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Research Article

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Protective effects of graph peel extracts on cardiac hypertrophy in 2-kidney/1-clip hypertensive rats

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ABSTRACT

Aim of this study was investigate to protective effects on cardiac hypertrophy by graph peel extracts (GP) in 2-kidney/1-clip (2K1C) hypertensive rats model. For this purpose we constructed 2K1C rat models and orally administrated GP two times per day for 7 weeks. For determine of anti-hypertensive effects, we measured blood pressure (BP) by tail cuff methods. At the first weeks, observed no changes of BP in all groups, At the 4 and 7 weeks, BP was markedly elevated on 2K1C group to 30 and 50% than sham control group, respectively. Whereas, sham + GP group not observed changes of BP. In 2K1C + GP administrated group, BP was reduced in dose dependent manner but not significant. In contrast, administration of GP was induced reducing of fibronectin expression on cardiac muscle tissue and then suggested cardioprotective effect. These results suggested that graph peel extracts have anti-hypertensive function through reducing of hypertrophy marker on hypertension and its complications.

Keywords: 2-kidney/1-clip (2K1C), cardiac hypertrophy, hypertension, blood pressure, fibronectin, graph

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INTRODUCTION

The renin angiotensin system (RAS) play a key role in the regulation of blood pressure in growth of the cardiac muscle[1].RAS is also involved in the homeostasis of body fluid and sodium balance by the angiotensin II (Angotensin II), the vasoactive peptide. Increase of serum concentration of angiotensin II leads to a increase in blood pressure by temporary vasoconstriction, and involved in cell growth, motility, changes of cellular membrane, and inflammatory[2]. Effect on cells of angiotensin II is known to be via the G-protein coupled receptor, and reported various signaling molecules such as Src, phosphatidylinositol-3-kinase (PI3K)/Akt, phospholipase C (PLC), inositol triphosphate (IP_3), and cADPR formation by ADPR-cyclase activation [3,4]. The abnormal angiotensin II-induced hypertension are activates T lymphocytes in the blood and its ADPR-cyclase is activated. In other report, cardiac hypertrophy promoted by increase of intracellular cADPR formation and sequential increase of intracellular calcium mobilization. rdiac hypertrophy[2,5]. Chronic sustained hypertension was induced cardiac hypertrophy and initially looks like a normal cardiac function, but leads to increases of afterload in heart and blood vessel. Thereafter, pathologic changes shown in cardiac muscle tissue and may progress to functional damage to the heart[6].

Grapes have been reported to be effective in prevention and ameliorates materials in cardiovascular disease, including atherosclerosis in many countries as well as South Korea. In particular, well known components including grape polyphenols such as anthocyanin, procyanidin, resveratrol has been reported about s having efficacy against various diseases like metabolic disorder. [7]. Resveratrol is contained in the grape peels, and reported that inhibition of lipid peroxidation which are the cause of cancer. Indeed, function of resveratrol reported to scavenging of free radicals, anti-inflammation, and the inhibition of cancer growth [8-12].Anti-oxidative effects of polyphenols reported that prevention of cardiac hypertrophy, anti-proliferative effects of vascular smooth muscle cells and kidney messangial cells[13-15]. In many reports, anti-oxidative effects by extracts of grape seeds [16-19], grape tree

leafs[20,21], and whole grapes [22-24]. However, they used the whole grape extracts for functional assessment and not reported effects of grape peel extract in hypertension animal model, especially 2-kidney/1-clip rats (2K1C) model. In present study, we selectively collected only the grape peels to obtain a hot water extracts, and the effect on cardiac hypertrophy was identified and suggested the mechanism and its use.

EXPERIMENTAL SECTION

2.1. Animals

Male Sprague-Dawley rats (8 weeks) were purchased from Orient Bio (Seong-Nam, Korea). Animals were acclimated to the facility for 5 d and fed and ad libitum with regular chow. Animal Research Committee of Chonnam National University approved the animal study in accordance with the guidelines of the National Institutes of Health (NIH publication #85-23, 1985).

