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Original Article

Cardioprotective Effects of *Salvia Miltiorrhiza* Radix on the Pressure Overloaded Heart Failure Model by Transverse Aortic Constriction-induced Mice

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Objectives: The objective of this study is to demonstrate the cardioprotective effects of *Salvia Miltiorrhiza* Radix (SMR) on the pressure overload (PO)-induced heart failure (HF) by transverse aortic constriction (TAC) in C57BL/6 mice through possible antioxidant effects.
Methods: The mortality, body and heart weights, antioxidant defense system of the heart and histopathology of heart were analyzed. The obtained results were compared with resveratrol, in which potent cardioprotective effects on TAC mice model were already proved at a dose level of 10 mg/kg by antioxidant effects, as reference in this experiment.
Results: Significant increases of mortalities, heart weights, and hypertrophic, lytic and focal fibrotic histological changes in the left ventricles were found with defects of heart antioxidant defense systems – the increases of heart cortex MDA contents, decreases of GSH contents, SOD and CAT activities in TAC control mice as compared with sham vehicle control mice, respectively. However, these HF signs induced by TAC surgery through PO and destroys heart antioxidant defense systems were significantly and dose-dependently inhibited by 14 days continuous oral treatment of SMR 500, 250 and 125 mg/kg, similar to those of resveratrol 10 mg/kg in SMR 125 mg/kg.
Conclusions: The results obtained in this study propose that oral administration of SMR potently alleviates PO-induced HF by TAC, through augmentation of heart antioxidant defense system.

Key Words : Cardioprotective Effects, *Salvia Miltiorrhiza* Radix, Pressure Overloaded Heart Failure

Introduction

Heart failure (HF) is a chronic syndrome which can induce the heart fail to work properly and be unable to meet the metabolic needs of tissues[1]. HF follows left ventricular hypertrophy in return for pressure overload (PO). Even though cardiac hypertrophy is an adjustment that is adventageous to the stressed heart in the early stages wherein cardiomyocytes expand in size to get adequate function under chronic pathological stress[2], this compensatory stage is temporary because in the presence of continued stress, the heart enters into a decompensatory stage at last[3]. This change from compensatory to decompensatory phase is characterized by marked increases in cardiac fibrosis, apoptosis, and hypoxia that cause irreversible functional changes and HF[1,4].

Salvia Miltiorrhiza Radix (SMR, 丹蔘), a dried root of *Salvia miltiorrhiza* Bunge (Labiatae), is a herbal medicine traditionally used as an anti-inflammatory agent and antipyretic[5]. Recently, it

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has been reported that extracts of SMR have protective effect on ethanol-induced hepatotoxicity using hepatic lipid peroxidation, blood ethanol concentration as well as alcohol dehydrogenase and aldehyde dehydrogenase activity as indicators[6]. Up to now, many experiments have indicated that SMR has potent antioxidant-based cardioprotective effects against various animal or *in vitro* models, mainly myocardial infarctions[7-9], however, no study has examined the effects of SMR on preventing chronic pathological changes within cardiac structure and function in PO-induced HF in C57BL/6 mice.

The objective of this study is to find out the cardioprotective effects of SMR on the PO-induced HF by TAC in C57BL/6 mice[10,11] with possible antioxidant effects. The changes on the mortality, body and heart weights, indicators of antioxidant defense system and histopathology of heart were investigated.

Materials and Methods

1. Animals and husbandry

Six groups, total eight sham or 40 TAC operated mice were chosen based on the body weights at 14 days after TAC surgery (Mean body weights: 21.56±1.08 g, ranged in 19.40-23.80 g), and used in this study as follows(Table 1).

2. Preparations and administration of test materials

Three different dosages, 50, 25 and 12.5 mg of SMR were directly dissolved in distilled water 1 ml, and administered in a volume of 10 ml/kg as equivalence to 500, 250 and 125 mg/kg using gastric gavages by oral, once a day for 14 days from 14 days after TAC operation, and resveratrol also dissolved in distilled water and orally administered at a dose level of 10 mg/kg, in a volume of 10 ml/kg. In sham and TAC control mice, instead of test materials, same volumes of distilled water as vehicle were administered once a day for 14 days from 14 days by oral after TAC surgery, respectively (Table 1).

