Kami-bang-pung-tong-sung-san is Involved in Regulating Physiological Parameters Associated with Hypertension in Spontaneously Hypertensive Rat

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KBPTS is the fortified prescription of Bang-pung-tong-sung-san(BPTS) by adding Spatholobi Clulis and Salviae Miltiorrzae Radix. BPTS prescription has been utilized in oriental medicine for the treatments of vascular diseases including hypertension, stroke, and arteriosclerosis. Yet, the overall mechanism underlying its activity at the cellular levels remains unknown. Using spontaneously hypertensive rat (SHR) model, we investigated whether the KBPTS has an effect on the pathophysiological parameters related to hypertension. Pretreatment of SHR with KBPTS was found to lower blood pressure and heartbeat rate. Levels of aldosterone, dopamine, and epinephrine were found to be significantly reduced in the serum of KBPTS-treated SHR. Histological examination of adrenal cortex and superior aorta showed that tissues from KBPTS-treated SHR rats were more intact and cleaner compared to saline-treated control. Levels of superoxide dismutase (SOD) protein in adrenal gland, aorta, myocardial tissue, and kidneys were higher in KBPTS-treated animals than control group. The present data suggest that KBPTS may play a role in normalizing cardiovascular function in SHR by controlling hypertension-related blood factors and superoxide stressors

Key words : Kami-bang-pung-tong-sung-san, spontaneously hypertensive rat (SHR), hypertension, blood, catecholamine, superoxide dismutase (SOD)

Introduction

Bang-pung-tong-sung-san(BPTS), which has its original description in Sun-myung-non-bang, a classical treatise of the oriental medicine, is known to be effective in lowering blood viscosity, alleviating inflammatory and allergic reactions, promoting blood circulation, and regulating the autonomic nervous system. BPTS has been used for the treatments of stroke, hypertension, arteriosclerosis, constipation, and cutaneous diseases. Studies using experimental animals have further documented that physiological effects of BPTS are related to attenuating analgesic immune responses[18] and pyrogenic reaction in the body. Similarly, the effects of BPTS on obesity or on the hypertension and hyperlipidemia were reported.

Spontaneously hypertensive rat (SHR) is a genetic model for primary hypertension in humans. The angiotensin II system of SHR is hyperactive as a result of an increased expression of angiotensin I receptors[20,22,24], which is associated with an increased turnover and synthesis of catecholamines by angiotensin II[10,27]. A similar hyperactivity of the angiotensin system has been reported in a renin transgenic model of hypertension[16]. These studies lead to the hypothesis that a hyperactive angiotensin system is a result of a heightened stimulation of signaling kinases by angiotensin II. SHR models have been also used by others[5,11,6] and have been characterized extensively over extended time course evaluations, for the consistency of ischemic cortical blood flow effects and infarction[2,3], neurological deficits[4], increased cytokine expression and influence on tissue injury, cellular infiltration, inflammation, tissue changes, and resolution of injury[2,3,9]. More recently, it has been shown that elevated levels of superoxide in SHR are involved in the activation of endothelial cell-dependent vasodilation via the activation of nitric oxide signaling pathway[19]. Increased production of reactive oxygen species including superoxide activated endothelial cells of the blood vessels as well as cardiac muscle. Overexpression of superoxide dismutase (SOD), an enzyme converting superoxide to hydrogen peroxide, in hypertension-prone animal or SHR models was reported to improve endothelial cell dysfunction, suggesting the potential importance of SOD in regulating

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vasodilation and hypertension.

The principal reagent, Kami-bang-pung-tong-sung-san (KBPTS) which was used in the present study, is the modified prescription of BPTS in which Spatholobi Clulis and Salviae Miltiorrzae Radix were added in order to eliminate congestive morphology in diverse tissues due injuries. Abnormal regulations of cardiovascular system including altered levels of catecholamines in the blood have been noted in SHR. Here, we examined the possible effects of KBPTS as a 'blood clearing agent' on the cardiovascular system of SHR. To investigated the potential role of KBPTS on hypertension, we examined whether there are any changes in parameters related to hypertension in KBPTS-treated SHR from control animals. We determined whether KBPTS affects on the blood pressure and hormones in the SHR. We also examined superoxide dismutase (SOD) levels in blood vessels, adrenal cortex, and kidney by immunohistochemical staining method. Our data suggest that KBPTS has an effect for the cardiovascular system by regulating pathophysiological parameters related with hypertension.