2.2. Preparation of grape peel extracts and administration

Grape was purchased from downtown market in Jeonju, Korea. Grape peels obtained under aseptic condition and 100 g of grape peels were grinded with dry ice. Then the homogenates were mixed with cold distilled water (1,000 ml) and extracted at 80 ℃ for 3 h. The crude extracts were centrifuged in 15,000 ×g at 4℃ for 20 min and supernatants were freeze dried. Dried powder was stored in -80℃ until use and re-suspended extracts with phosphate-buffered saline (PBS, pH 7.4) were filtrated before use. The schematic diagram of animal experiments shown in Fig 1.

2.3. Surgical processes

Rats were anaesthetized with ketamine (100 mg/kg, intraperitoneally) and xylazine (5 mg/kg, intraperitoneally). The left kidney was exposed through the median abdominal incision, and the renal artery was separated from the renal vein with caution. Then, a silver clip with 0.15 mm slit was placed around the renal artery. The sham procedure was performed, including the entire surgery with an exception of arterial clipping. To examine the effect of extracts in the 2K1C model, we orally administrated for 7 weeks. Sham group and Control 2K1C received vehicle treatment.

2.4. Blood pressure measurement

Animals were maintained for indicated times and end-systolic blood pressure was measured by tail cuff plethysmography (Power Lab 2/20, AD instruments, Australia). The blood pressure measurements required restraint during the tail-cuff procedure and measured at every week.

2.5. Immunohistochemistry.

Tissue sections (4 μm) were deparaffinized and rehydrated. Antigen retrieval was performed by incubation in target retrieval solution (DakoCytomation, Carpinteria, CA) at 95°C for 20 min. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 min, and slides were rinsed with TTBS. Sections were blocked with the appropriate preimmune serum and then incubated with avidin/biotin blocking solutions (DakoCytomation). Slides were then incubated overnight at 4°C with a primary antibody for fibronectin (1:100, Goat anti-fibronectin antibody; SantaCruz, USA). The stained sections were then incubated with secondary antibody (anti-goat IgG-HRP conjuagated, Sigma-Aldrich, USA), and theimmune complexes were detected using a chloronaphthol - diaminobenzidine (CN-DAB, Advanced Biochemicals Inc. Korea) with 0.03% hydrogen peroxide. The sections were finally counterstained with hematoxylin solution (Dako Cytomation) before being mounted and obtained images by DC200 digital camera with microscope (Leica, Germany)

2.6. Statistical analysis

Data are expressed as mean±S.E.M. Statistical comparisons were performed using oneway ANOVA. Significant differences between groups were determined using the Student's *t* test. Statistical significance was set at *P*/0.05.

RESULTS

3.1. Effects of grape peels extracts on blood pressure in 2K1C hypertensive rats

To identify whether the extracts have any physiological relevance, we investigated the effects of grape peel extracts, on an Ang II-dependent model of hypertension (2K1C) with fixed schedule (Fig.1). In the untreated 2K1C rats, hypertension was established in 1–2 weeks after clipping process. The extracts was administered for 7 weeks to both sham-operated and 2K1C rats 1 week after the surgery. As a results, no significant relevance shown in both group at 1 weeks (Fig. 2A and B).