3. TAC operation

A 3 mm center thoracotomy was conducted. The transverse aortic arch was bound (7–0 Prolene) between the innominate and left common carotid arteries using an overlying 28-gauge needle and after that, the needle was eliminated, leaving a discrete region of stenosis. Some mice underwent a sham operation in which the aortic arch was visualized but not ligated.

4. Mortalities

All abnormal mortalities were recorded before and after administration twice a day from the first day of administration to the last drug treatment day.

Table 1. Experimental Design Used in This Study

Groups	Surgery	Group identification	Treatment
Control	Sham	Sham control	Distilled water 10 ml/kg/day
Control	TAC	TAC control	Distilled water 10 ml/kg/day
Reference	TAC	Resveratrol	Resveratrol 10 mg/kg/day
Active	TAC	SMR 500	SMR 500 mg/kg/day
Active	TAC	SMR 250	SMR 250 mg/kg/day
Active	TAC	SMR 125	SMR 125 mg/kg/day

SMR = *Salvia Miltiorrhiza* Radix aqueous extracts [Lyophilized Powder]
HF = Heart failure
TAC = Transverse aortic constriction

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5. Body weight measurements

Changes of body weight were evaluated at 1 day before initial test article administration, the day of first test material administration, 1, 7, 13 and 14 days after initial resveratrol or SMR administration with an automatic electronic balance (Precisa Instrument, Dietikon, Switzland).

6. Heart weight measurement

At sacrifice, the weights of heart in all survived animals were evaluated at g levels, individually with an automatic electronic balance (Precisa Instrument, Dietikon, Switzland), and to reduce the differences from individual body weights, the relative weights (% of body weights) were also calculated using body weight at sacrifice and absolute heart weight as follow **E**quation [**2**] based on previously established methods with some modifications[12].

EQUATION [**2**]. **R**elative **H**eart **W**eights (%) = (Absolute heart weights / Body weight at sacrifice) × 100

7. Analysis of Antioxidant defence system

The total amount of proteins for the determination of lipid peroxidation, GSH and enzyme antioxidant assays was evaluated with the Lowry protein assay[13].

1) Analysis of lipid peroxidation : To quantify levels of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) made during acid-heating reactions were evaluated as described by Draper and Hadley[14]. TBARS absorbance was spectrophotometrically analyzed at optical density (OD) 535 nm with a UV/VIS spectrophotometer (OPTIZEN POP, Mecasys, Daejeon, Korea) and results are showed as MDA equivalents (nM/g of protein).

2) Analysis of CAT and SOD activity : The CAT activity level was measured by the decay of the hydrogen peroxide OD at 240 nm, spectrophotometrically using a UV/VIS spectrophotometer[15] (OPTIZEN POP, Mecasys, Daejeon, Korea). Results were showed as U/g protein. SOD activity was evaluated at OD 560 nm by the degree of inhibition of this reaction, and was shown as U/g protein.

3) Analysis of GSH levels : Total GSH (reduced and disulphide forms) levels in tissue homogenate samples were evaluated with the method described by Tietze[16].

8. Histological process

Approximated same regions of individual hearts, brought from survived animals at sacrifice (five mice in each group), were crossly cut based on the ventricles as one part in each heart. To investigate more detail changes, total thicknesses of left ventricle from endocardium to pericardium (μm/heart,), mean numbers of lytic necrotic cardiac muscle fibers (myofibers/1000 myofibers) and diameters of cardiac muscle fibers (μm/fiber) were estimated for histomorphometrical analysis using a computer -assisted image analysis program (*i*Solution FL ver 9.1, IMT *i*-solution Inc., Quebec, Canada) under H&E stain with the mean percentages of perivascular and interstitial collagen fiber occupied regions in left ventricle (%/mm^2 of field) under Sirius red stain.

9. Statistical analyses

All Data were shown as mean ± standard deviations (SD). Multiple comparison tests for different dose groups were carried out. Variance homogeneity was examined using the Levene test[17]. If the Levene test showed no significant deviations from variance homogeneity, the obtained data were analyzed by one way ANOVA test followed by least-significant differences (LSD) multi-comparison test to make a decision which pairs of group comparison were considerably different. When massive deviations

from variance homogeneity were found at Levene test, Kruskal-Wallis H test, a non-parametric comparison test, was conducted. When a significant difference was found in the Kruskal-Wallis H test, the Mann-Whitney U (MW) test was used to decide the specific pairs of group comparison, which are considerably different. Statistical analyses were conducted using SPSS for Windows[18] (Release 14.0K, IBM SPSS Inc., Armonk, NY, USA).

