

Antioxidant Synergism among Component Herbs of Traditional Chinese Medicine Formula, ShengMai San Studied *In Vitro* and *In Vivo*

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ShengMai San (SMS) comprising three herbal components (*Panax Ginseng*, *Ophiogon japonicus* and *Schisandra chinensis*) is one of the most popular traditional Chinese medicine prescriptions, having been used for more than 3000 years in China to treat symptoms related to Qi depletion and cardiac disorders. In the present study, antioxidant synergism among the component herbs of SMS was precisely examined *in vitro* using extracts prepared from respective component herbs and their possible combinations under high hydrostatic pressure (600 Mpa). Expression of antioxidant enzymes in brain was also studied in rats administered those extracts by western blotting method. It was revealed that complete SMS formula had the highest potential both in scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals *in vitro*, and also in the expression of heme oxygenase-1 (HO-1), superoxide dismutase (SOD) and cytosolic glutathione peroxidase (GPx) proteins in brain of rats administered the extract for five days. Certain synergistic interactions among the component herbs were observed in the antioxidant activity *in vitro* and also in the antioxidant enzyme expression *in vivo*. Correlation analysis between *in vivo* and *in vitro* data revealed that expression of HO-1 and SOD were highly related to *in vitro* DPPH and superoxide radical scavenging activities with significant correlation coefficients of 0.899 and 0.758, respectively. Expression of GPx showed weak correlation with all the *in vitro* antioxidant indicators. It was concluded that the combination of three herbs are essential to confer the maximal antioxidant potential to the SMS formula, and that SMS exerts potential antioxidant activity *in vivo* through its inherent radical scavenging activity and also the modulation of expression level of antioxidant enzymes.

Key words— traditional Chinese medicine, ShengMai San, antioxidant synergism, high pressure preparation, antioxidant enzyme

INTRODUCTION

Traditional Chinese Medicine (TCM) is a natural therapeutic agent used under the guidance of the theory of traditional Chinese medical science. It has been widely used for the prevention and treatment of complex disorders since antiquity in China. TCM is usually prescribed with several herbs, at least two in combination and is provided in the form of granule or concentrated extract for easy use and quality control.^{1,2)} However, factors in the process of the TCM modernization policy in China

is not satisfactorily controlled, such as low quality control criteria, unknown combination mechanisms, unsteady processing technology, harvest and storage conditions, and so on.^{3,4)} Chemotaxonomy has been used as one approach to control the quality but such activity as antioxidant activity can be an alternate criterion of TCM. Elucidation of the underlying mechanism involved in synergism among component herbs in the formula is also one of the most important targets of study for making better usage of TCM, since understanding of the mechanism of herbal combinations can explain the role of every herbal constituent or its dose in the pharmacological activity of certain TCM preparations and is advantageous for controlling its inherent qualities.^{5–8)}

ShengMai San (SMS) is a typical traditional TCM prescription comprising three herbal compo-

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nents, *Panax Ginseng*, *Ophiopogon japonicus* and *Fructus schisandrae* in a 2:2:1 ratio. It has been clinically used for the treatment of shock caused by cardiovascular diseases, contractile heart failure and myocardial ischemia.⁹⁾ Our recent research has proved that SMS is a strong antioxidant both *in vitro* and *in vivo*, and it can effectively prevent brain oxidative injury caused by ischemia-reperfusion in rats.^{9, 10)} In the present study, extracts were prepared from each component herb and their four possible combinations under a high hydrostatic pressure condition at 600 mega Pascal (MPa), and their *in vitro* antioxidant potentials and the modulation effects on cellular antioxidant enzymes in rats were examined in detail to analyze the synergistic interaction among the component herbs in the SMS prescription.

MATERIALS AND METHODS

Chemicals and Herbal Materials—Dimethylpyrroline oxide (DMPO) was purchased from LABOTEC Co. Ltd. (Tokyo, Japan) and FeSO₄ from Kanto Chemical Co., Ltd. (Osaka, Japan), respectively. Xanthine oxidase (XOD, 20 u/ML, from cow's milk) and 6-hydroxyl-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were obtained from Boehringer Mannheim Co. (Phoenix, AZ, U.S.A.), Germany and Aldrich Chem. Co. (Saint Louis, MO, U.S.A.), respectively. All other chemical reagents including diethylene triamine-*N,N,N',N'',N'''*-penta acetic acid (DTPA) and 1,1-diphenyl-2-piclyhydrazyl (DPPH) were purchased from Wako Pure Chemical Industries Co. Ltd. (Osaka, Japan). BCA protein assay reagent was purchased from Pierce Chemical Co., Ltd. (Rockford, IL, U.S.A.).

