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ORIGINAL ARTICLE

Antihypertensive effects of *Ocimum basilicum* L. (OBL) on blood pressure in renovascular hypertensive rats

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Ocimum basilicum **L. (OBL), sweet basil, is a medicinal herb used in traditional Chinese medicine to treat cardiovascular diseases including hypertension. The objective of the study was to investigate the possible antihypertensive effects of OBL extract in renovascular hypertensive rats. The two-kidney one-clip (2K1C) Goldblatt model of renovascular hypertension was used in Wistar rats. Rats were randomized into sham, untreated 2K1C, captopril- (30 mg kg^{-1} per day orally) and OBL- (100, 200, 400 mg kg^{-1} per day orally) (low (L)-, medium (M)-, high (H)-OBL) treated 2K1C groups (n=10–12 per group), followed up for 4 weeks. Blood pressure, heart weight/body weight, plasma angiotensin-II and endothelin (ET)-1 were studied. OBL reduced systolic and diastolic blood pressure by about 20 and 15 mm Hg, respectively, compared with 35 and 22 mm Hg for captopril, from the lowest dose tested with no dose dependency. Cardiac hypertrophy was reduced from 3.6 ± 0.7 mg g^{-1} for untreated 2K1C to 3.0 ± 0.6, 2.9 ± 0.6 and 2.4 ± 0.4 mg g^{-1} for L-, M- and H-OBL, respectively, compared with 2.6 ± 0.5 for sham and 3.1 ± 0.4 mg g^{-1} for captopril (P<0.05). Renal function was improved with captopril. Angiotensin was reduced to a lesser extent than with captopril. ET was reduced to lower concentrations (78 ± 15, 80 ± 22, 82 ± 15 pg ml^{-1} for L-, M-, H-OBL, respectively) than in sham (116 ± 31 pg ml^{-1}), untreated 2K1C (174 ± 72 pg ml^{-1}) or captopril (117 ± 72 pg ml^{-1}) groups. The effects of OBL on blood pressure, cardiac hypertrophy and ET, are consistent with an effect on ET-converting enzyme, and warrant further exploration.**
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Keywords: angiotensin-II; endothelin-1; left ventricular hypertrophy; *Ocimum basilicum*; 2K1C experimental hypertension

INTRODUCTION

Ocimum basilicum L. (OBL), sweet basil, is used in traditional Asian medicine to treat chronic effects related to the cardiovascular system, in particular hypertension and coronary heart disease. Sweet basil is also a major constituent of traditional recipes in the Mediterranean diet, which has been associated with reduced cardiovascular morbidity.

We have shown in previous studies that OBL possesses an inhibitory effect on human platelet aggregation induced by adenosine diphosphate and thrombin, which is dose dependent and results in an antithrombotic effect *in vivo*.[1] It also has effects on experimental hyperlipidemia and cholesterol metabolism.[2,3] Hypertension is with dyslipidemia and platelet activation a major contributor to cardiovascular risk[4] with clinical consequences, such as heart failure, acute coronary syndrome and ischemic stroke.[5,6] It was therefore thought useful to test whether OBL also had any effects on blood pressure in a model of experimental hypertension and cardiac hypertrophy.

Of the various experimental or genetic models of hypertension, the Goldblatt chronic two-kidney, one-clip hypertension (2K1C) is a classical model of renovascular angiotensin-II-dependent hypertension,[7–10] which has been described as very close to human mature hypertension.[11,12] Drugs acting on the renin–angiotensin system are major factors in the treatment of hypertension. We therefore chose this model to investigate the effect of OBL on blood pressure.

METHODS

Renovascular hypertension

To induce hypertension according to the Goldblatt 2K1C model,[7–9,13] 200 ± 20 g male Sprague–Dawley rats were anesthetized with sodium pentobarbital (50 mg kg^{-1}, intraperitoneally). The left renal artery was exposed by retroperitoneal flank incision and dissected free of the renal vein and connective tissue. A silver clip with a lumen of 0.22 mm was placed around the artery for partial occlusion;[14] in sham operations, the artery was not clipped. The animals

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were kept under constant temperature (20 ± 1 °C) and illumination (12-h light, 12-h dark cycle) until the day of experiment, with free access to food and tap water.