Results

1. Survivability

No unscheduled mortalities were found in sham-operated control mice throughout the whole 14 days of experimental periods, but total two mice (2/8; 25.0%) in TAC control were died within 14 days of experimental periods; Each of one mouse at 3 and 5 days after initial administration, respectively. Furthermore, each of one mouse (1/8; 12.5%) administered with resveratrol 10 mg/kg and SMR 125 mg/kg was died at 9 and 10 days after initial administration, respectively. However, no unscheduled mortalities were detected in SMR 500 and 250 mg/kg treated mice throughout the 14 days of experimental periods(Table 2).

2. Changes on the body weight

No meaningful or significant changes on the body weight and gains during 14 days of experimental periods were demonstrated in all TAC operated mice, and also no significant changes on the body weight and gains were demonstrated in all three different dosages of SMR or resveratrol 10 mg/kg treated mice, respectively (Table 3).

3. Heart weight changes

Marked hypertrophic changes were found in TAC control mice as compared with sham control mice; consequently, noticeable ($p<0.01$) increases of absolute and relative heart weights were found in TAC control mice, at 14 days after end of administration (28 days after TAC surgery). However, these heart hypertrophic changes induced by TAC were markedly inhibited by treatment of SMR 500, 250 and 125 mg/kg, dose-dependently, and also by resveratrol 10 mg/kg; significant ($p<0.01$) and dose-dependent decreases of heart absolute and relative weights were found in all three different dosages of SMR administered mice, and also in resveratrol 10 mg/kg treated(Fig 1, 2).

Table 2. Mortalities Observed in Sham or TAC Operated Mice

Times Groups	Days of administration														Total*	Survival (%)
	0	1	2	3	4	5	6	7	8	9	10	11	12	13		
Controls																
Sham	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/8	100.00
TAC	0	0	0	1	0	1	0	0	0	0	0	0	0	0	2/8	75.00
Resveratrol																
10 mg/kg	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1/8	87.50
SMR treated																
500 mg/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/8	100.00
250 mg/kg	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1/8	100.00
125 mg/kg	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1/8	87.50

Values are expressed as number of died animals
* Total mortalities during 14 days of observation periods - died animals/total observed animals (eight mice in each group).
SMR = *Salvia Miltiorrhiza* Radix aqueous extracts [Lyophilized Powder]
TAC = Transverse aortic constriction

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Table 3. Body Weight Gains Observed in Sham or TAC Operated Mice

Times Groups	Body weights at		Weight gains [B-A]
	First administration [A]	Sacrifice [B]	
Controls			
Sham	19.35±1.38	20.45±1.30	1.10±0.37
TAC	19.45±1.32	20.37±1.79	1.08±0.44
Resveratrol 10mg/kg	19.30±0.81	20.54±0.70	1.09±0.39
SMR treated			
500 mg/kg	19.60±1.46	20.68±1.52	1.08±0.60
250 mg/kg	19.39±0.81	20.48±0.89	1.09±0.74
125 mg/kg	19.50±1.22	20.60±1.25	1.06±0.26

Values are expressed as Mean ± S.D. of variable numbers of mice according to mortality, g
SMR = *Salvia Miltiorrhiza* Radix aqueous extracts [Lyophilized Powder]
TAC = Transverse aortic constriction
Sacrifice means at 14 days after first test material treatment, 28 days after TAC surgery

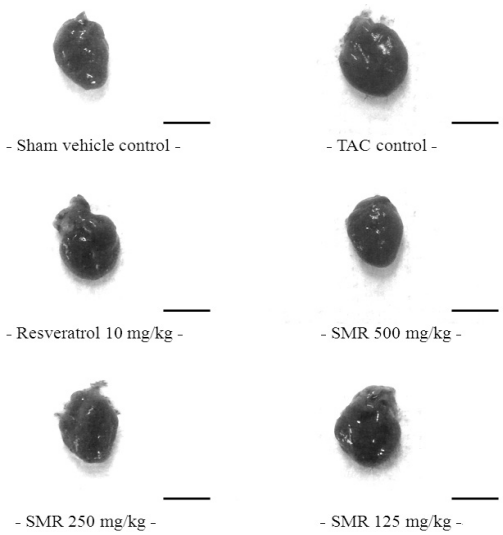

Fig. 1. The Representative Gross Images of Heart, Taken from Sham or TAC Operated Mice
SMR = *Salvia Miltiorrhiza* Radix aqueous extracts [Lyophilized Powder]
TAC = Transverse aortic constriction
Scale bars = 5 mm