Prestained sodium dodecyl sulfate-polyacrylamido gel electrophoresis (SDS-PAGE) standards (broad range) were purchased from Bio-Rad Laboratories (Sandiego, CA, U.S.A.). HyperfilmTM, ECLTM western blotting analysis system kit and HybondTM-ECLTM nitrocellulose membrane were purchased from Amersham Biosciences, Ltd. (Chalfont, U.K.). The primary antibodies for rabbit anti-heme oxygenase-1 (HO-1) and mouse anti-tubulin (internal control protein) were purchased from Stressgen Biotechnologies (Columbia, Canada) and Sigma Co. (Saint Louis, MO, U.S.A.), respectively. The primary antibodies for rabbit anti-superoxide dismutase (SOD) and

mouse anti-glutathione peroxidase (GPx) were obtained from EMD Biosciences Inc. (Darmstadt, Germany) and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, U.S.A.), respectively. All the corresponding secondary antibodies used in this experiment were from Zymed Laboratories Inc. (San Francisco, CA, U.S.A.).

Constituent herbs of SMS, *Ophiopogon japonicus* (M), *Panax Ginseng* (R) and *Schisandra chinensis* (W) were generously provided by Kotaro Kampo Pharmaceutical Co. Ltd., Osaka, Japan (Their Chinese names are Maimendong, Renshen and Wuweizi so that M, R and W were given as their abbreviations, respectively).

Preparation of SMS and Other Incomplete Formula under High Hydrostatic Pressure

Defined amounts of each component herb and their possible combinations described in Table 1 were suspended in 15 ml of distilled water, degassed by argon blow and sealed under vacuum in a plastic bag. After soaking overnight at 4°C, the samples were subjected to high hydrostatic pressure treatment at 600 MPa for 5 min using a high pressure experimental system made by Kobe Seiko Co. (Kobe, Japan). Then samples were filtered through a double layer of gauze and the filtrates were stored at 4°C until use. For the analysis of antioxidant activity, the sample concentration was adjusted to 0.33 g/ml for *in vitro* and 0.67 g/ml for the *in vivo* experiments, respectively.

Animal Treatment—Male ddY mice (6 weeks old and 28–30 g body weight) were purchased from SLC Inc. (Shizuoka, Japan). Animals were maintained under 12 hr light/dark cycle at a temperature approximately 24 ± 1°C. During the experiments, all animals were fasted from 9:00 AM to 7:00 PM and were allowed free access to a pellet diet and water *ad libitum* from 7:00 pm to 9:00 am every day. In this experiment, animals were randomly assigned

Table 1. Herbal Composition of SMS and Related Incomplete Formulae

Group	Herbal composition and dose
W	W (1 g)
R	R (2 g)
M	M (2 g)
WR	W (1 g) + R (2 g)
WM	W (1 g) + M (2 g)
RM	R (2 g) + M (2 g)
SMS	W (1 g) + R (2 g) + M (2 g)

SMS: ShengMaiSan (W + R + M); W: *Schisandra chinensis*; R: *Panax Ginseng*; M: *Ophiopogon japonicus*

into 8 groups ($n = 6$ for each group). Group 1 (control group): the same volume of water as SMS preparation was given orally by incubation as the vehicle. Group 2 to group 8 (TCM-treated groups): SMS and related preparations (10 g herb/kg body weight for complete SMS and the equivalent dose for other preparations as described in Table 1) were orally given by intubation at 7:00 pm for 5 days. 12 hr after the last administration of the test samples, brain was removed under anesthesia induced by pentobarbital, washed in a saline and immediately snap-frozen in liquid nitrogen. All the tissue samples were stored at -80°C before use.

