Screening of the Positive Inotropic Activity of Medicinal Plants Used in Oriental Medicine

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Aqueous extracts of medicinal plants traditionally used in the East Asia such as China, Korea, and Japan were screened for inotropic activity using isolated rabbit atria. Among the twenty-one aqueous-extracts from medicinal plants, the aqueous extracts of *Convallaria keiskei* (ACK) and rhizome of *Coptis chinensis* (ACC) were found to exhibit distinctive positive inotropic activity. The aqueous extracts of *C. keiskei* and rhizome of *C. chinensis* significantly increased atrial stroke volume and pulse pressure in beating rabbit atria. These findings suggest that the aqueous extracts of *C. keiskei* and rhizome of *C. chinensis* enhance the cardiac muscle contractility and then could be useful for the treatment of cardiac failure.

Key words : inotropic activity, atrial stroke volume, pulse pressure, cardiac failure, Oriental medicines

Introduction

The term "cardiac failure" means simply failure of the heart to pump enough blood to satisfy the needs of the body[1]. The heart failure is not only indirectly caused by chronic hypertension, the damage of heart valves, and myocardial infraction, but also directly evoked by essential disorder of cardiac muscle or ischemic heart disease[2]. In the state of higher total peripheral resistance caused chronically throughout hypertension and the damage of heart valves, heart is fibrillated and hypertrophied[2]. These transformations of cardiac muscle are termed such as "heart remodeling". This heart remodeling is at first beneficial but ultimately becomes maladaptive by causing negative effects like that arrhythmia and decrease in heart contractility. Ultimately, the patients with diverse cardiac failure accompany the decrease of myocardial contractility resulted from diminished coronary blood[3].

It is well appreciated that treating heart failure patients with drugs those augment pump function by increasing the contractility of cardiac myocytes can improve hemodynamics and exercise tolerance[4-6]. There are several cardiac glycosides such as digitoxin, strophanthin, ouabain, convallatoxin, and proscillaridin A have been isolated from plant medicines such as *Digitalis purprea, Strophanthus kombe, Strophanthus gratus, Convallaria majalis, and Scilla maritima,* respectively[4,7]. Especially, the cardiac glycosides such as digoxin and digitoxin isolated from *Digitalis purprea* have been used as stimulants for treatment patients with congestive heart failure in clinical medicine[8].

The present study is also aimed at a preliminary screening of inotropic activity of the aqueous extracts from some medicinal plants used in Oriental medicines (Table 1) using beating rabbit atria.

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Materials and methods

1. Reagents

N-2-hydroxyehtylpiperazine-N'-2-ethanesulfonic acid (HEPES), bovine serum albumin (BSA), sodium chloride, potassium chloride, calcium chloride, magnesium chloride, sodium bicarbonate, glucose were purchased Sigma Chemical Co. (St. Louis, MO, USA).

2. Plant materials and extraction

All medicinal plants in Table 1 were commercially available and purchased from herbal market in Iksan, Jeonbuk Province, and authenticated by professor Tae-Oh Kwon, College of Life Sciences and Natural Resources, Wonkwang University. Herbarium voucher specimens were prepared and deposited in the herbarium of the Professional Graduate School

of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk, South Korea. The dried medicinal plants (600 g) boiled to extract with 3000 ml of distilled water for 2 hours. The extract was filtered through Whatman No. 3 filter paper and concentrated using freeze dryer and then used in this experiment.