Figure 1. Time schedules of animal experiments

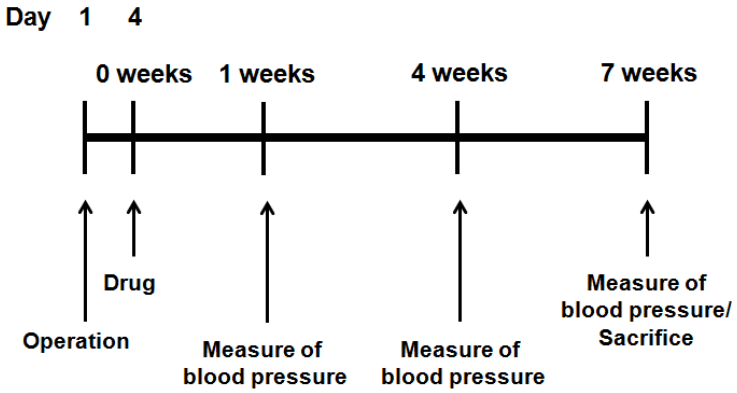
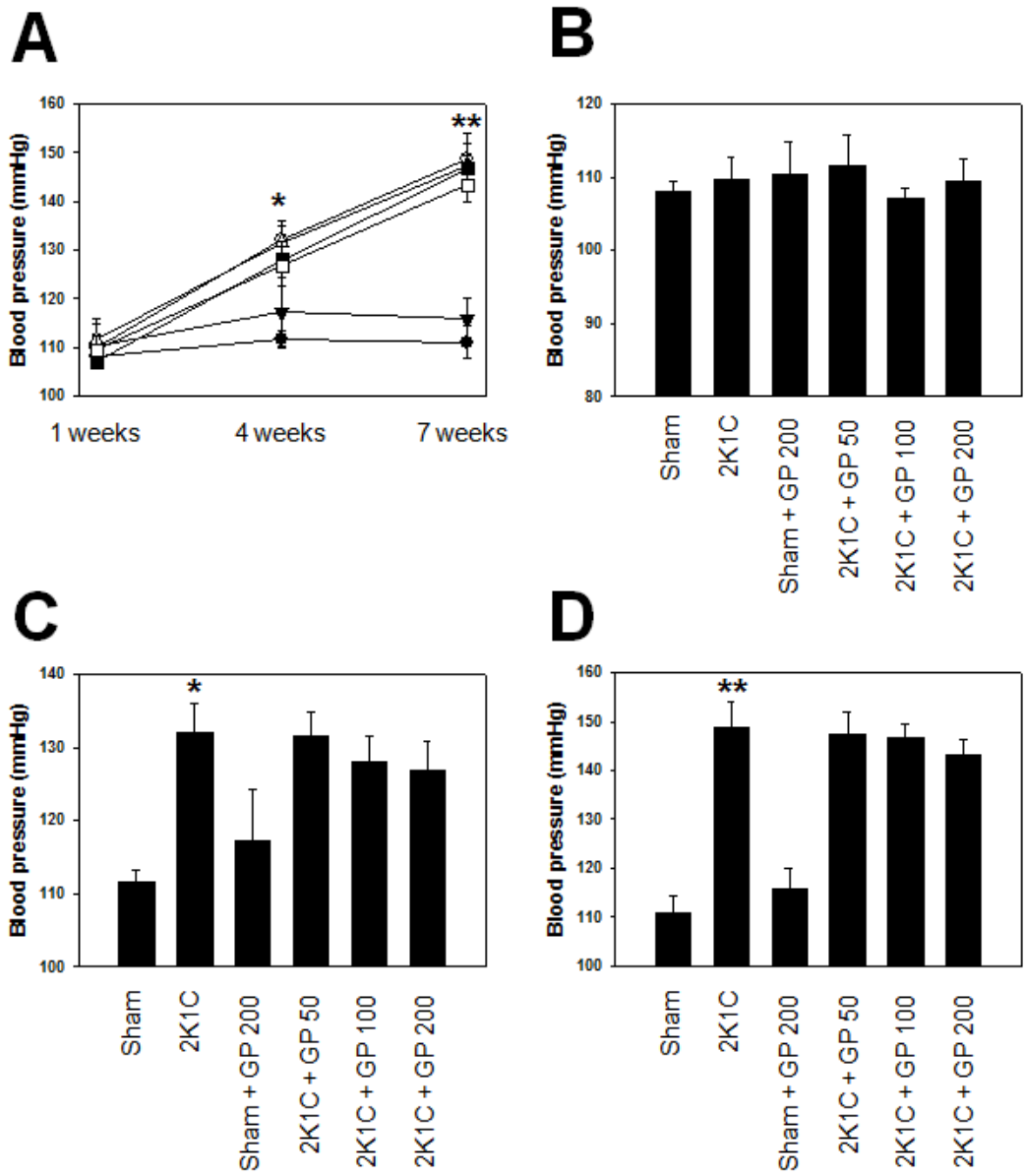