Tissue Homogenate Preparation— Brain tissue (0.1 g) was suspended in 1 ml cold 0.05 M phosphate buffer and then homogenized at 0°C using an ULTRA-TURRAX homogenizer (KIKA Labortechnik, Hamburg, Germany). Protein concentrations of the homogenates were determined by BCA protein assay kit (Pierce Co.,) using bovine serum albumin (BSA) as a standard.

In Vitro Antioxidant Assays— Hydroxyl radical scavenging activity was determined as previously reported using Fenton reaction as the hydroxyl radical source.¹¹⁾ The DMPO-OH signal was determined by ESR for the reaction mixture (300 μl total volumes) containing 20 mM DMPO, 10 mM H_2O_2 and 0.1 mM FeSO_4 in the presence or absence of defined amounts of test samples.

Superoxide scavenging activity was measured according to the method of Finkelstein *et al.*¹²⁾ with a slight modification, using the hypoxanthine/XOD system as the superoxide radical generator. DMPO-OOH signals were determined by ESR for the reaction mixture (300 μl total volume) containing 1 mM DTPA, 0.5 mM hypoxanthine, DMPO and 0.1 U/ml XOD in 0.2 M phosphate buffer (pH 7.8) in the presence or absence of defined amounts of test samples.

DPPH radical scavenging activity was measured according to the method of Yoshida *et al.*¹³⁾ with minor modification as described.¹¹⁾ A defined amount of SMS preparations was incubated with 0.1 mM DPPH in 2 ml methanol/0.05 M phosphate (1 : 1, v/v) buffer (pH 7.6) for 10 min at room temperature. The kinetic decrease in DPPH absorbance

at 520 nm was determined for the first 10 min after addition of test samples.

In Vivo Antioxidant Assays— SDS solubilized protein samples (60 μg) were loaded on 14% (w/v) SDS-polyacrylamide gel, separated by electrophoresis, and then transferred onto a nitrocellulose membrane at 4°C . The membranes were treated in blocking buffer containing 5% fat-free milk powder, 10 mmol/l Tris-HCL (pH 7.6), 100 mmol/l NaCl and 0.1% Tween-20 (Tris Buffered Saline-Tween buffer) for 1 hr at room temperature and then incubated overnight at 4°C with corresponding primary antibody dilution (1 : 1000 for HO-1 and tubulin; 1 : 200 for GPx; 1 : 500 for SOD). The membranes were washed with TBS-T buffer, incubated with the 1 : 10000 dilution of the secondary antibodies for 1 hr at room temperature. The ECL Western blotting system was used for chemiluminescence observation of proteins according to the manufacturer's instructions, and the blots were then exposed to photographic film.

RESULTS

Antioxidant Activities of SMS Component Herb and Their Combinations

Herbal extracts from the three constituent herbs, R, M and W of SMS, and their possible combinations as shown in Table 1 were prepared by high-pressure extraction method described in Methods section, and their antioxidant potentials were assayed by DPPH quenching method. First, the extract preparation condition was examined. As shown in Table 2, DPPH scavenging activities of the extracts prepared under different pressure conditions were rather pressure-dependent so that the extract prepared at 600 MPa showed the highest activity comparable to the activity of decoction preparation. Thus the pressure level at 600 MPa was used for extract preparation in the following experiments. When the activity of the 600 MPa extract was evaluated by trolox as a reference antioxidant, the activity of the 600 MPa extract at 0.33 mg herb/ml concentration was equivalent to that of 0.15 mM trolox.

Table 2. DPPH Radical Scavenging Activity of SMS Extracts Prepared by High Pressure Method

Samples	SMS extracts prepared at different pressures (MPa)				SMS decoction
	0	200	400	600	
Relative trolox unit (mM)	0.114 \pm 0.02	0.099 \pm 0.03	0.123 \pm 0.03	0.150 \pm 0.02	0.148 \pm 0.02

Data were given as an average of five independent experiments.

Thus, data shown in Table 2 are expressed as relative trolox units.