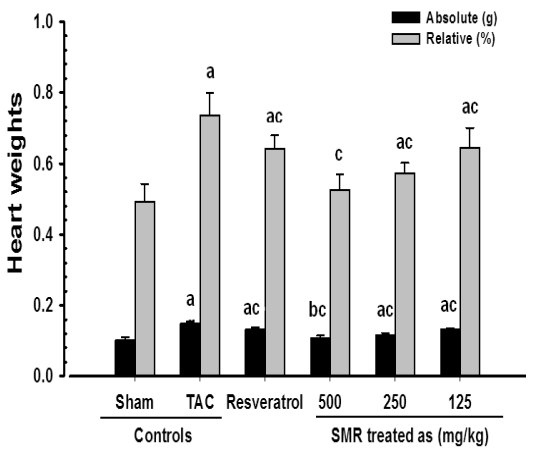

Fig. 2. Changes on the Heart Weights in Sham or TAC Operated Mice
Values are expressed as Mean ± S.D. of variable numbers of mice according to mortality, g
SMR = *Salvia Miltiorrhiza* Radix aqueous extracts [Lyophilized Powder]
TAC = Transverse aortic constriction
[a] $p<0.01$ and [b] $p<0.05$ as compared with sham control by LSD test
[c] $p<0.01$ as compared with TAC control by LSD test

4. Effects on the heart antioxidant defense system

1) Changes on the heart MDA levels : Marked ($p<0.01$) increases of heart lipid peroxidation, elevation of the MDA levels, were demonstrated in TAC control mice. However, these elevation of MDA levels were significantly ($p<0.01$) decreased by treatment of all three different dosages of SMR, dose-dependently. The heart lipid peroxidation in resveratrol 10 mg/kg treated mice were also markedly ($p<0.01$) decreased(Table 4).

2) Changes on the heart GSH contents : A marked ($p<0.01$) decrease of heart endogenous antioxidant,

the GSH content was found in TAC control. However, these decreases of heart GSH contents induced by TAC surgery were significantly ($p<0.01$) and dose-dependently inhibited by treatment of 14 days continues oral treatment of SMR 500, 250 and 125 mg/kg, respectively. Furthermore, the heart GSH contents in resverastrol 10 mg/kg treated mice were also markedly ($p<0.01$) and significantly increased (Table 4).

3) Changes on the heart CAT activity : A marked ($p<0.01$) decrease of heart endogenous antioxidative enzyme, the CAT activity was found in TAC control mice. However, these decreases of heart CAT activities were significantly ($p<0.01$) and dose-dependently inhibited by treatment of 14 days continues oral treatment of SMR at any dose examined. Furthermore, the heart CAT activities in resveratrol 10 mg/kg treated mice were also markedly ($p<0.01$) increased (Table 4).

4) Changes on the heart SOD activity : Significant ($p<0.01$) decreased of heart endogenous antioxidative enzyme, the SOD activities were detected in TAC control mice, but significant ($p<0.01$) increases of SOD activities were found in resveratrol 10 mg/kg, SMR 500, 250 and 125 mg/kg administrated mice, respectively(Table 4).

5. Effects on the heart histopathology

1) Changes on the total thicknesses of left ventricle: In TAC control mice, markedly ($p<0.01$) increased total thicknesses of the left ventricle from endocardium to pericardium were detected. However, 14 days continuous oral treatment of resveratrol 10 mg/kg, SMR 500, 250 and 125 mg/kg in TAC -operated mice significantly and markedly ($p<0.01$) inhibited the TAC associated ventricle hypertrophic changes, respectively. In addition, SMR detected clear dose-dependent effects(Table 5, Fig 3).

