Phytomedicine 20 (2013) 1048–1054

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Effects of imperatorin, the active component from Radix Angelicae (Baizhi), on the blood pressure and oxidative stress in 2K,1C hypertensive rats

Yan-Jun Cao, Xu He, Nan Wang, Lang-Chong He*

School of Medicine, Xi'an Jiaotong University, Xi'an 710061, PR China

ARTICLE INFO

Keywords:
Angelica dahurica
Imperatorin (IMP)
Oxidase stress
NADPH oxidase
2K,1C hypertension

ABSTRACT

The 2-kidney, 1-clip (2K,1C) model of hypertension was used to investigate the potential antihypertensive and antioxidant effect of imperatorin extracted from the root of radix angelicae. After 10 weeks treatment of imperatorin, mean blood pressure (MBP) of 2K,1C hypertensive rats was obtained, and superoxide dismutase (SOD), nitric oxide (NO) and nitric oxide synthase (NOS) were measured. Malondialdehyde (MDA) and glutathione (GSH) levels, catalase (CATA), xanthine oxidase (XOD), angiotensinII (Ang II) and endothelin (ET) levels of kidney were evaluated with commercial kits. Nicotinamide adenine dinucleotidephosphate (NADPH) oxidase subunits of the renal cortial tissues were determined by RT-PCR and Western blot. 8-Iso-prostaglandin F2α (8-iso-PGF2α) of 24 h urinary excretion was also measured by ELISA. MBP was significantly reduced by treatment with IMP (6.25, 12.5 and 25 mg/kg/day, i.g.) in 2K,1C hypertensive rats. Meanwhile, we found that renal CATA and XOD activities, GSH levels, plasma NO and NOS contents were significantly increased in IMP-treated groups. Plasma ET, renal Ang II levels, MDA and the 24 h urinary excretion of 8-iso-PGF2α in the IMP treated group were lower than control SD group. After that, we found the mRNA expressions and protein levels of NADPH oxidase subunits in the clipped kidney were markedly reduced after IMP treated in 2K,1C hypertensive rats. IMP showed antihypertensive and antioxidant effects in the renal injury of renovascular hypertensive rats, suggesting that IMP could be of therapeutic use in preventing renal injury related hypertension.

Introduction

In recent years, attention of drug research had focused on the role of oxidative stress in the pathophysiology of hypertension and cardiovascular disease. Oxidative stress was caused by reactive oxygen species (ROS), which was an important mediator of the progression of renal injury in different animal models of hypertension (Datla and Griendling 2010). ROS were generated by normal respiration of cells and by xanthine oxidase (XOD), nicotinamide adenine dinucleotidephosphate (NADPH) oxidase, glucose oxidase, cyclooxygenase (COX), and nitric oxide synthase (NOS), all of which were found in the hypertension (Nishiyama et al. 2004; Mansour et al., 2011). Under pathological conditions, increased ROS bioactivity lead to endothelial dysfunction, increased contractility, vascular smooth muscle cell (VSMC) growth, lipid peroxidation and increased deposition of extracellular matrix proteins, which were important factors in hypertensive vascular and renal damage (Diep et al. 2002). If not kept in balance by naturally occurring enzymes such as SOD and CATA, ROS can oxidize and destroy proteins (Cowley 2008), membrane lipids, and nucleic acids, diminish the biological half-life of nitric oxide, and generate new vasoconstrictors such as 8-ISO (Bowers et al. 2005). The major source of ROS in the kidney was elevated NADPH oxidase (Cowley 2008). An inducible model of high blood pressure was the 2-kidney, 1-clip (2K,1C) hypertension model, where the increased blood pressure was induced after clipping of one renal artery. Long-term 2K,1C hypertension was followed by organ damage enlarged heart and damage of the nonclipped kidney with increased urinary protein excretion and declining glomerular filtration rate (Bivol et al. 2005; Helle et al. 2009; Iversen et al. 1983).

