CA4P), the most potent of this class, is being investigated in clinical studies to treat advanced cancers [4–9]. Despite its promise, CA4P has been associated with transient hypertension in animal models [4,10–14] and patients enrolled in clinical trials have developed significant but transient increases in blood pressure (BP) [5,8,15–17]. CA4P treatment resulted in a concentration dependent increase in BP without significant myocardial damage in healthy rats, and the hypertensive effect of CA4P was effectively blocked by both nitroglycerin and diltiazem [12]. However, despite the fact that healthy patients and animals appear to tolerate this transient increase in BP without significant myocardial damage, most of the clinical trials administering CA4P target elderly cancer patients with multiple underlying medical conditions, such as coronary artery disease and hypertension. In fact, although rare, some patients have developed acute coronary syndromes as a result of CA4P administration [18–20]. Thus, the clinically relevant issue of potential cardiotoxicity due to CA4P-induced hypertension may not be represented by the normal healthy rat model. In this study, we examined potential cardiotoxicity and hypertensive effects of CA4P in moderately hypertensive rats, and examined the effect of CA4P-induced hypertension in these rat hearts. In addition, we studied to identify the most effective pharmacological inhibition of CA4P-induced hypertension in these rats.

2. Materials and methods

2.1. Materials

Chemicals were obtained from following sources: D-cis-diltiazem, enalapril and nitroglycerin (Sigma-Aldrich, St. Louis, MO). CA4P was provided by Oxigene. CA4P was stored and protected from light.

2.2. Animal model

Pathological cardiac hypertrophy was generated in female Dahl salt sensitive (DSS) rats (Harlan Sprague Dawley, Indianapolis, IN) as described previously by our laboratory [21–23]. Control group was age-matched DSS females rats fed normal rat chow. All animals

Abbreviations: ACEI, angiotensin converting enzyme inhibitor; CA4P, Combretastatin A-4 phosphate; DSS, Dahl salt sensitive; HS, high salt; MAP, mean arterial blood pressure; ND, normal low salt diet.

* Corresponding author at: Cardiovascular Institute, Beth Israel Deaconess Medical Center, 3 Blackfan Circle, CLS 910, Boston, MA 02215, USA. Tel.: +1 617 735 4290; fax: +1 617 735 4202.
E-mail address: pkang@bidmc.harvard.edu (P.M. Kang).

[1] Contributed equally to the study.

http://dx.doi.org/10.1016/j.vph.2015.05.004
1537-1891/

were housed in pathogen-free conditions at 20 °C and a reverse daily 12:12 h light:dark cycle. The animal care standards were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and all experimental procedures were carried out with approval from the Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center.

2.3. In vivo hemodynamic measurements

In vivo hemodynamic measurements were performed as described previously [12]. Animals were anesthetized with intraperitoneal (*i.p.*) ketamine/xylazine, stabilized in a supine position, and the right common carotid artery surgically exposed and isolated by blunt dissection between the sternohyoid, sternomastoid and omohyoid muscles. An occluding ligature was placed on the artery as far anterior as possible and a loose posterior ligature placed approximately 5 mm away. A small clamp (micro aneurysm clip, 6 mm in length) was placed on the most distal portion of the carotid artery to stop the flow of blood. A small incision was made using microscissors for insertion of a 1.4-Fr high-fidelity pressure catheter (SPR-671, Millar catheters). Then, the posterior ligature was loosely tightened around the artery and the catheter so that the clamp could be removed safely and as the inserted catheter was advanced into the aorta. The catheter was secured in place with 5-0 silk suture and arterial blood pressure (AP) measured. The catheter was calibrated before each experiment. Heart rate and AP were recorded at 2 kHz for 60 min and analyzed using a built-in analytic program in PowerLab software Chart 5 (ADInstruments, CO). After recording, the rat was euthanized by an overdose of Ketamine/Xylazine (100 mg/mL, 90 mg of ketamine and 10 mg of xylazine) given at 0.2 mL/100 gm.

2.4. Serum troponin I measurement

To check for myocardial damage, serum levels of troponin I were measured. Tail vein blood was collected at baseline (before medication) and 24 h after CA4P *i.p.* administration. Serum troponin I levels were measured using Rat Troponin I ELISA kit (Assaypro LLC, MO) according to manufacturer instructions.

2.5. Drug administration

After stabilization of BP, 3, 10 or 30 mg/kg CA4P was administered by *i.p.* injection. Constant intravenous (*i.v.*) infusion of anti-hypertensive drugs was achieved using a microinfusion pump via a cannulated jugular vein. An anesthetized and surgically prepared animal was positioned under a dissecting scope in dorsal recumbency. A 2 cm ventral cervical skin incision was made right of the midline with its caudal terminus at the level of the clavicle. Underlying salivary and lymphatic tissues were separated by means of blunt dissection to visualize the right common jugular vein. A sterile i.v. catheter line was inserted into the vessel and secured in place with suture.