2) Changes on the mean numbers of lytic necrotic cardiac muscle fibers in left ventricle: In TAC control mice, markedly ($p<0.01$) increased the mean numbers of lytic necrotic cardiac muscle fibers among 1000 myofibers were detected. However, 14

Table 4. Heart Antioxidant Defense Systems Detected in Sham or TAC Operated Mice

Groups \ Items (Unit)	MDA (nM/g protein)	GSH (nM/g protein)	CAT (U/g protein)	SOD (U/g protein)
Controls				
Sham	0.96±0.21	4.81±0.70	0.69±0.17	7.37±1.27
TAC	4.28±0.53^{c}	1.11±0.35^{a}	0.18±0.05^{c}	2.18±0.28^{c}
Resveratrol 10 mg/kg	2.59±0.60^{ce}	2.72±0.42^{ab}	0.34±0.05^{ce}	4.29±0.49^{ce}
SMR treated				
500 mg/kg	1.41±0.17^{de}	3.72±0.58^{ab}	0.49±0.03^{e}	5.85±0.50^{e}
250 mg/kg	1.92±0.15^{ce}	3.40±0.44^{ab}	0.41±0.06^{de}	5.25±0.48^{de}
125 mg/kg	2.56±0.65^{ce}	2.73±0.34^{ab}	0.34±0.05^{ce}	4.30±0.47^{ce}

Values are expressed as Mean ± S.D. of five mice, g
SMR = *Salvia Miltiorrhiza* Radix aqueous extracts [Lyophilized Powder]
TAC = Transverse aortic constriction
MDA = Malondialdehyde
GSH = Glutathione
CAT = Catalase
SOD = Superoxide dismutase
[a] $p<0.01$ as compared with sham control by LSD test
[b] $p<0.01$ as compared with TAC control by LSD test
[c] $p<0.01$ and [d] $p<0.05$ as compared with sham control by MW test
[e] $p<0.01$ as compared with TAC control by MW test

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days continuous oral treatment of resveratrol 10 mg/kg, SMR 500, 250 and 125 mg/kg in TAC-operated mice significantly and markedly ($p<0.01$) inhibited the TAC related increases of degenerative myofibers, respectively. In addition, SMR detected clear dose-dependent effects(Table 5, Fig 3).

3) Changes on the diameters of cardiac muscle fibers in left ventricle: In TAC control mice, markedly ($p<0.01$) increased the mean diameters of cardiac muscle fibers were demonstrated. However, 14 days continuous oral treatment of resveratrol 10 mg/kg, SMR 500, 250 and 125 mg/kg in TAC-operated mice significantly and markedly ($p<0.01$) inhibited the TAC related increases of the diameters of cardiac muscle fibers, respectively. In addition, SMR detected clear dose-dependent effects(Table 5, Fig 3).

4) Changes on the mean percentages of perivascular collagen fiber occupied regions in left ventricle: Significant and marked ($p<0.01$) increases of the mean percentages of perivascular collagen fiber occupied regions in left ventricle were found in TAC control mice. SMR dose-dependently and significantly ($p<0.01$ or $p<0.05$) restored these TAC-related perivascular fibrosis, respectively. Furthermore, resveratrol 10 mg/kg also markedly ($p<0.01$) decreased the mean percentages of perivascular collagen fiber occupied regions in left ventricle (Table 5, Fig 3).

5) Changes on the mean percentages of interstitial collagen fiber occupied regions in left ventricle: Significant and marked ($p<0.01$) increases of the mean percentages of interstitial collagen fiber occupied regions in left ventricle were detected in TAC control mice. SMR dose-dependently and significantly ($p<0.01$ or $p<0.05$) restored these TAC-related interstitial fibrosis, respectively. Furthermore, resveratrol 10 mg/kg also markedly ($p<0.01$) decreased the mean percentages of interstitial collagen fiber occupied regions in left ventricle (Table 5, Fig 3).