Materials and Methods

1. Materials

1) Experimental animals

Spontaneously hyperactive rat (SHR) was purchased from the Korea Experimental Animal Center. Animals were fed with food pellets (; composed of crude protein 21%, crude fats 8%, crude carbohydrate 5%, mineral 8%, calcium 0.6%, phosphorous 0.4%; Samyang animal food Co.) and water, and adapted for at least 2 weeks before the experiment in the animal room with 22 ± 2℃, relative humidity of 50 ± 10%, with 12 hours of day and night cycle and an luminescence of 150 - 300 Lux. Only healthy animals showing normal body weight increase were used for the experiment.

2) Drugs

KBPTS used in the present study was obtained from Daejeon University Oriental Medicine Hospital. One seal of KBPTS has the composition as shown in the Table 1. Augmented KBPTS was prepared by adding 4 g of Spatholobi caulis (Gye-Hyul-Deung) and Salviae miltirrhizae radix (Dan-Sam) each. These two components are recognized to remove effectively congestive damages caused by various insults.

Ten seals of KBPTS were suspended in 2 liters of water, heat-extracted for 3 hr, and filtered three times. The filtered fluid was distilled using the rotary vacuum evaporator. Concentrated solution was frozen at -70℃ for 4 hr, and freeze-dried for 24 hr. The yield of the powder after freeze-drying was an average of 10.75 g per each seal. The powder was diluted with physiological saline and used for the present experiment.

Table 1. The Compositions of KBPTS Extracts

Conventional name	Herb name	amount (g)
Cnidii Rhizoma	川芎	4.0
Ledebouriellae Radix	防風	4.0
Angelicae gigantis Radix	當歸	4.0
Paeonia Radix Alba	芍藥	4.0
Menthae Herba	薄荷	4.0
Forsythiae fructuse	連翹	4.0
Ephedrae herba	麻黃	4.0
Natrii sulfas	芒硝	4.0
Rhei Radix ET Rhisoma	大黃	4.0
Gypsum Fibrosum	石膏	4.0
Platycodi Radix	桔梗	4.0
Scutellariae Radix	黃芩	4.0
Atractylodis Macrocephalae Rhizoma	白朮	3.0
Nepetae Herba	荊芥	3.0
Talcum	滑石	10.0
Gardeniae Fructuse	山梔子	3.0
Glycyrrhizae Radix	甘草	2.0
Spatholobi Clulis	鷄血藤	6.0
Salviae Miltiorrzae Radix	丹參	6.0
Total Amount		81

3) Chemicals and Instruments

Chemicals were obtained from following sources; normal saline and gerorane (Enflurane reagent, Joong-Wei Pharmaceutical Co. Inc., Korea), xantopren VL (Bayer Dental, Japan), optosil-Xantopren activator (Bayer Dental, Japan), aldosterone RIA diagnostic kit (Abbott Co., U.S.A.), superoxide dismutase (Stressgen Co., U.S.A.), histostain plus kit (Zymed Co., U.S.A), Following chemicals were all purchased from Sigma (USA); sodium citrate, 2,3,5-triphenyl-2H-tetrazoliumchloride, cresyl fast violet, paraformaldehyde, H_2O_2, formalin, glutaraldehyde, OsO_4, toluidine blue, hematoxylin, and eosin.

Experimental instruments and apparatus used in this study were as follows; serum separator (Green Cross Co., Korea), minus-ST (Cobras Co., France), centrifuge (Beckman Co., U.S.A.), rotary vacuum evaporator (Buhl 461, Switzerland), deep freezer (Sanyo Co., Japan), freeze dryer (Eyelid Co., Japan), autoclave (Hirayama, Japan), ultrasonic cleaner (Branson ultrasonics Corp., U.S.A.), roller mixer (Gowon scientific technology Co., Korea), vortex (Vision Co., Korea), brain matrix (ASI Instrument, U.S.A.), Royal Multi-Plus (Royal Medical Co., Korea), ACL-100 (Instrumentation Laboratory, U.S.A.), physiograph Model 7 (Grass Instrument Co., U.S.A.), optical microscope (Olympus BH-2., Japan), and scanning electron microscope (Hitachi S-2500, Japan).