Table 1. Botanical names, family, plant parts, and uses in Oriental medicines

Botanical names	Family	Plant parts	Uses in Oriental medicine
Crataegus pinnatifida Bunge	Malaceae	Fruit	Depressor, anti-hyperlipidermia
Pueraria lobata Ohwi	Leguminosae	Root	Anti-hypertensive, anti-pyretic
Uncaria rhynchophylla Jack.	Rubiaceae	Stem	Anti-hypertensive, anti-pyretic
Polygonatum sibiricum Redoue	Liliaceae	Root	Anti-hypertensive, anti-microbial
Alisma canaliculatum Al.	Alismataceae	Root	Dinuretic, anti-pyretic
Artemisiae capillaris Thunb.	Compositae	Whole	Anti-hyperlipidermia, anti-pyretic
Rheum coreanum Nakai	Polygonaceae	Root	Hemostatic, dinuretic
Ganoderma japonicum Lyoyd	Polporaceae	Mushroom	Sadative, tonic, anti-hypertensive
Morus alba L.	Moraceae	Leaf	Anti-pyretic, anti-hyperglycemia
Paeonia suffruticosa Andr.	Paeoniaceae	Stem bark	Anti-hypertensive, anti-pyretic
Prunella vulgaris L.	Labiatae	Whole	Anti-hypertensive, anti-pyretic
Cassia tora L.	Leguminosae	Seed	Anti-hypertensive, anti-pyretic
Siegesbeckia orientalis L.	Compositae	Whole	Anti-bleeding, anti-hypertensive
Chrysanthemum indicum L.	Compositae	Flower	Anti-hypertensive, anti-pyretic
Scutellaria baikalensis Georgi	Labiatae	Root	Anti-inflammation, anti-pyretic
Coptis chinensis Franch.	Ranunculaceae	Root	Anti-pyretic, cardiotonic
Phellodendron amurense Rupr.	Rutaceae	Stem bark	Dinuretic, anti-hyperglycemia
Eucommia ulmoides Oliv.	Eucommiaceae	Stem bark	Dinuretic, anti-hypertensive
Convallaria keiskei Miq.	Liliaceae	Leaf	Dinuretic, cardiotonic
Polygala tenuifolia Willd.	Polygalaceae	Root	Sadative, anodyne
Lespedeza cuneata G. Don	Leguminosae	Whole	Dinuretic, anti-hyperglycemia

3. Beating perfused rabbit atrial preparation and determination of atrial stroke volume and pulse pressure

New Zealand white rabbits weighing 2 kg were used. Rabbits were anesthetized by injecting ketamine-HCl, and the chest was opened. An isolated perfused atrial preparation was prepared by the Cho's method[9] with some modification (Fig. 1), allowing atrial pacing and measurements of changes in atrial volume during contraction (stroke volume). Briefly, the hearts were rapidly removed and placed in oxygenated warm saline. The left atrium was then dissected. A calibrated transparent atrial cannula (8 cm long, 4 mm OD) containing two small catheters within it was inserted into the left atrium through the atrioventricular orifice. The cannula was secured by ligatures around the atrioventricular sulcus. The outer tip of the two catheters located in the atrium was for inflow, the other catheter was used to record pressure changes in the atrium. The cannulated atrium was then transferred to an organ chamber containing 3 ml buffer at 34 ℃. The pericardial space of the organ chamber was open to the air so as not to restrict atrial dynamics. The atrium was immediately perfused with HEPES buffer solution by means of a peristaltic pump (1 ml/min). The composition of the buffer was as follows (in mM); 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 10.0 glucose, and 10.0 HEPES (adjust to pH 7.4 with 1 M NaOH) and 0.1% BSA. Soon after setup of the perfused atrium, transmural electrical field stimulation with a luminal electrode was started at 1.3 Hz (duration, 0.3 - 0.5 ms voltage, two times threshold intensity, 20 - 30 V; 6.1 cmH_2O distention). The organ chamber was fixed so as to make an axial rotation to change the height of the atrial cannula and intra-atrial pressure. The perfusate was prewarmed to 34 ℃ by passage through silicone tubing in a gas mixing chamber. The buffer in the organ chamber was oxygenated by passing oxygen through silicone tubing coils located inside the chamber. The changes in atrial stroke volume were monitored by reading the lowest level of the water column in the calibrated atrial cannula during end diastole. Atrial pulse pressure was measured via a pressure transducer connected to the intra-atrial catheter and recorded on a Power Lab / 8sp (model ML785, AD Instruments, Australia).