After 6 weeks the systolic blood pressure (SBP) was measured using the tail-cuff method in conscious rats. Only hypertensive rats (SBP above 150 mm Hg) were used in the experiments.

Product tested

OBL is an aqueous extract of *Ocimum basilicum* prepared by the Phytochemistry Department of Xinjiang Medical University, Urumqi, Xinjiang, PR China. OBL was dissolved in 0.9% saline, to obtain a primary solution of 0.2 g l^{-1}. Yield for extract is 50 g dry extract per kg dried *Ocimum basilicum* leaves and stalks. The primary solution was diluted with 0.9% saline to obtain the various treatment solutions, administered under the same volume by daily gavage.

Treatment

At 6 weeks after the surgery, when hypertension was established, the 2K1C rats were divided into five groups of 10–12 rats: (1) an untreated model group, (2) a group treated with low-dose OBL (L, 100 mg kg^{-1} per day), (3) middle-dose OBL (M, 200 mg kg^{-1} per day), (4) high-dose OBL (H, 400 mg kg^{-1} per day), (5) a group treated with captopril group (30 mg kg^{-1} per day). In addition, a group of sham-operated control rats was also followed up. Rats were treated for 4 weeks with daily oral administration of the products or the same volume of vehicle (0.9% saline). Doses of OBL were chosen in reference to doses commonly used in man, and doses used in previous experiments. (1) All rats were weighed and their blood pressure measured once a week for 4 weeks.

Blood pressure measurement, angiotensin, endothelin and heart weight

SBP and diastolic blood pressure (DBP) were measured by the tail-cuff method (BP-6 noninvasive Electro-Sphygmomanometer, ChengduTaimeng Science and Technology, Chengdu, PR China) in awake rats. Each value was the average of three consecutive readings.

At the end of the experiment, after weighing and measurement of blood pressure, rats were anesthetized with sodium pentobarbital (30 mg kg^{-1}, intraperitoneally). Blood samples (8 ml) were taken from the abdominal aorta, handled as follows: 4 ml were collected on aprotinin (40 µl) and 10% EDTA disodium (30 µl), then centrifuged at 4 °C, at 3000 r.p.m. for 10 min, and the plasma retrieved and stored for determination of plasma angiotensin-II level using a commercial radioimmunnoassay kit purchased from Beijing North Institute of Biological Technology, Beijing, PR China.

In all, 4 ml were centrifuged at 4 °C, at 3500 r.p.m. for 10 min; resulting serum was used to measure endothelin (ET) concentrations using a commercial radioimmunnoassay kit purchased from the Beijing North Institute of Biological Technology, Beijing, PR China.

The heart was removed, blotted, weighed and stored at −20 °C.

All samples were stored at −20 °C until assays.

Renal function

The animals were placed in individual metabolic cages for 24-h urine collection. Creatinine and urea were measured with a commercial enzyme-linked immunosorbent assay (WAK Chemie, Bad Soden, Germany) as described by the manufacturer.

Statistical analysis

Results are expressed as means ± s.e.m. Two-way analysis of variance, followed by Bonferroni's *post hoc* test with adjustment for multiple comparisons, was used to test the significance of differences between groups. A value of $P<0.05$ was considered significant. The procedures were carried out with Statistica software (Stat Soft, Tulsa, OK, USA).

RESULTS

Blood pressure and cardiac hypertrophy

Untreated (model) renovascular hypertensive rats had markedly higher SBP and DBP than the sham controls (Table 1). This hypertension was stable over the 4 weeks of the experiment. In the captopril and OBL groups, the blood pressure, which was initially the same as that of the hypertensive controls, decreased progressively over the course of the treatment ($P<0.05$), without obvious difference between the OBL dose groups. Blood pressure was reduced by captopril to a greater extent than by any of the OBL doses, although it was still significantly greater than in sham-operated rats (Figure 1).