A growing number of newly discovered drugs were produced from simple plant and mineral sources (De Souza et al., 2011; Huang and Chen, 2013; Somova et al., 2003; Takei et al., 2004). The enormous growth in the market for herbal medicinal products over the last few years has been one of the most interesting aspects of healthcare in the developed world. Many herbal drugs based on ethnomedicinal use were used traditionally in Chinese medicine

Abbreviations: MBP, mean blood pressure; BP, bloodpressure; SOD, superoxide dismutase; NO, nitric oxide; NOS, nitric oxide synthase; MDA, malondialdehyde; GSH, glutathione; CATA, catalase; XOD, xanthine oxidase; AngII, angiotensinII; ET, endothelin; NADPH, nicotinamide adenine dinucleotidephosphate; 8-Iso-PGF2α, 8-iso-prostaglandin F2α; RHR, renovascular-hypertensive rats, two kidneys one clip model; SD rats, Sprague-Dawley rats.

* Corresponding author. Tel.: +86 29 82656264; fax: +86 2982655451.
E-mail address: helc@mail.xjtu.edu.cn (L.-C. He).

0944-7113/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.phymed.2013.04.021

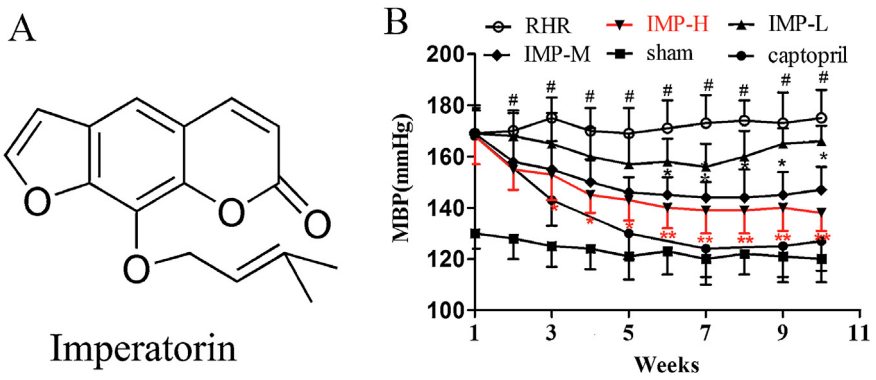

Fig. 1. Chemical structure and antihypertensive effects of IMP in 2K,1C renovascular hypertensive rats. (A) Chemical structure of IMP. (B) Effects of IMP on the MBP. IMP-H (▼), IMP-M (□), IMP-L (▲), Captopril (●), RHR group (○), sham (■).*$p < 0.05$, **$p < 0.01$, compared with RHR group. #$p < 0.05$, compared with sham group.

for the treatment of conditions such as myocardial hypertrophy, antioxidant, anti-tumor, ventricular remodeling, heart disease and hypertension (Huang et al., 2012; Somova et al., 2003; Zhang et al. 2011). IMP (9-(3-methylbut-2-enyloxy)-7H-furo [3,2-g] chromen-7-one, Imp; Fig. 1A), a dietary furocoumarin, was wide spread and found not only in the medicinal plant such as *Cnidium monnieri (L) cusson and Angelica dahurica (Fisch. ex Hoffm) Benth, et Hook. f* (Baek et al. 2000; Konga et al. 1986), but also in popular culinary herbs such as *parsley*, and *fennel*. IMP had been shown to reduce blood pressure as calcium antagonist and inhibit myocardial hypertrophy in spontaneously hypertensive rats (SHR) (Zhang et al. 2011, 2010a,b). Despite a growing understanding of the mechanisms by which imperatorin acted on the hypertension, the effects on oxidative stress and blood pressure in 2K,1C hypertensive rats were not reported.