2. Methods

1) Measurement of blood pressure and heartbeat rate in spontaneously hypertensive rat (SHR).

KBPTS (358 mg/kg) or saline vehicle was injected on a daily basis for 5 weeks into SHR (n = 5 for each group), and then the animal was placed in the cage for 2 hr for relaxation. Blood pressure was measured using 7P8 channel of the physiograph Model 7 (Grass Instrument Co. Quincy, Mass., USA) and quantitatively analyzed using the chart papers.

2) Measurement of plasma aldosterone and catecholamines

SHRs were sacrificed with an overdose of ether, and 1 ml of blood was collected from the collarbone vein. Blood samples in the presence of EDTA (0.5 ml of 3 mg/ml) were centrifuged at 3000 rpm at 4℃ for 15 min to separate plasma. Aldosterone was labeled with I^{125} isotope by using aldosterone Diagnostic kit (DPC Co., USA), and the emitted gamma radioactivity was quantitated with Gamma count Cobra II (Packard Co. USA). Catecholamines were extracted from blood as follows. After removing the plasma protein by 0.1 M $HClO_4$ treatment, the sample was adsorbed to clean alumina, washed with distilled water, and extracted with 0.1 M $HClO_4$. Then, the extract (20 μl) was injected into the HPLC (High Performance Liquid Chromatography; Waters Model U6K Injector, 510 pump) to measure norepinephrine, epinephrine, and dopamine contents. These chemicals were quantitated by using the Data module instrument (Waters model 745). The voltage applied to C18 stainless steel column was +0.63 volts. All reagents including water were the highest quality available.

3) Histological examinations

(1) Animal surgery and tissue preparation

The animals were starved for 12 hr before the experiment. Animals were then anesthetized with ether, and the blood was collected from the abdominal artery. The heart, aorta, kidney, and adrenal gland were dissected, washed with saline, minced into the small pieces, and fixed in 10% paraformaldehyde solution for 48 hr. After washing with water and dehydrating with ethanol, tissues were histocleared with xylene treatment and penetrated into paraffin to prepare 4 μm thickness sections. Sections were stained with hematoxylin and eosin (H&E), observed under bright-field microscope, and photographed.

(2) Immunohistochemistry; SOD immunostaining

Sections (4 μm thickness) were thaw-mounted onto the glass slide, hydrated, and washed with PBS for 5 min. They were treated with 3% H_2O_2 solution for 5 min to block endogenous hydrogen peroxide, washed with PBS, and incubated with serum blocking solution for 10 min at room temperature. Primary antibody reaction was performed by adding anti-superoxide dismutase (SOD) antibody (1:500 dilution) for 1 hr at room temperature and washed with PBS. Sections were then incubated with biotinylated anti-mouse secondary antibody for 10 min, washed with PBS, and reacted with streptavidin-conjugate for 10 min. After washing with PBS for 10 min, sections were treated with DAB chromogen, and counterstained with Mayer hematoxylin. The cover-slipped slides were examined under the bright-field microscope (Olympus BH-2).

(3) Scanning electron microscopy (SEM)

The superior aorta was dissected from 5 mm segments inferior to the arch of aorta. Isolated aorta was rinsed with saline solution, fixed with 2.5% glutaraldehyde and washed with PBS three times for 20 min. The tissues were postfixed using 1% OsO_4 for 2 hr, dehydrated with increasing concentrations of ethanol (from 60% to 100%), and freeze-dried using Eko-1. The sample was coated with gold and observed under scanning electron microscope (Hitachi S-2500).

4) Statistical analysis

Number data among groups were compared by unpaired Student's t-test or analysis of variance (ANOVA) using SPSS/PC statistical program. A criterion for statistical significance was assessed at $p < 0.05$, $p< 0.01$, and $p < 0.001$.

Results

1. Effects of KBPTS on cardiovascular functions in the spontaneously hypertensive rat (SHR)

To examine whether KBPTS affects on cardiovascular function, several pathophysiological parameters including blood pressure, heartbeat rate, and several blood hormones were measured in SHR. As shown in Table 2, values of blood pressure and the heartbeat rate of KBPTS-treated groups were decreased compared to the corresponding vehicle-treated SHR animals.