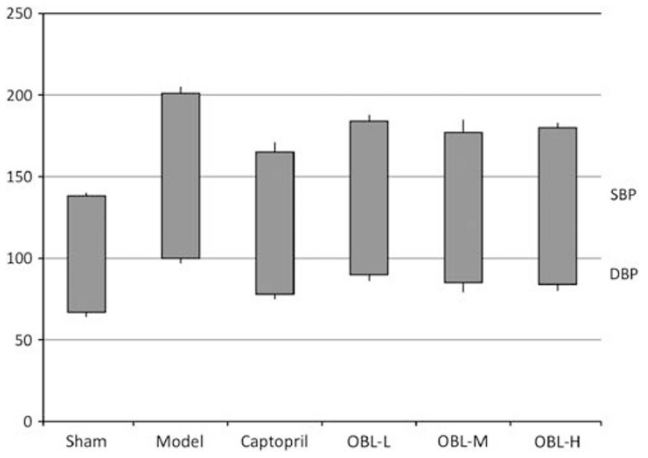

Figure 1 Effect of captopril and OBL at low, medium and high doses (OBL-L, OBL-M, OBL-H) on systolic (SBP) and diastolic (DBP) blood pressure in two-kidney, one-clip Goldblatt hypertensive rats, compared with sham-operated rats and untreated control (model) rats. $P<0.05$, all treated and model *vs.* sham; all treated *vs.* model.

Table 1 Cardiovascular parameters in renovascular hypertensive rats after 4 weeks of daily oral treatment with captopril (30 mg kg^{-1} per day), low-dose OBL (100 mg kg^{-1} per day) (OBL-L), medium-dose OBL (200 mg kg^{-1} day) (OBL-M) and high-dose OBL (400 mg kg^{-1} per day) (OBL-H), compared with untreated controls (model) and sham-operated rats (sham)

	Sham	*Model*	*Captopril*	*OBL-L*	*OBL-M*	*OBL-H*
SBP (mm Hg)	138 ± 2	201 ± 4*	165 ± 6*,#	184 ± 4*,#,†	177 ± 8*,#,†	180 ± 3*,#,†
DBP (mm Hg)	67 ± 3	100 ± 3*	78 ± 3*,#	90 ± 4*,†	85 ± 6*,#,†	84 ± 4*,#,†
BW (g)	583 ± 84	580 ± 110	497 ± 54#	550 ± 44	536 ± 74†	556 ± 47†
HW (g)	1.46 ± 0.17	2.01 ± 0.27*	1.50 ± 0.17#	1.67 ± 0.31*,†	1.55 ± 0.18*,#	1.33 ± 0.14*,#,†
LHW (mg)	268.2 ± 0.03	396.2 ± 0.09*	239.3 ± 0.02*,#	290.9 ± 0.07*,#,†	234.4 ± 0.03*,#	256.1 ± 0.04*,#,†
HW/BW	2.55 ± 0.45	3.56 ± 0.72*	3.05 ± 0.44*,#	3.04 ± 0.61*,#	2.93 ± 0.55*,#	2.41 ± 0.38*,#,†
LVAWTh	0.27 ± 0.04#	0.4 ± 0.09†	0.24 ± 0.02#	0.29 ± 0.07#	0.23 ± 0.03#	0.26 ± 0.04#

Abbreviations: BW, body weight; DBP, diastolic blood pressure; HW, heart weight; HW/BW, heart weight to body weight ratio; LHW, left heart weight; LVAWTh, left ventricular anterior wall thickness; SBP, systolic blood pressure.
Values are mean ± s.e.m.
*$P<0.05$ *vs.* sham; #$P<0.05$ *vs.* model; †$P<0.05$ *vs.* captopril.

The heart weight/body weight ratio was markedly lower in the captopril and OBL groups than that in the untreated hypertensive group (Figure 2). The values in the OBL groups showed dose dependence. The heart weight to body weight ratio in the highest dose group for OBL was not different from that of the sham-operated normotensive rats.

Renal function

Blood urea nitrogen (BUN) and serum creatinine in each group are shown in Table 2. In the untreated (model) group, the BUN was higher than in the other groups ($P<0.05$). There was no difference between the treatment groups.