2.6. Statistics

All data were expressed as mean ± SEM. Between groups and among groups comparisons were conducted with unpaired Student *t* tests and one-way ANOVA analyses, respectively. Probability (p) values of <0.05 were considered significant.

3. Results

3.1. Hypertensive effects of CA4P were exaggerated in DSS rats fed a high-salt (HS) diet

The DSS rat is previously characterized HS diet-induced model of hypertension and heart failure [21–23]. At the age of 6 weeks MAP of DSS rats averaged 79 mmHg. When fed a HS diet (6% NaCl), MAP gradually increased over time to 165 mm Hg by week 12 (6 weeks of HS diet) (Fig. 1A). In addition, by the age of 12 weeks, we have previously demonstrated that animals fed the HS diet develop significant cardiac hypertrophy [22–24]. We first tested the effect of CA4P at different time points to determine the duration of HS diet needed to observe a potential pathological response to CA4P—one that might mimic what is observed in a clinical setting with patients receiving CA4P. We first tested rats fed a HS diet for 2 weeks, 2.5 weeks (18 days), and 3 weeks. There were progressive increases in MAP corresponding to the duration of HS diet (Fig. 1B). Those fed a HS diet for 2 weeks, however, developed a non-significant increase in BP compared to the baseline. Those rats fed a HS diet for 2.5 or 3 weeks showed a significant increase in BP compared to the baseline. After 2.5 weeks on a HS diet, the BP of DSS rats was significantly increased to 106 ± 2.6 mm Hg compared with those fed a normal diet (ND) (79 ± 2.6 mm Hg) without any mortality. However, a preliminary study demonstrated an increase in mortality rate after 30 mg/kg of CA4P (~20%), administration in the 3-week group (data not shown). Therefore, we chose 2.5-week-HS diet group, which showed a significant increase in BP compared to the ND diet group without significant mortality after high dose CA4P infusion, for further experiments.

These hypertensive rats fed 2.5 weeks of HS diet were randomly divided to receive either vehicle or CA4P by *i.p.* injection. In normotensive rats, there was a significant 44% increase in MAP after 30 mg/kg injection of CA4P (Fig. 2A and B). In hypertensive rats, 3 and 10 mg/kg CA4P resulted in significantly exaggerated MAP increases of 53% and 55% from the hypertensive baseline (data not shown). In fact, the MAP of DSS rats was elevated from 106 ± 2.8 to 193 ± 5.6 mm Hg (86%) after *i.p.* injection of 30 mg/kg CA4P (Fig. 2E). The pressure amplitude in this group also increased from 46 ± 1.9 mm Hg to 82 ± 4.3 mm Hg with a dramatic peak systolic pressure of 247 ± 3.8 mm Hg (Fig. 2D).

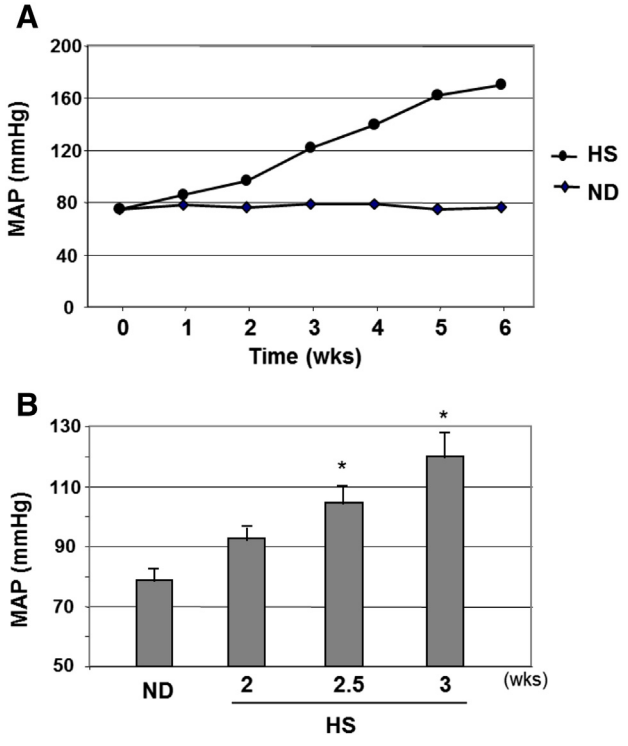

Fig. 1. Hypertensive DSS rat model. A. The time-dependent elevation of mean arterial pressure (MAP) in normal diet (ND) and high salt (HS) diet DSS rats. B. Baseline MAP after specific duration of HS diet. Control rats were fed a normal diet for 2.5 weeks. N = 3-4 animals. * $p < 0.05$ compared to the ND group.