Aldosterone levels in the plasma of KBPTS-treated and control SHRs were 10.9±0.2 pg/㎖ and 27.2±1.1 pg/㎖ respectively, indicating a significant decrease in KBPTS-treated SHR. Measurement of catecholamines showed some differences between KBPTS- and saline-treated control animal groups (Table 3). Dopamine levels in KBPTS-treated animals and saline-treated animals were 74.9±0.5 pg/㎖ and 106.4±8.8 pg/㎖ respectively. Norepinephrine levels in the plasma were 628.2±101.3 pg/㎖ for KBPTS-treated group and 589.6±163.2 pg/㎖ for saline controls, indicating no significant difference between two groups. In contrast, levels of epinephrine in the KBPTS group (787.2±50.0 pg/㎖) were significantly lower than that of the control group (5060.4±670.2 pg/㎖). These data

suggest that treatment of KBPTS in SHR contributes to decrease levels of aldosterone and catecholamine hormones such as dopamine and epinephrine

Table 2. The Effects of KBPTS on blood pressure and heart rate

	Control	KBPTS
Blood pressure (㎜Hg)	173.3±3.3	157.5±2.5
Heartbeat rate (pulse/min)	400.0±30.6	375.0±25.0

Control : Saline treated group, KBPTS : KBPTS (358 ㎎/㎏)-treated group

Table 3. The Effects of KBPTS on the plasma levels of catecholamines in SHR

Catecholamines	Control (pg/ml)	KBPTS (pg/ml)
Aldosterone	27.2±1.1	10.9±0.2***
Dopamine	106.4±8.8	74.9±0.5**
Norepinephrine	589.6±163.2	628.2±101.3
Epinephrine	5060.4±670.2	787.2±50.0**

Control: Saline treated group, KBPTS: KBPTS (358 ㎎/㎏)-treated group,
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

2. Effects of KBPTS on rat adrenal cortex morphology

In saline-injected SHR animals, cell sizes in the adrenal cortical layer were small and spindle-shaped forming 10-13 laminated layers. Cells were forming groups and dilated capillaries were observed between cells. Nuclei were small and irregularly shaped with heavily-stained particularly in the nucleosomes. Numerous fats and small granular bodies were found in the cytoplasm. In KBPTS animals, organization of 10 - 13 spindle cell layers of the adrenal cortex was similar to that of saline control. The nucleus was a little bit swollen, but the overall shape including nucleosomes was similar. Any capillary dilation was not observed. Levels of fat granules in the cytoplasm were found to be significantly reduced compared to saline control(Fig. 1).

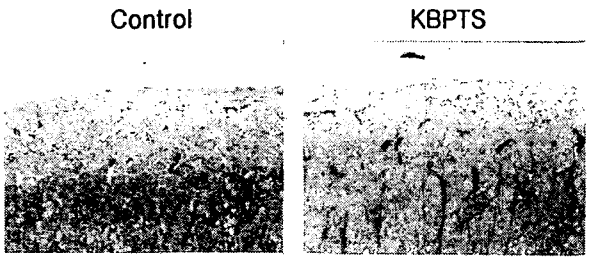

Fig. 1. Histological comparison of zona glomerulosa of adrenal cortex of SHR treated with either saline (Control) or KBPTS. Bright-field photographs were taken after staining tissues with 0.5% toluidine blue (400x amplification).

3. Comparison of SOD immunoreactivity between KBPTS-and saline- treated SHRs

Levels of superoxide dismutase (SOD) were investigated in KBPTS treated animals in several tissues of SHR. In adrenal gland, strong DAB chromogenic reaction was found in some adrenal medullary cells in both saline- and KBPTS-treated animals. Yet, any chromogenic reactions were not observed in the adrenal cortex. Staining intensity was stronger in the nucleus of KBPTS groups than in saline, but the staining pattern was similar in the cytoplasm in both groups. Overall staining intensity was slightly stronger in KBPTS group than in saline control (Fig 2A and B). In the aorta, SOD immunoreactivity was evenly high throughout the cytoplasm of vascular smooth muscle cell layer in both groups, but overall staining intensity was higher in KBPTS group compared to control group (Fig 2C and D). SOD immunostaining showed strong DAB immunochrogenic reactivity in cardiac muscle cells of both left- and right ventricles. Signals were observed in the vesicles peripheral to the nucleus, but the antigen-antibody reactivity was not detected in myocardial cells. Overall positive reactivity was stronger in KBPTS group than saline control (Fig 2E and F). SOD immunoreactivity in the kidneys was slightly positive in the cytoplasm of some of the glomerular mesangial cells. Positive reactivity was found in a few cytoplasms and all nuclei of the proximal tubules, but only in the cytoplasm of the distal tubules. Strong reactivity was also detected in the nucleus and cytoplasm of epithelial cells of the proximal tubules in the vicinity of the border between cortex and medula, but any clear signals were not observed in medullary tubules. Overall, stronger signals were observed in KBPTS group compared to control group (Fig 2G and H).