Antioxidant activities of SMS and the related incomplete formulae were studied *in vitro* by three different antioxidant assays (DPPH quenching, hydroxyl radical scavenging and superoxide anion radical scavenging activities). The results were normalized against the activity of complete SMS formula in each assay and given in Fig. 1 as the activity profiles of each preparation relative to the complete SMS formula. It was noticed that the activity profile of these test samples was different in three different antioxidant assays. Each component herb has a rather characteristic feature in its radical scavenging profile: W showed strong activity toward DPPH and superoxide radicals but not toward hydroxyl radical. On the other hand, R showed moderate activity toward hydroxyl and superoxide radicals but not toward DPPH; M showed moderate activity toward all three radicals. Moreover, synergistic interactions were observed when these component herbs were mixed in combination. For example, R tended to suppress the activity of M and W in the case of DPPH and superoxide radical scavenging activities. Therefore, the DPPH scavenging activity of the RM combination showed the lowest activity, although the activity of M was moderate. R interacted additively with M and R in the hydroxyl radical scavenging activity so that RM and RW combinations showed rather stronger hydroxyl radical scavenging activity than each of them alone. There was no significant interaction between M and W in hydroxyl radical and DPPH radical scavenging activities but some additive interaction was observed in superoxide radical scavenging activity.

It was noted, however, that the complete SMS formula showed the highest antioxidant activity among all the preparations in DPPH and hydroxyl radical scavenging assays. Even in the superoxide radical scavenging assay, the complete SMS formula showed higher activity relative to other combinations or component herbs, although the highest activity was obtained in the WM combination.

Effect of SMS Components Herbs and Their Combinations on Antioxidant Enzyme Expression in Mouse Brain

SMS, its component herbs and their combination extracts prepared by high-pressure method were orally administered to mice for 5 days with doses equivalent to the complete SMS dose (10 g herb/kg body weight), and then the expression lev-

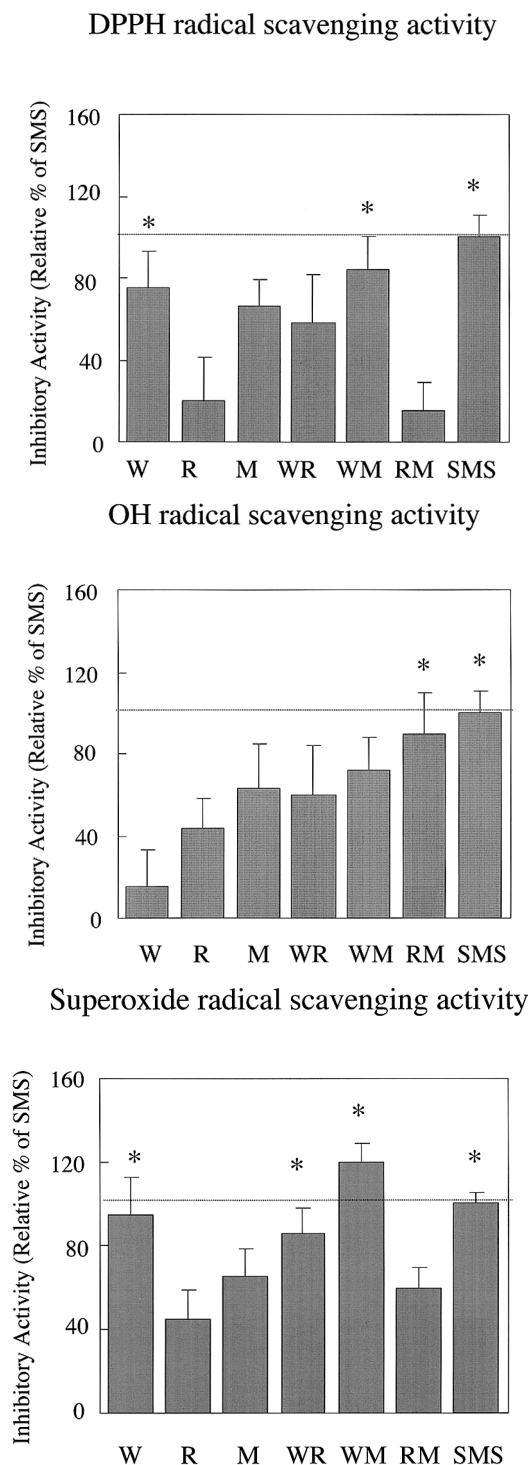


Fig. 1. *In Vitro* Antioxidant Activities of SMS Component Herbs and Their Combinations

The antioxidant activities of SMS and related formulae were determined by five different assay methods. Data are shown as relative % of the activity of complete SMS (as 100%) and given as an average of five independent experiments for each herb and combination. * $p < 0.05$, compared with control group.

els of antioxidant enzymes at the protein level were analyzed in brain by Western blot method using cor-

responding antibodies. Six Western blot determinations were carried out for each brain sample and averaged for six animals in a group. The expression level was normalized against tubulin as a reference. The results are shown in Fig. 2 as the relative expression level of control mice together with typical Western blot results for respective antioxidant enzymes.