Discussion

According to Korea national health insurance report,

Table 5. Heart Histomorphometrical Analysis Calculated in Sham or TAC Operated Mice

Items (Unit) / Groups	Total left ventricle thickness (μm/heart)	Lytic and necrotic myofiber numbers (fibers/1000 fibers)	Myofiber mean diameters (μm/fiber)	Collagen occupied regions (%/mm^2)	
				Perivascular regions	Interstitial regions
Controls					
Sham	1198.79±103.92	62.20±17.37	13.76±1.04	2.33±0.53	1.27±0.75
TAC	2067.91±293.04^{c}	609.00±106.44^{c}	30.03±2.88^{a}	26.00±5.74^{c}	25.22±5.04^{c}
Resveratrol 10 mg/kg	1510.20±101.55^{ce}	332.80±44.81^{ce}	22.20±1.75^{ab}	7.15±1.68^{ce}	15.86±2.74^{ce}
SMR treated					
500 mg/kg	1367.03±58.26^{de}	184.60±32.16^{ce}	18.49±1.29^{ab}	3.93±0.62^{ce}	8.44±3.08^{ce}
250 mg/kg	1403.26±81.53^{de}	280.00±61.09^{ce}	19.36±2.17^{ab}	4.93±0.58^{ce}	10.79±2.76^{ce}
125 mg/kg	1504.08±132.89^{de}	320.40±70.52^{ce}	22.12±1.20^{ab}	7.13±1.42^{ce}	15.85±3.60^{cd}

Values are expressed as Mean ± S.D. of five mice, g
SMR = *Salvia Miltiorrhiza* Radix aqueous extracts [Lyophilized Powder]
TAC = Transverse aortic constriction
[a] $p<0.01$ as compared with sham control by LSD test
[b] $p<0.01$ as compared with TAC control by LSD test
[c] $p<0.01$ and [d] $p<0.05$ as compared with sham control by MW test
[e] $p<0.01$ and [f] $p<0.05$ as compared with TAC control by MW test