Figure 2. Effects of grape peel extracts on 2-kidney/1-clip (2K1C) rats. Blood pressures were measured at the 1, 4, and 7 weeks after surgical operation. (A) Changes of blood pressure at 1, 4 and 7 weeks displayed with line/scatter graph, and compared each group at (B) 1 weeks, (C) 4 weeks and (D) 7 weeks. Data are expressed as mean ± S.E.M. n=4 of each group. *p<0.01 and **p<0.001 vs sham group. GP : grape peel extracts

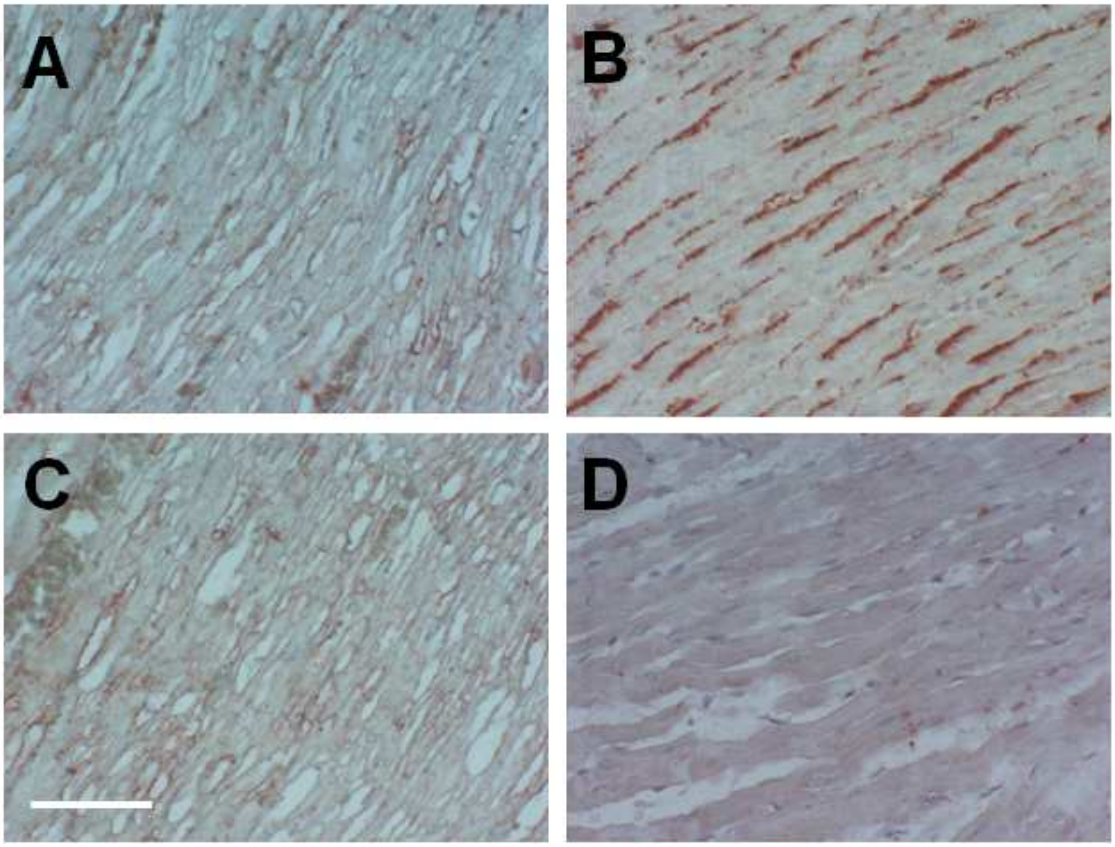


Seven weeks after clipping, the systolic blood pressure in the vehicle-treated 2K1C rats was significantly increased(Fig. 2A, C, and D), whereas the systolic blood pressure in the extracts-treated 2K1C rats was slightly reduced at 200 mg/kg treat group but not significant. Although no significant difference in the systolic blood pressure was observed in 2K1C rats treated with low and high dose ofextracts compared with vehicle-treated 2K1C rats (Fig. 2D), this extract was not purified components and then very complexed form. Therefore, we should be solve this questions with purified substances in same experimental model in further study.

3.2. Effects of grape peels extracts on cardiac fibronectin expression in 2K1C hypertensive rats

To further evaluate the inhibitory effects of extracts on cardiac hypertrophy, fibronectin protein expression were measured as markers of cardiac hypertrophy. 2K1C rats showed an increase in fibronectin protein expression in cardiac tissues as compared with sham controls (Figure 3). On the other hand, high dose of extracts-treatment (200 mg/kg) blocked fibronectin protein expression in 2K1C rats. Indeed, this high dosage of was not induced any changes of fibronectin expression in sham control group (Fig. 3D).

Figure 3. Grape peel extracts reduced expression of myocardial fibronectin expression on 2-kidney/1-clip (2K1C) rats. The data shown are representative of the (A) Sham control, (B) 2K1C, (C) 2K1C + grape peel, (D) Sham + grape peel only. n=4 of each group. Scale = 100 μm