4. SEM microscopic observation

To further investigate changes in tissue morphologies in KBPTS-treated SHR, we examined the structures of the superior aorta using scanning electron microscopy (SEM). In saline control group, endothelial cells in the superior aorta were elevated above the surface. Also, cells were detached and some of endothelial cells, platelet, and white blood cells were sloughed or lost. Damaged endothelial cells were partly lost and the basement membrane area was exposed with forming various sizes of pores between epithelial cells. Damaged area occupied one third of the lumen surface (Fig 3A). In KBPTS-treated animals, epithelial layer of superior aorta was mostly elevated and composed of squamous cells. Although some damages in the vascular epithelia cells were observed, overall surface structure was clean. Platelet aggregation and adhesion of while blood cells were observed in some areas where injury was observed (Fig 3B). Thus, it was concluded that overall damage in KBPTS treated animals was much reduced compared to control animals.

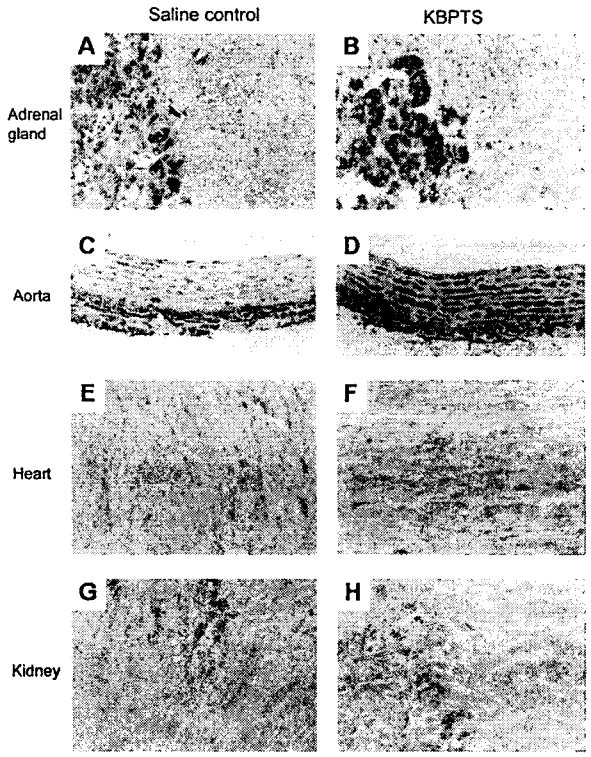

Fig. 2. Immunohistochemical staining of different tissues of SHR with anti-SOD antibody. Sections of adrenal gland (A, B), aorta (C, D), heart (E, F), and kidney (G, H) tissues were prepared from SHR treated with either saline (A, C, E, and G) or KBPTS (B, D, F, H). Bright-field photographs (200x amplification) were taken from sections after immunostaining (see Materials and Methods).

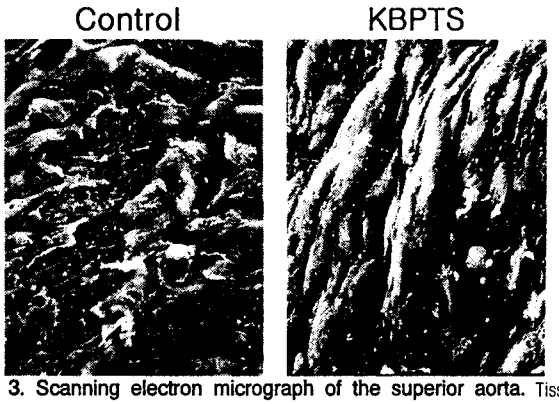

Fig. 3. Scanning electron micrograph of the superior aorta. Tissues were dissected from SHR treated with either saline (Control) or KBPTS and photographed (1000x amplification)

Discussion

KBPTS has been used in the oriental medicine for the treatment of several diseases associated with inflammatory abnormalities in cardiovascular and nervous systems. In the present study, we found that KBPTS has an effect on decreasing blood pressure and heartbeat rate and also lowering hormonal levels in blood of spontaneously hypertensive rats (SHR). Immunohistological examinations revealed increased levels of SOD protein in heart, blood vessels, kidney, and adrenal cortex which are all directly or indirectly related with the hypertension incidence.