The aim of the present study was to determine whether long-term IMP administration ameliorate the blood pressure and oxidative stress in 2K,1C hypertensive rats. Blood pressure, renal excretory function and excretion of 8-iso-PGF2α in SD and 2K,1C hypertensive rats would be evaluated after 10 weeks IMP administration. Then, to discover the possible contribution of IMP to ROS generation, and to clear the effects of IMP on membrane components of NADPH oxidase, we also measured renal cortical mRNA expressions of p22phox, p47phox, p67phox, gp91phox and proteins of p47phox, gp91phox.

Materials and methods

Drugs and chemicals

Imperatorin (purity ≧98% by high-performance liquid chromatography and thin-layer chromatography) was from the supercritical fluid extraction of C. monnieri Cuss, and purificated by ethanol recrystallization as patent described (patent number: ZL200610042997.2, China). Captopril was purchased from Changzhou Pharmaceutical Company Limited (Changzhou, China). The NOS, NO, SOD, MDA, XOD, GSH, CATA and proteinuria assay kits were all purchased from the Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China). Enzyme linked immunosorbent assay (ELISA) kits for 8-iso-PGF2α was from Cayman Chemical Company (Ann Arbor, MI, USA). Antibodies for p47phox and GAPDH were purchased from Santa Cruz Biotechnology (California, USA) and gp91phox was purchase from Epitomics (California, USA). ECL reagent was from Pierce (IL, USA). IMP was dissolved in 0.5% sodium carboxymethyl cellulose.

Surgical preparation for chronic study

All the experimental procedures and protocols on animals were approved by the Ethical Committee of Xi'an Jiaotong University for Animal Research. Young male Sprague-Dawley rats (200–220 g) were anesthetized with pentobarbital sodium (2%). A silver clip (0.2 mm) was placed around the left renal artery (2K,1C). Sham operated rats (sham) were prepared similarly without clip placement, and all surgeries were performed under aseptic conditions. At the end of 3 weeks, only animals showing definite hypertension, i.e. SBP > 140 mmHg were selected for further studies (Mansour et al., 2011). Treatment with IMP (or vehicle) was maintained for 10 weeks. The rats were randomly divided into seven groups ($n = 8$/group), named: sham (sham surgery-treated with vehicle), RHR (2K,1C surgery-treated with vehicle), IMP-H (2K,1C surgery-treated with imperatorin, 25 mg/kg/day), IMP-M (2K,1C surgery-treated with imperatorin, 12.5 mg/kg/day), IMP-L (2K,1C surgery-treated with imperatorin, 6.25 mg/kg/day) and Cap (2K,1C surgery-treated with captopril, 5 mg/kg/day), SD (the SD rats without any surgery-treated with vehicle) were treated for 10 weeks with a daily gavage of vehicle (0.5% sodium carboxymethyl cellulose as placebo), imperatorin or captopril. The rats were housed ($n = 8$ per cage) in room with a relative humility of 50% and a 12/12 h light/dark cycle at 22–24 °C, and had unrestricted access to standard rodent chow and water.

Body weight and tail mean blood pressure (MBP) was assessed weekly by tail-cuff plethysmography (Coda-VPR, Kent Scientific, Torrington, CT, USA). To minimize the effects of stress induced by this method on blood pressure measurement, the animals were trained for a week before drug administrations. 24 h urine samples and blood were harvested at the end of 10 weeks. After decapitation, the kidneys were removed, snap-frozen in liquid nitrogen, and stored at −80 °C until processing for protein or mRNA extraction.

Measurement of 8-iso-PGF2α and proteinuria

After 10 weeks of drug administration, the rats were placed in metabolic cages for a 24 h urine collection. Urine was collected in containers with 10 μl of 2 mmol/l EDTA to prevent ex vivo production of 8-iso-PGF2α. Urine was centrifuged at 1000 × *g* for 10 min at 4 °C and stored in aliquots at −80 °C until assayed (Schnackenberg and Wilcox 1999). Whole-body oxidative stress was assessed from the excretion of 8-iso-PGF2α. 8-Iso-PGF2α was measured by ELISA assay kit on extracted 24 h urine samples. Proteinuria was also measured by chemical assay kits on extracted 24 h urine.