DISCUSSION

Hypertension is a well-known disease that progressed by genetics, obesity, over-nutrition, changes of lifestyle like Western diet in almost countries. This hypertension can accelerate by various abnormal mechanism such as abnormal calcium signaling in kidney, mesangial cell proliferation, increases of angiotensin II production[1-4]. To care of hypertension, calcium channel blocker (CCB), Angiotensin receptor blockers (ARB), Angiotensin converting enzyme inhibitors (ACEi) were reported in medicinal purpose and can choice followed by severity, susceptibility of patients etc.

Cardiac hypertrophy is major complication of hypertension and it is different to hyperplasia. Ang II was rapidly induces intracellular calcium mobilization and ADPR-cyclase activation in cardiomyocyte and mesangial cells. Ang II-induced calcium mobilization was completely blocked by pretreatment of cardiomyocytes with a cADPR antagonist, 8-Br-cADPR. These reports indicated that the Ang II-induced sustained calcium signal in cardiomyocytes was mediated by cADPR[3,4,25].

In 2K1C hypertensive animal model, fibronectin, extracellular matrix protein, is primary marker and particularly expressed in cardiomyocyte [26,27]. In other reports, mRNA level of fibronectin was increased in L-triiodothyronine-induced hypertrophy model experiments [28], and involved in fibrosis of cardiomyocytes in hypertension model [29]. Then, several research group tried screening of novel drug candidate from natural products[30]

CONCLUSION

The grape peel extracts have ameliorative effects on hypertension and its complicationvia reduces of blood pressure and fibronectin expression in 2K1C rats model. These results suggest that these extracts may provide the tool for prevention and care to hypertension in medicinal/functional food purpose.