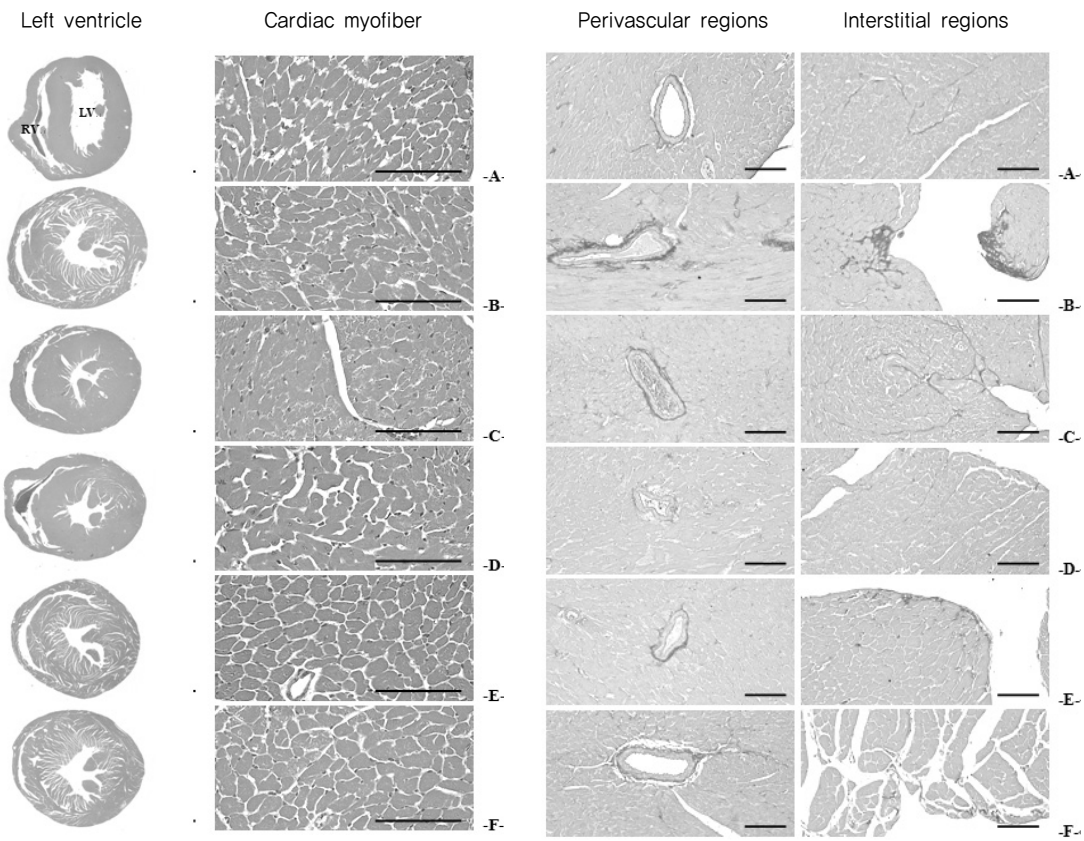

Fig. 3. The Representative General and Collagen Stained Histological Images of Heart, Taken from Sham or TAC Operated Mice
A = Sham vehicle control
B = TAC control
C = Resveratrol 10 mg/kg
D = SMR 500 mg/kg
E = SMR 250 mg/kg
F = SMR 125 mg/kg
SMR = *Salvia Miltiorrhiza* Radix aqueous extracts [Lyophilized Powder]
TAC = Transverse aortic constriction
RV = Right ventricle
LV = Left ventricle
General Histological Images : Hematoxylin-Eosin stain
Collagen Stained Histological Images : Sirius red stain
Scale bars = 100 μm

HF patients' number increases annually from 94,000 in 2009 to 115,000 in 2013[19]. One of the most significant risk factors for the development of HF is chronic hypertension, as reported by Levy et al.[20], which puts long-standing PO to the heart. PO results in compensated cardiac hypertrophy at an early phase, which may lead to decompensated HF at the later phase[21]. Couples of mechanisms have been suggested to play a part in the transition from cardiac hypertrophy to HF, like increasing cardiomyocyte death and interstitial fibrosis, mitochondrial energetic failure, relative ischemia of the hypertrophic heart, and the ROS mediated oxidative stresses[22,23].

SMR, also known as Danshen, is a traditional

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Korean medicine[24]. SMR affects blood viscosity, improves blood circulation, removes blood stasis, promotes blood flow in menstruation, resolves mental uneasiness and restlessness, nourishes the blood, tranquilizes the mind, eliminates and breakes stone, treats gurgling in the intestines, relieves fullness, and resolves swelling[25]. Several active components in SMR such as tanshinones, D(+)3,4-dihydroxyphenol lactic acid, protocatechuic aldehyde, salvianolic acids (A, B, C, D, E, F) and rosmarinic acid[26,27], have been isolated and identified[28]. In addition, seven phenolic compounds isolated from SMR as active components have a strong protective action against oxidative damage to liver microsomes, hepatocytes, and erythrocytes[29]. These active chemical components of SMR are well distributed into the tissues including heart in human subject and also in experimental animals[30,31]. So screening of the biological active compounds in SMR should be conducted with more detail mechanism studies in future.

In the present study, the cardioprotective effects of SMR were evaluated on the PO induced HF by TAC in C57BL/6 mice[10,11] through possible antioxidant effects. The results were compared with resveratrol, in which potent cardioprotective effects on TAC mice model through antioxidant effects were already proved, as reference[10].

Well corresponded to the previous studies[10,11], significant increases of mortalities, heart weights, and hypertrophic, lytic and focal fibrotic histological changes in the left ventricles were found with defects of heart antioxidant defense systems − the increases of heart cortex MDA contents, decreases of GSH contents, SOD and CAT activities in TAC control mice as compared with sham vehicle control mice, respectively. However, these HF signs induced by TAC surgery through PO and destroys of heart antioxidant defense systems were significantly and dose-dependently inhibited by 14 days continuous oral treatment of SMR 500, 250 and 125 mg/kg, respectively. It, therefore, is considered as direct evidences that SMR potently attenuates PO-induced HF by TAC, through augmentation of heart antioxidant defense system, in a condition of this study at least. The overall effects of SMR 125 mg/kg were shown similar to those of resveratrol 10 mg/kg, in the present study.

According to the previous TAC experiments[11,32], survivability of animals from TAC surgery amounted to 60 ~ 80%, and also showed as 75.0% in TAC control mice in the present study. However, potent increases of survivabilities were detected by oral treatment of SMR 500, 250 and 125 mg/kg as 100.0, 100.0 and 87.5%, dose-dependently and also by resveratrol 10 mg/kg as 87.5%. Especially, SMR 125 mg/kg demonstrated similar increases of the survival percentages as comparable to those of resveratrol 10 mg/kg, and no unscheduled mortalities were found in SMR 500 and 250 mg/kg treated mice throughout the whole 14 days of experimental periods, as similar to those of sham vehicle control. This finding on the survival percentages can be regarded as direct evidences which mean SMR can inhibit mortality due to PO-induced HF, as comparable to those of resveratrol 10 mg/kg in a dose level of 125 mg/kg, in TAC mice.