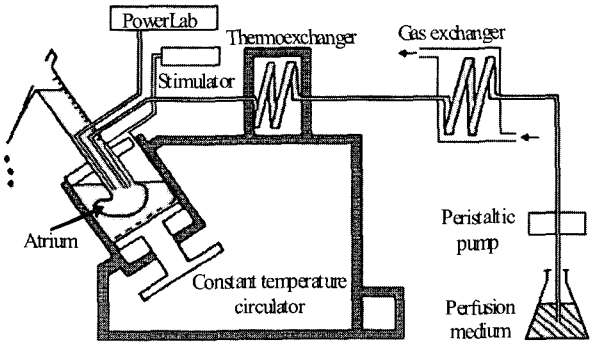

Fig. 1. Schematic illustration of experiment apparatus using the perfused beating rabbit atrium. The atrium was electrically driven by transmural field stimulation and was provided with HEPES buffer by peristaltic pump. Axial rotation of the organ chamber permits changes in intra-atrial pressure.

4. Statistical analysis

Significant difference was compared using repeated measures ANOVA followed by Bonferroni's multiple-comparison test. Student's t-test for unpaired data was also applied. Statistical significance was defined as $P<0.05$. The results are given as means ± SE.

Results

1. Effect of Oriental medicines on the atrial dynamics

Atrial stroke volume and pulse pressure were steady and stable during the entire control periods in perfused beating rabbit atria (Fig. 2A and 3A).

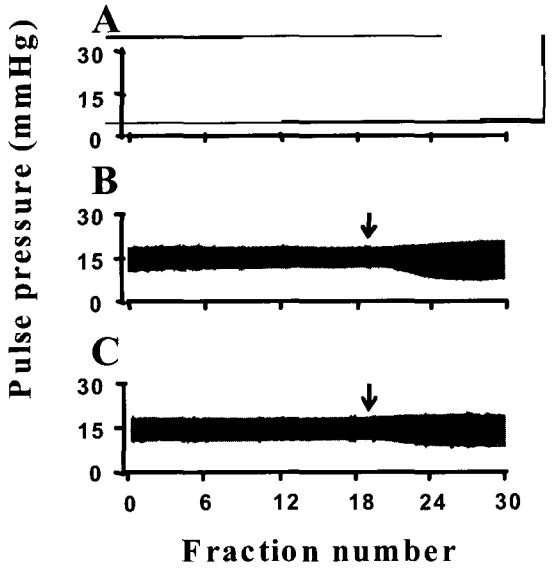

Fig. 2. Representative trace of time-matched control for pulse pressure (A) and effects of the aqueous extracts of *Convallaria keiskei* (B) and rhizome of *Coptis chinensis* (C) on pulse pressure in beating rabbit atria (1.3 Hz). Arrow indicates perfusion of drugs

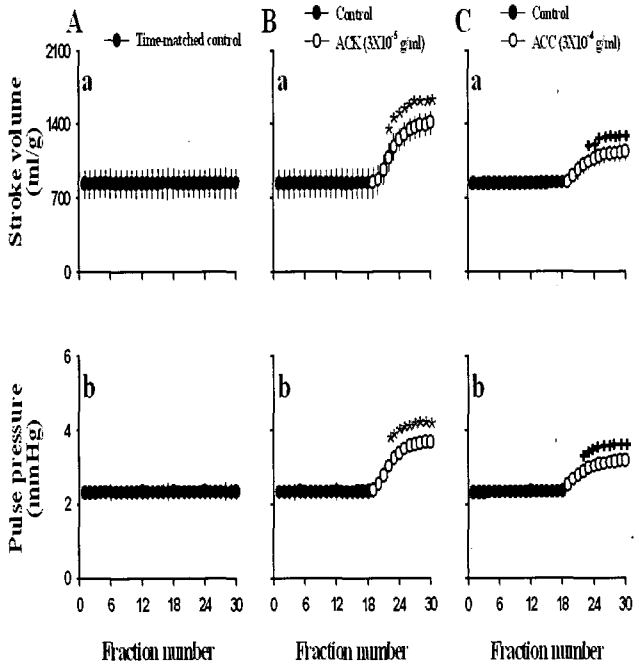

Fig. 3. Time-matched control (A), the positive inotropic effects of the aqueous extracts of *Convallaria keiskei* (B) and rhizome of *Coptis chinensis* (C) on stroke volume and pulse pressure in beating rabbit atria (1.3 Hz). Values are mean ± SE (n=4); *P<0.001, +P<0.01 vs. control (compare with values of the last each 3 fraction of control).