Since KBPTS is a mixture prescription containing more than 10 kinds of herbs (see Table 1), its effects on cardiovascular system cannot be easily predicted. Our experimental approach was therefore to examine its effect using well-characterized experimental model system. SHR is a genetic model for primary hypertension in humans, which has shown that the angiotensin II system is hyperactive as a result of an increased expression of angiotensin I receptors[16,20,21,22,24,27]. Therefore, SHR has been widely used to understand pathophysiological factors related to hypertension. Abnormal regulation of angiotensin system in SHR is associated with alterations of catecholamine levels in the nervous system as well as in the circulation system. Since KBPTS has been recognized for its efficacy for the treatments of cardiovascular diseases, a role of KBPTS in alleviating physiological abnormalities in SHR is the possibility. We found that in rats administered with KBPTS, blood pressure and heartbeat rate were significantly reduced compared to the control animals, and KBPTS lowered levels of aldosterone, dopamine, and epinephrine in blood. Adrenal medulla secretes epinephrine and norepinephrine in response to sympathetic stimulation, and adrenal cortex secretes steroid hormones including aldosterone. Histological comparison of adrenal gland between KBPTS- and saline-treated SHRs showed that they were overall similar except that less fat granules in KBPTS-treated animals were noted, raising the possibility that reduced epinephrine levels in blood might be associated with decreased neuronal inputs, rather than changes in adrenal tissues (see below). Epinephrine, dopamine, and norepinephrine are important for regulating cardiovascular activity and enhancing neuronal activity[7,10,1,13]. Both cardiac output and blood pressure are increased by sympathetic stimulation via the action of epinephrine and norepinephrine. Aldosterone increases blood pressure by increasing blood vessel contraction and also by increasing sodium retention in the kidney. Thus, decreased levels of these hormones in blood could contribute to decrease in the blood pressure and heartbeat rate.

Superoxide dismutase (SOD) is an enzyme which converts reactive superoxide to hydrogen peroxide, thereby reducing the free radical toxicity[15]. Previous studies have shown that superoxide is increased in the vessel wall of SHR, and if the superoxide levels are blocked, vasodilation occurs via the activation of nitric oxide synthesis pathways. Other studies showed abnormal regulation of activation of angiotensin II-dependent oxidative stress in SHR, which causes

reduced efficiency of oxygen usage[25]. These and other studies strongly suggest that increased production of superoxide in SHR plays a key role in activation of endothelial cells and thus vasodilation of the blood vessels, one of the pathophysiolgical causes of hypertension. Our immunohistochemical study showed that KBPTS treatment increased SOD levels in adrenal gland, aorta, heart and kidney compared to saline-treated SHR. These data imply that KBPTS might play a role in reducing basal levels of reactive oxygen species in several tissues and may protect tissues from cytotoxic insults, which is consistent with previous reports[12,14,17,26]. This interpretation is also supported by our electron microscopic observation of aorta tissues; basal levels of tissue damage in superior aorta were found to be lower in KBPTS-treated rats than in saline control group. Alternatively, reduced levels of superoxide by increased SOD activity in SHR could decrease angiotensin II activity and reduce vasodilation. Thus, decreases in epinephrine levels in blood, heartbeat rate, and blood pressure all could be related to changes in SOD enzyme activity.