It was found that, although W and R themselves rather suppressed HO-1 expression in the brain (38% and 69%, respectively), the expression of HO-1 was considerably enhanced in the mice group administered with complete SMS (170%), followed by WM and WR combinations (149% and 169%, respectively).

In the case of SOD expression, the expression level in the mice administered either respective component herb, R or M was low compared to control but WM and WR groups showed significant enhancement (220% and 187%, respectively). It was noted that complete SMS did not give rise

to significant effect on the SOD expression level. Interestingly, the SOD protein expression profile of seven combinations of SMS component herbs demonstrated here was rather similar to the superoxide radical scavenging activity profile of test samples shown in Fig. 1.

On the other hand, GPx expression was remarkably enhanced in the mice group administered complete SMS formula (193% of control). W itself and WR combination also significantly enhanced the GPx expression but the extents were smaller than that by SMS (142% and 125%, respectively). The profile of SOD protein expression by SMS and related preparations was quite similar to the activity profile of superoxide radical scavenging shown in Fig. 1.

Correlation between *In Vitro* Antioxidant Activity and *In Vivo* Antioxidant Enzyme Expression

The *in vitro* antioxidant activities of SMS component herbs and all their combinations were cor-

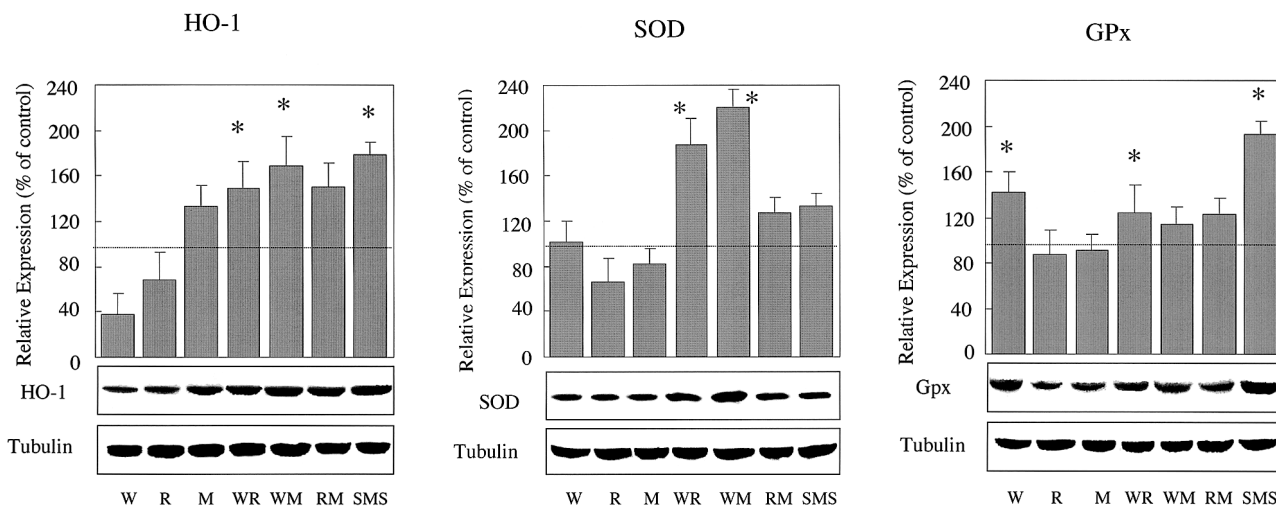


Fig. 2. Effects of SMS and the Related Incomplete Formulae on Antioxidant Gene Expression in Rat Brain
 Typical western blot results are given under the graph figures. Data are shown as relative % of expression of control group (as 100%) and given as an average of six animals in a group for each herb and combination. * *p* < 0.05, compared with control group.