As shown in Table 2, among the twenty-one aqueous-extracts from medicinal plants, the aqueous extracts of *Convallaria keiskei* and rhizome of *Coptis chinensis* were found to exhibit distinctive positive inotropic activity. In beating rabbit atria, perfusion of the aqueous extracts of *C. keiskei* (3×10^{-5} g/ml) significantly increased atrial stroke volume by 75.8 ± 2.4% compared with that of vehicle-treated period ($P<0.001$, Fig. 2B and 3B). Atrial pulse pressure was also increased by perfusion of the aqueous extracts of *C. keiskei* ($P<0.001$, Fig. 3B). Perfusion of rhizome of *C. chinensis* (3×10^{-5} g/ml) also significantly increased atrial stroke volume and pulse pressure compared with those of vehicle-treated period ($P<0.01$, Fig. 2C and 3C).

Table 2. The inotropic activity of aqueous extracts of Oriental medicines in beating rabbit atria

Botanical names	Voucher specimen number	Atrial dynamics (%)	
		Stroke volume	Pulse pressure
Crataegus pinnatifida Bunge	BDR-2	10.6 ± 0.7	1.1 ± 0.1
Pueraria lobata Ohwi	BDR-3	-7.3 ± 0.4	1.0 ± 0.1
Uncaria rhynchophylla Jack.	BDR-4	-9.7 ± 0.7	-7.7 ± 0.5
Polygonatum sibiricum Redoue	BDR-5	-3.3 ± 0.5	-4.2 ± 0.2
Alisma canaliculatum Al.	BDR-6	-7.1 ± 0.3	-2.2 ± 0.3
Artemisiae capillaris Thunb.	BDR-7	3.1 ± 0.5	0.2 ± 0.1
Rheum coreanum Nakai	BDR-8	5.3 ± 0.4	6.7 ± 0.4
Ganoderma japonicum Lyoyd	BDR-9	-6.3 ± 0.3	-6.3 ± 0.2
Morus alba L.	BDR-10	0.1 ± 0.1	0.1 ± 0.1
Paeonia suffruticosa Andr.	BDR-12	-9.3 ± 0.7	-5.3 ± 0.1
Prunella vulgaris L.	BDR-13	0.2 ± 0.3	0.5 ± 0.1
Cassia tora L.	BDR-14	-12.0 ± 0.6	-13.0 ± 0.4
Siegesbeckia orientalis L.	BDR-15	2.2 ± 0.3	0.2 ± 0.1
Chrysanthemum indicum L.	BDR-16	-3.8 ± 0.1	-4.4 ± 0.3
Scutellaria baikalensis Georgi	BDR-17	0.2 ± 0.1	0.1 ± 0.1
Coptis chinensis Franch.	BDR-18	48.1 ± 0.5	35.9 ± 0.4
Phellodendron amurense Rupr.	BDR-19	11.3 ± 0.5	11.6 ± 0.6
Eucommia ulmoides Oliv.	BDR-20	0.2 ± 0.3	2.7 ± 0.3
Convallaria keiskei Miq.	BDR-21	75.8 ± 2.4	60.4 ± 1.2
Polygala tenuifolia Willd.	BDR-22	3.9 ± 0.4	1.0 ± 0.2
Lespedeza cuneata G. Don	BDR-23	-4.0 ± 0.2	-4.0 ± 0.3

Discussion

The heart is a pulsatile two chamber pump composed of an atrium and a ventricle, and pumps the blood through the peripheral organs and the lungs. The atrium functions principally as a primer pump for the ventricle, helping to move the blood into the ventricle. The mechanical contraction and distension of cardiac muscle repeatedly evoked from electrical stimulation is possible function of heart as pump. In the cardiac muscle, generally, there are excitation-contraction coupling mechanisms, essential motivation itself and external motivation like nerve system for the regulation of cardiac muscle contractility[10]. As factors related with the mechanism for cardiac muscle regulation, first of all, Ca^{2+} play an pivotal

role in the regulation of cardiac contractility and is implicated with functional mechanism of a various agents involved in modulation of the cardiac action[11].