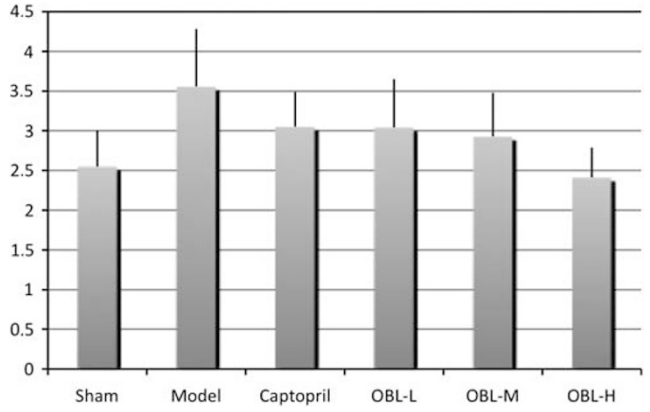

Figure 2 Effect of captopril and OBL at low, medium and high doses (OBL-L, OBL-M, OBL-H) on heart weight to body weight ratio in two-kidney, one-clip Goldblatt hypertensive rats, compared with sham-operated rats and untreated control (model) rats. $P<0.05$, all treated and model *vs.* sham; all treated *vs.* model; OBL-H *vs.* captopril.

Table 2 Renal parameters in renovascular hypertensive rats after 4 weeks of daily oral treatment with captopril (30 mg kg^{-1} per day), low-dose OBL (100 mg kg^{-1} per day) (OBL-L), medium-dose OBL (200 mg kg^{-1} per day) (OBL-M) and high-dose OBL (400 mg kg^{-1} per day) (OBL-H), compared with untreated controls (model) and sham-operated rats (sham)

	Sham	*Model*	*Captopril*	*OBL-L*	*OBL-M*	*OBL-H*
BUN	46 ± 7$^{\#}$	69 ± 15*,†	51 ± 10$^{\#}$	52 ± 15	41 ± 13$^{\#}$	47 ± 12$^{\#}$
SCr	38 ± 13	52 ± 7	47 ± 4$^{\#}$	49 ± 7	48 ± 9$^{\#}$	47 ± 9$^{\#}$

Abbreviations: BUN, blood urea nitrogen; SCr, serum creatinine.
Values are mean ± s.e.m.
$^{*}P<0.05$ *vs.* sham; $^{\#}P<0.05$ *vs.* model; $^{\dagger}P<0.05$ *vs.* captopril.

Angiotensin-II and ET-1

The changes in ET and angiotensin-II in each group are shown in Table 3. ET was higher in the model than in sham rats. In the OBL groups, ET was lower than that in the model group ($P<0.05$) and even lower than that in sham rats (Figure 3). Angiotensin-II was higher in model than in sham rats, but returned to about the same level as sham rats in the captopril and medium-dose OBL groups.

DISCUSSION

Sweet basil, OBL, is a well-known medicinal herb in traditional Chinese or Asian medicine preparations, used in a variety of chronic diseases, including hypertension and atherosclerosis. It is also widely used as a culinary herb in the Mediterranean area, where the cardiovascular morbidity is very low.

We demonstrated an action on platelets in an earlier study,[1] and others have found effects on lipid metabolism.[2,3] In this study, we tested the effects of OBL on a rat model of hypertension, on determinants of the hypertension (angiotensin, ET) and its consequences (renal function, myocardial hypertrophy). Our results show that OBL may decrease hypertension in this Goldblatt 2K1C model, but to a lesser degree than captopril. We found no dose dependence of this effect, which thus is probably already maximal for this drug. This was accompanied by a variable decrease of angiotensin-II, again not to the same degree as with captopril. In contrast to the small effect on angiotensin-II, OBL reduced considerably ET concentrations, to less than half of hypertensive control rat values, and to lower values than that in sham rats. There was no clear dose dependence, the lowest dose of OBL having the same effect as the highest, fourfold greater dose.