Table 3. Correlation Coefficients between *In Vitro* Antioxidant Activities and *In Vivo* Antioxidant Enzyme Gene Expression

	<i>in vitro</i> antioxidant indicators		
	DPPH radical	Hydroxyl radical	Superoxide anion radical
HO-1	0.267	0.899*	0.346
SOD	0.396	0.344	0.758*
GPx	0.606	0.380	0.542

* *p* < 0.05, correlation is significant at the 0.05 level.

related to their effects on *in vivo* expression of antioxidant enzymes. Correlation analysis was performed by SPSS Software for Windows 98/NT (Version 11.0 from Statistical Product and Service Solutions, Chicago, IL, U.S.A.). As seen by the results summarized in Table 3, the expression profiles of HO-1 and SOD showed highly significant correlation with hydroxyl and superoxide radical scavenging activities, respectively (correlation coefficients of 0.899 and 0.758, respectively). However, GPx expression did not show any significant correlation with any of the three *in vitro* antioxidant indicators.

DISCUSSION

Traditional Chinese or Kampo medicines are prepared by decoction for use but the method includes several disadvantages such as humble processing and difficulty in controlling activity. In the present study, the application of high hydrostatic pressure was examined first for the preparation of SMS extracts. When the aqueous suspensions of herbal compositions of SMS were treated at different pressures (0, 200, 400 and 600 MPa), DPPH quenching activity of the extract was increased as applied pressure was elevated and the SMS extract obtained at 600 MPa showed almost the same magnitude of antioxidant activity as that of the decoction preparation (see the data in Table 2). The method was thus applicable to prepare a series of SMS related extracts under the same condition for the analysis of herbal interactions in SMS formulae.

We previously studied antioxidant activities of decoction preparations of SMS, its component herbs and their possible combinations *in vitro*, and discussed the synergism among the component herbs.¹⁴⁾ The antioxidant activity profile of the series of high-pressure extracts of SMS and the related preparations observed in the present study were essentially the same as that of the decoctions in that the complete SMS formula showed stronger than or the strongest activity among other SMS related incomplete formulae, and W played rather an important role in the antioxidant activity of SMS, WM and WR combinations, however, showed rather stronger activity than the complete SMS formula toward hydroxyl radicals in the present study. In addition to these characteristics of *in vitro* antioxidant activity, the present study further revealed that the expression level of antioxidant enzymes in the brain was modulated in the rats administered high pres-

sure extracts of SMS and related formulae, and the strongest modulating effects on HO-1 and GPx proteins expression were attained by the complete SMS formulation. These results suggest again that complete formulation is the best combination of component herbs for the SMS activity. Reliability of the complete SMS prescription was also supported by the antioxidant enzyme regulation characteristics in that SMS enhanced GPx expression but did not give rise to any enhancing effect on SOD, whereas incomplete combinations such as WM and WR remarkably enhanced SOD expression without enhancing GPx. Considering that the cooperative function of SOD and GPx is necessary for the sequential processing of Reactive Oxygen Species under physiological conditions, the SMS function observed here seems more physiological. It further suggests that the high-pressure extraction method is useful for the preparation of active extract of SMS and probably of other formulae at least in terms of antioxidant activity.

As we know, chemical constituents are the material basis for the efficacy in SMS. There are a variety of saponins and polysaccharides in R and M, and lignans in W. It has been reported that saponins in M and lignans in W showed certain antioxidant effects.¹⁵⁻¹⁸⁾ Further, it has been reported that levels and types of saponins released from R were different in all combinations, and some of them were easily decomposed by acidic constituents from W, while combination with M made R resistant to this decomposition and the presence of M also increased the level of effective constituents in W.^{19,20)} These observations are somewhat reflected in SOD and HO-1 protein expression profiles of the extracts *in vivo* and also *in vitro* DPPH and superoxide scavenging profiles observed here.