No significant or meaningful changes on the body weight were demonstrated in TAC control mice as compared with sham vehicle control mice; consequently, TAC did not influenced on the body weight gains during 14 days of continues oral administration periods. In addition, SMR 500, 250 and 125 mg/kg and also resveratrol 10 mg/kg did not influence on the body weight and gains as compared with those of sham vehicle and TAC control mice, throughout all experimental periods.

GSH is representative endogenous antioxidants and prevents tissue from damage by keeping at low levels and at certain cellular concentrations of the ROS. GSH is also accepted as protective antioxidant factors in tissues[33]. SOD is a kind of the antioxidant

enzymes that help to enzymatic defense mechanisms also in heart[17,34], and CAT is an enzyme catalyzes the conversion of H_2O_2 to H_2O[35]. So the inhibition of increased lipid peroxidation, ROS and the increase in GSH contents, SOD and CAT activities in the damaged heart tissue are secondarily significant in terms of assisting protection for heart damages in HF[32,34]. In this study, TAC surgery also markedly increased the heart lipid peroxidation, as elevation of MDA contents, decreases of ROS levels eliminating enzymes' activity like SOD and CAT, and GSH in heart tissue. SMR treatments were shown to noticeably and dose-dependently inhibit heart lipid peroxidation, and also increase SOD, CAT activity and GSH levels in comparison with TAC control mice, similar or slightly higher than those of resveratrol 10 mg/kg in SMR 125 mg/kg. These results proposed that the cardioprotective effects of SMR, in part, mediated by augmentation of antioxidant defense systems.

Left ventricular hypertrophy is followed by HF in response to PO. Left ventricular hypertrophy compensates for the PO, but adverse remodeling impairs left ventricular function[10]. At histopathological analysis in TAC animals, increases of left ventricle thicknesses, lytic necrosis or focal apoptosis on the cardiac myofibers, hypertrophy of the myofibers with focal interstitial and perivascular fibrosis, have been demonstrated as summarized by other investigators[10,11]. In the present study, potent increases of heart weights detected in TAC control mice with hypertrophic, lytic and focal fibrotic histological changes in the left ventricles were showing transition from compensatory to decompensatory stage in TAC-induced PO HF. However, these increases of heart weights and hypertrophic, lytic and focal fibrotic histological changes in the left ventricles were significantly and dose-dependently inhibited by 14 days continuous oral treatment of SMR 500, 250 and 125 mg/kg, respectively. Especially, SMR 125 mg/kg demonstrated good enough effects on the heart weights and histopathological changes − the total thicknesses of left ventricle from endocardium to pericardium, mean numbers of lytic necrotic cardiac muscle fibers, diameters of cardiac muscle fibers, mean percentages of perivascular and interstitial collagen fiber occupied region of the left ventricle as comparable to those of resveratrol 10 mg/kg. It, therefore, is regarded as direct evidences that SMR potently alleviates PO-induced HF by TAC as comparable to those of resveratrol 10 mg/kg in a dose level of 125 mg/kg.

There are some herbal formulations which include SMR often used for palpitation and anxiety, Doinhonghwa-jeon[36], Hyeonsamdansam-eum[37], and Cheonwangbosim-dan[38]. There are reports about vasodilative, antihypertensive and antioxidative effects of these herbal formulations[39,40]. Therefore, it may be possible that herbal formulations including SMR which have cardioprotective effects are used for cardiac diseases such as HF, which is part should be studied more in future.

Conclusion

This study investigated the cardioprotective effects of SMR on the PO-induced HF by TAC in C57BL/6 mice[20,22] through possible antioxidant effects.

Therefore, oral administration of SMR reasonably alleviates PO-induced HF by TAC, through augmentation of heart antioxidant defense system. Based on this document, more detail mechanism studies about the screening of the biological active compounds in SMR and application of herbal formulations such as Cheonwangbosim-dan including SMR for cardiac diseases like HF should be carried out in future.

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