There are three important mechanisms responsible for positive inotropic activity in heart. At first, cardiac glycosides accentuate cardiac contractile force by increasing cytosolic Ca^{2+} concentration associated with the activation of Na^{+}-Ca^{2+} exchanger caused by Na^{+}-K^{+}ATPase inhibition in the myocardial cell[12,13]. Besides cardiac glycosides, phosphodiesterase III inhibitors like amrinone and milrinone used for treatment of congestive heart failure also augment cardiac contractility by increasing intracellular cAMP level[14]. Dobutamine has also been used in the remedy of patients with heart failure by improving of heart contractility associated with an increase in cAMP level via beta-adrenergic receptor activation[15,16].

In the present study, we screened inotropic activity of various Oriental medicines including fructus of *Crataegus pinnatifida*, radix of *Pueraria lobata*, ramulus of *Uncaria rhynchophylla*, rhizome of *Polygonatum sibiricum*, rhizome of *Alisma canaliculatum*, herba of *Artemisiae capillaris*, radix of *Rheum coreanum*, *Ganoderma japonicum*, folium of *Morus alba*, cortex of *Paeonia suffruticosa*, herba of *Prunella vulgaris*, semen of *Cassia tora*, herba of *Siegesbeckia orientalis*, flos of *Chrysanthemum indicum*, radix of *Scutellaria baikalensis*, rhizome of *Coptis chinensis*, cortex of *Phellodendron amurense*, and cortex of *Eucommia ulmoides*, herba of *Convallaria keiskei*, radix of *Polygala tenuifolia*, herba of *Lespedeza cuneata* in perfused beating rabbit atria. As a result of screening, the aqueous extracts of *Convallaria keiskei* and rhizome of *Coptis chinensis* significantly increased atrial pulse pressure and stroke volume in beating rabbit atria.

Rhizome of *C. chinensis*, called 'hwuangryun' in Korea, 'Huanglian' in China, 'Oren' in Japan, is one of the most well known and widely used herbs in traditional Oriental medicine. It is officially listed in the Chinese Pharmacopoeia and used as a bacteriostatic, antipyretic, antiphlogistic for the treatment of gastroenteritis, diarrhea, vomiting, and for the treatment of cardiovascular diseases[17]. From the rhizome of *C. chinensis*, berberine, coptisine, jatrorhizine, palmatine, worenine were isolated and identified[17]. Among them, berberine, an isoquinoline alkaloid, is the most well-known component of the rhizome of *C. chinensis*. The compound is known to exhibit multiple pharmacological activities such as antibiotic[18], antitumor[19], antiarrhythmic[20], antidiarrheal activities[21]. In addition, the clinical effects of berberine on severe congestive heart failure[22] have also been reported. In vivo experiment, berberine also lowers blood pressure in mammals[23,24]. Recently, we also have reported that berberine induced endothelium-dependent relaxation aortic tissues isolated from rats and inhibited the activity of angiotensin converting enzyme[25]. Base on the previous reports, we suggest that berberine, at least in part, might play an important role in the positive inotropic activity induced by rhizome of C. chinensis.

The aqueous extract of *C. keiskei*, called 'Youngran' in Korea and 'linglan' in China, has been used as diuretics and heart stimulants in Oriental medicine. From the herba of *C. keiskei*, convallatoxin, covallatoxol, convalloside, convallasaponin, desglucocheirotoxin, and convallagenin have been isolated[26-29]. Among them, convallatoxin, a main constituent of ACK, is a well known as a digitalis-like compound. The previous report demonstrated that convallatoxin could inhibit Na^{+}-K^{+} ATPase activity[30]. Therefore, the aqueous extract of C. keiskei might increase cardiac contractility by increasing intracellular Ca^{2+} concentration through the inhibition of Na^{+}-K^{+} ATPase and then subsequently activation of Na^{+}-Ca^{2+} exchanger by convallatoxin as a cardiac glycoside.

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