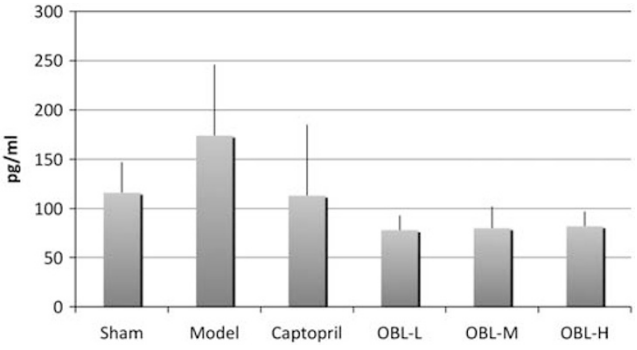

Figure 3 Effect of captopril and OBL at low, medium and high doses (OBL-L, OBL-M, OBL-H) on plasma endothelin concentrations (pg ml^{-1}) in two-kidney, one-clip Goldblatt hypertensive rats, compared with sham-operated rats and untreated control (model) rats. $P<0.05$ *vs.* model for OBL-L, OBL-M, OBL-H.

Table 3 Endothelin and angiotensin-II in renovascular hypertensive rats after 4 weeks of daily oral treatment with captopril (30 mg kg^{-1} per day), low-dose OBL (100 mg kg^{-1} per day) (OBL-L), medium-dose OBL (200 mg kg^{-1} per day) (OBL-M) and high-dose OBL (400 mg kg^{-1} per day) (OBL-H), compared with untreated controls (model) and sham-operated rats (sham)

	Sham	*Model*	*Captopril*	*OBL-L*	*OBL-M*	*OBL-H*
ET (pg ml^{-1})	116 ± 31	174 ± 72	113 ± 72	78 ± 15$^{\#}$	80 ± 22$^{\#}$	82 ± 15$^{\#}$
Ang-II (pg ml^{-1})	1064 ± 88$^{\#}$	1244 ± 145*,†	1070 ± 75$^{\#}$	1223 ± 142*,†	1086 ± 79$^{\#}$	1132 ± 74

Abbreviations: Ang-II, angiotensin II; ET, endothelin.
Values are mean ± s.e.m.
$^{*}P<0.05$ *vs.* sham; $^{\#}P<0.05$ *vs.* model; $^{\dagger}P<0.05$ *vs.* captopril.

This differential effect on angiotensin and ET is mirrored by the effect on myocardial hypertrophy: despite a greater reduction in blood pressure, the effect of captopril on myocardial hypertrophy, one of the major end points in the treatment of hypertension, was less than that of OBL. In the highest OBL dose group, in fact, the heart weight to body weight ratio was even lower than in that sham rats, even though the SBP and DBP remained higher. Although captopril-treated rats had lower absolute heart weight, left heart weight and anterior wall thickness, they also had lower body weight, so that the effect of captopril on the heart weight to body weight ratio was less marked than that with OBL.

A dissociation between the effect on blood pressure and myocardial hypertrophy has already been described,[12] for instance, in the other direction with hydralazine, which reduces blood pressure without affecting myocardial hypertrophy.

Considering the overall picture, one would be tempted to link the effect on cardiac hypertrophy to the decreased ET concentrations: the effect is greater than expected from the decreased blood pressure, and the decrease in serum ET is quite important.

These results suggest that OBL might act at least in part by reducing ET release, in a manner that might be akin to that of Et-converting enzyme inhibitors.[15]

ET is one of the more potent vasoconstrictors, and has been implicated in cardiac and vascular remodeling. Experimentally, the effects of ET inhibitors has been less convincing, with little effect on blood pressure, and variegated effects on hypertrophy, fibrosis or remodeling, depending probably on the models used.[15] ET-converting enzyme inhibitors, which oppose the production of ET, reduce cardiac remodeling and hypertrophy in animal models of heart failure[16] and in hypertension[17] to a greater degree than ACE inhibitors.[17]

OBL also has an effect on platelets and lipid metabolism, but whether this could be related to possible inhibition of ET is unclear. The effects of ET antagonists or convertase inhibitors on platelet function are ambiguous, possibly related to the opposite effects of endothelin-A and endothelin-B receptors.[18]

Similar to all herbals, OBL certainly contains many components. Much study is still needed to identify the components involved in the present effect, and whether it is the same or different compounds that are also responsible for OBL's effect on platelets.

Clearly the effects we found on blood pressure and on end-organ damage, added to the antiplatelet effects, warrant further exploration of the cardiovascular effects of OBL in other models of hypertension, and in human disease.

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