At this moment, we do not know by what mechanism(s) KBPTS increases SOD protein levels. Although it is a possibility that the elevated levels of SOD immunoreactivity in several tissues could be contributed to increased SOD gene expression, that is, increased transcriptional activity and/or increased mRNA stability, increased protein stability, or decreased degradation rates, further studies are essential to define molecular targets of KBPTS in SOD production machinery in several cell types including blood vessel cells. Finally, KBPTS treatment could increase SOD levels in brain tissues[23]; KBPTS, by regulating neuronal activity, might be involved in controlling autonomic nervous system related to contraction and relaxation of cardiac and smooth muscle cells of the blood vessels. Indeed, our studies on KBPTS effects on the neuronal cells showed that KBPTS protects neuronal cells from excitotoxic stimulation or by middle cerebral artery occlusion (Na et al., in this issue). Taken together, KBPTS seems to be important for maintaining several tissues less susceptible to intrinsic or extrinsic noxious stimulations, which could further contribute to homeostatic control of organ functions.

Conclusion

By using genetic animal model of hypertension, we examined whether KBPTS affects physiological parameters in the cardiovascular system. It was found that KBPTS lowered blood pressure and heartbeat rate, elevated SOD enzyme levels in several tissues including cardiovascular vessels. While these data are interesting and imply potential role for improving cardiovascular abnormalities in human, further basic investigation is mandatory. By characterizing major chemical compositions of KBPTS first, potentially useful chemical components should be sorted out from other dispensible components and be used for molecular studies. For instance, potential target site of KBPTS on the angiotensin system in SHR can be examined by using in vitro primary culture of vascular epithelial cells. Future studies would be essentially required to understand interactions of KBPTS or its defined chemical components with molecular factors on the target tissues.

References

1. Arai N, Furukawa N, Miyamae T, Goshima Y, Sasaki Y, Ohshima E, Suzuki F, Fujita K, Misu Y. : DOPA cyclohexyl ester, a competitive DOPA antagonist, protects glutamate release and resultant delayed neuron death by transient ischemia in hippocampus CA1 of conscious rats. Neurosci Lett. 299(3):213-6, 2001.
2. Barone FC, Price WJ, White RF, Willette RN, Feuerstein GZ. : Genetic hypertension and increased susceptibility to cerebral ischemia. Neurosci Biobehav Rev 16:219-233, 1992.
3. Barone FC, Schmidt DB, Hillegass LM, Price WJ, White RF, Feuerstein GZ, Clark RK, Griswold DE, Sarau HM. : Reperfusion increases neutrophil and LTB4 receptor binding in focal ischemia. Stroke 23:1337-1348, 1992.
4. Barone FC, Clark RK, Price WJ, White RF, Storer BL, Feuerstein GZ, Ohlstein EH. : Neuron specific enolase increases in cerebral and systemic circulation following focal ischemia. Brain Res 623:77-82, 1993.
5. Brint S, Jacewicz M, Kiessling M, Tanable J, Pulsinelli W. : Focal brain ischemia in the rat: methods for reproducible neocortical infarction using tandem occlusion of the distal middle cerebral artery and ipsilateral common carotid arteries. J Cereb Blood Flow Metab 8:474-485, 1988.
6. Buchan AM, Xue D, Slivka A. : A new model of temporary focal neocortical ischemia in the rat. Stroke 23:273-279, 1992.
7. Calabresi P, Centonze D, Pisani A, Cupini L, Bernardi G. : Synaptic plasticity in the ischaemic brain. Lancet Neurol. 2(10):622-9, 2003.
8. Chan P, Liao SS, Hsu CT, Lee YS, Tomlinson B, Kuo JS, Cheng JT. : Superoxide dismutase gene expression and activity in the brain of spontaneouslyhypertensive rats and normotensive rats. Chin Med J(Engl). 112(12): 1119-24. 1999
9. Clark RK, Lee EV, Fish CJ, White RF, Price WJ, Jonak ZL,

Feuerstein GZ, Barone FC. : Progression of cerebral changes following middle cerebral artery occlusion in the rat: a quantitative planimetric, histologic, and immunohistochemical study. Brain Res Bull 31:565-572, 1993.