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REFERENCES

[1] KK Parsons;TM Coffman,*J. Clin. Invest*.,**2007**, 117, 873-876.
[2] BC Berk;K Fujiwara; S Lehoux, *J. Clin. Invest*.,**2007**, 17, 568-575.
[3] R Gul; JH Park;SY Kim;KY Jang;JK Chae*et al*., *Cardiovasc. Res*., **2009**, 81(3), 582-591.
[4] SY Rah;KH Park;MK Han;MJ Im;UH Kim,*J. Biol. Chem*. **2005**, 280, 2888-2895.
[5] JD Molkentin;JR Lu;CL Antos;B Markham;J Richardson *et al*., *Cell*,**1998**, 93, 215-228.
[6] DL Mann;MR Bristow,*Circulation*, **2005**, 111(21), 2837–2849.
[7] EN Frankel;J Kanner;JB German;E Parks; JE Kinsella,*Lancet*, **1993**,.341 (8843), 454-457.
[8] SK Lee;ZH Mbwambo;H Chung;L Luyengi;EJ Gamez *et al*., *Comb. Chem. High Throughput Screen*., **1998**, 1(1), 35-46.
[9] J Martinez;JJ Moreno, *Biochem. Pharmacol*., **2000**, 59(7), 865-70.
[10] AK Joe;H Liu; M Suzui; ME Vural;D Xiao*et al*., *Clin. Cancer Res*., **2002**, 8(3), 893-903.
[11] L Frémont,*Life Sci*., **2000**, 66(8), 663-673.
[12] KP Bhat;JM Pezzuto, *Ann. N.Y. Acad. Sci*., **2002**, 957, 210-229.
[13] HL Li;AB Wang;Y Huang; DP Liu;G Liu*et al*., *Free Radic. Biol. Med*.,**2005**, 38, 243-257.
[14] T Makino;T Ono;E Muso;H Yoshida;G Honda*et al*., *Nephrol. Dial. Transplant*., **2000**, 15, 1140-1145.
[15] K Iijima;M Yoshizumi;M Hashimoto;S Kim;M Eto*et al*., *Circulation*,**2000**, 101, 805-811.
[16] X Cui;X Liu;H Feng;S Zhao; H Gao, *Biol. Pharm. Bull*. **2012**, 35(12), 2192-2197.
[17] S Ulusoy;G Ozkan;FB Yucesan;Ş Ersöz;A Orem *et al*., *Nephrology (Carlton)*, **2012**, 17(4), 372-379.
[18] D Bagchi;CK Sen;SD Ray;DK Das; M Bagchi*et al*., *Mutat. Res*. **2003**, 523-524, 87-97.
[19] K Charradi; H Sebai; S Elkahoui; F Ben Hassine; F Limam*et al*., *Cardiovasc. Toxicol*. **2011**, 11, 28-37.
[20] JY Kim; HY Jeong; HK Lee; S Kim; BY Hwang *et al*., *Phytomedicine*,**2012**, 19(2), 150-159.
[21] L Sartor;E Pezzato;I Dell'Aica; R Caniato; S Bigginetal.*Biochem. Pharmacol*. **2002**, 64(2), 229-37.
[22] A Overman; A Bumrungpert; A Kennedy; K Martinez; CC Chuang *etal*., *Int. J. Obes*. (Lond) **2010**, 34(5), 800-808.
[23] CC Chuang; A Bumrungpert; A Kennedy; A Overman; T West*et al*., *J. Nutr. Biochem*. **2011**, 22(1), 89-94.
[24] M Belviranli; H Gökbel; N Okudan; S Büyükbaş, *Arch. Physiol. Biochem*., **2012**, 118(5), 237-243.
[25] TS Nam; SH Choi; SY Rah; SY Kim; WJ Kim*et al*., *Exp. Mol. Med*., **2006**, 38, 718-726.
[26] CG Brilla,*Curr. Opin. Cardiol*., **1994**, 9(suppl 1), S2–S10.
[27] KT Weber;JS Janicki; R Pick; J Capasso; P Anversa, *Am. J. Cardiol*.,**1990**, 65, 1G–7G.
[28] WS Mamuya; P Brecher;*J. Clin. Invest*.,**1992**, 89(2), 392–401.
[29] S Rosenkranz, *Cardiovasc. Res*., **2004**, 63(3), 423-432.
[30] HS Hwang;MO Boluyt; K Converso; MW Russell; BE Bleske, *Pharmacotherapy*, **2009**, 29(6), 639-648.