10. Ding H, Zhou Q, Deng J, Lao HY, Yang K. : Effect of the overactivated central renin-angiotensin system on the concentration of brain norepinephrine and epinephrine in stroke-prone spontaneously hypertensive rats and its significance. Sheng Li Hsueh Pao. 42:379-384, 1990.
11. Duverger D, MacKenzie T. : The quantification of cerebral infarction following focal ischemia in the rat: influence of strain, arterial pressure, blood glucose concentration, age. J Cereb Blood Flow Metab 8:449-461, 1988.
12. Fullerton HJ, Ditelberg JS, Chen SF, Sarco DP, Chan PH, Epstein CJ, Ferriero DM. : Copper/zinc superoxide dismutase transgenic brain accumulates hydrogen peroxide after perinatal hypoxia ischemia. Ann Neurol. 44(3):357-64, 1988.
13. Hashimoto N, Matsumoto T, Mabe H, Hashitani T, Nishino H. : Dopamine has inhibitory and accelerating effects on ischemia-induced neuronal cell damage in the rat striatum. Brain Res Bull. 33(3):281-8, 1994.
14. Hayashi T, Saito A, Okuno S, Ferrand-Drake M, Dodd RL, Nishi T, Maier CM, Kinouchi H, Chan PH. : Oxidative damage to the endoplasmic reticulum is implicated in ischemic neuronal cell death. J Cereb Blood Flow Metab. 23(10):1117-28, 2003.
15. Kensuke Murakami, Takeo Kondo, Makoto Kawase, Yibing Li, Shuzo Sato, Sylvia F. Chen, and Pak H. Chan : Mitochondrial Susceptibility to Oxidative Stress Exacerbates Cerebral Infarction That Follows Permanent Focal Cerebral Ischemia in Mutant Mice with Manganese Superoxide Dismutase Deficiency. J. Neurosci., 18: 205-213, 1998.
16. Langheinrich M, Lee MA, Bohn M, Pinto YM, Ganten D, Paul M. : The hypertensive Ren-2 transgenic rat TGR (mREN2)27 in hypertension research. Characteristics and functional aspects. Am J Hypertens 9:506-512, 1996.
17. Lee JM, Zipfel GJ, Choi DW. : The changing landscape of ischaemic brain injury mechanisms. Nature. 399(6738 Suppl):7-14, 1999.
18. Lee, YM : Effect of Bangpoongtongsungsan on the Hypertention induced by L-NAME injection and SHR in Rats), Dongeui University, Graduate School thesis, 1999.
19. Mazzon, S, Dugo, E, Di L, Paola R, Caputi AP, Salvemini D. : Superoxide: a key player in hypertension. FASEB J. 18(1): 94-101. 2004
20. Phillips MI. : Brain angiotensin. In: Circumventricular organs and body fluids, Vol III (Gross P, ed), pp 163-182, 1987.
21. Raizada MK, Lu D, Sumners C. : AT1 receptors and angiotensin actions in the brain and neuronal cultures of normotensive and hypertensive rats. In: Current concepts: tissue renin-angiotensin system as local regulators in reproductive and endocrine organs (Mukhopadhyay A, Raizada MK, eds), pp 331-348, 1994.
22. Raizada MK, Lu D, Yang H, Richards EM, Gelband CH, Sumners C. : Brain angiotensin receptor subtypes and their coupling to distinct signal transduction pathways. In: Advances in molecular and cellular endocrinology, Vol 3 (LeRoith D, ed), pp 73-79. Greenwich, CT: JAI, 1998.
23. Ruetzler CA, Furuya K, Takeda H, Hallenbeck JM. : Brain vessels normally undergo cyclic activation and inactivation: evidencefrom tumor necrosis factor-alpha, heme oxygenase-1, and manganese superoxidedismutase immunostaining of vessels and perivascular brain cells. J Cereb Blood Flow Metab. 21: 244-252. 2001
24. Sumners C, Raizada MK, Kang J, Lu D, Posner P. : Receptor mediated effects of angiotensin II on neurons. Front Neuroendocrinol 15:203-230, 1995.
25. Welch WJ, Mendonca M, Aslam S, Wilcox CS. : Roles of oxidative stress and AT1 receptors in renal hemodynamics andoxygenation in the postclipped 2K,1C kidney. Hypertension. 41: 692-669. 2003
26. Wengenack TM, Curran GL, Poduslo JF. : Postischemic, systemic administration of polyamine-modified superoxide dismutase reduces hippocampal CA1 neurodegeneration in rat global cerebral ischemia. Brain Res. 754(1-2):46-54, 1997.
27. Yang K, Ding H, Zhou Q, Luo HY, Wu ZY. : Central norepinephrine and angiotensin II contents in the brain regions of spontaneously hypertensive rats (SHR) and the interaction between them. Sheng Li Hsueh Pao 43:345-351, 1991.