From the Western blot analysis of antioxidant defense enzymes (HO-1, SOD and GPx) in the brain of mice administered high pressure extracts of SMS constituent herbs and their combinations, it was interestingly observed that the tendency of both SOD and HO-1 protein expression in all combinations was similar to those of *in vitro* superoxide anion and hydroxyl radical scavenging activities. Thus good correlation was observed between *in vitro* antioxidant potential profiles of SMS related formulae and their modulation effects on antioxidant enzyme expression in the brain, such as with the correlation coefficient of 0.889 between HO-1 expression activity and the hydroxyl radical scavenging activities, and also 0.758 for SOD and superoxide scav-

enging activities (see Table 3). Although the correlation was significant, the present finding that the higher the antioxidant activity of the formula was *in vitro*, the higher modulation activity on the antioxidant gene expression was attained by them is rather complicated because it is known that these antioxidant enzymes are inducible under an oxidative stress condition;^{21–23} thus it is unlikely the enhanced expression of antioxidant enzymes observed here was directly mediated by reactive oxygen radicals. It is more plausible to consider that certain ingredients showing radical scavenging activity *in vitro* or other ingredients present in the extracts had direct effects on the enzyme induction process. Further study is required to clarify the mechanism of antioxidant enzyme gene expression. It can, however, be concluded that SMS as an herbal combination attained cellular antioxidant protection by functioning not only as free radical scavenger but also as a modulator of antioxidant enzymes. We previously studied the protective effects of decoctions of SMS and their incomplete formulae in rats^{11,14} and observed a reasonable correlation between *in vitro* antioxidant activities including superoxide and DPPH radical scavenging activities of these decoctions and their preventive potentials against ischemia-reperfusion brain injury. The present study suggested that the oxidative damage protection attained by SMS was due to the dual effects of SMS as the radical scavenger and as the modulator of antioxidant enzymes.

Chemical constituents in SMS have been studied elsewhere,^{24,25} and it was reported the *in vivo* antioxidant potential of SMS was closely correlated to total saponins in SMS, (especially, saponins from R and M). Although further studies are required for characterization of the active principle in SMS and the mechanism of antioxidative enzyme modulation at the transcriptional level, the present study showed the reasonability of an herbal combination that affords maximal antioxidant property to TCM formula, and also that the hydrostatic high pressure extraction method is applicable to prepare an effective herbal extract comparable to the decoction in terms of antioxidant activity.

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REFERENCES

- 1) Zhou, H. J. and Zhang, W. (1997) *Guide on research and development on modern new drugs*. Science and Technology of Chinese Medical and Drugs Press, Beijing, pp.334–360. (in Chinese)
- 2) Xu, J. Q. and Wang, M. Z. (1995) *Science of TCM Prescriptions*. People's Medical Publishing House, Beijing, pp.306–310. (in Chinese)
- 3) Li, Y. J., Wang, R. and Bi, K. S. (2001) Study on quality control method of Traditional Chinese Medicine. *Tradit. Chin. Drug Res. & Clin. Pharmacol.*, **12**, 192–195. (in Chinese)
- 4) Li, Y. J. and Bi, K. S. (2006) Study on the therapeutic material basis of traditional Chinese medicinal preparation Suanzaoren decoction. *Chem. Pharm. Bull. (Tokyo)*, **56**, 847–851.
- 5) Wang, Z. M. and Xiao, S. Y. (2000) Viewpoints on standardization study of traditional Chinese medicine. *Chin. Crude Drugs*, **25**, 244–245.
- 6) Wang, Z. T. and Hu, Z. B. (2000) Method on standardization of Traditional Chinese Medicine. *Pharm. Pract.*, **18**, 270–271.
- 7) Zhang, F. and Liang, M. X. (1996) Discussion on traditional Chinese medicine combination principles of Junchenzuoshi. *J. Chin. Pharm. Sci.*, **31**, 43–45.
- 8) Wang, S. and Zhang, Q. X. (2002) The status and analysis of the study on the decomposed recipes of traditional Chinese medicine. *Chin. J. Exp. TCM Formula*, **8**, 56–58.
- 9) Wang, X. J., Ichikawa, H. and Konishi, T. (2001) Antioxidant potential of Qizhu tang, a Chinese herbal medicine, and the effect on cerebral oxidative damage after ischemia-reperfusion in rat. *Biol. Pharm. Bull.*, **24**, 558–563.
- 10) Wang, X. J., Magara, T. and Konishi, T. (1999) Prevention and repair of cerebral ischemia-reperfusion injury by Chinese herbal medicine, Shengmaisan in rats. *Free. Radic. Res.*, **31**, 449–455.
- 11) Ichikawa, H. and Konishi, T. (2001) In vitro antioxidant potentials of traditional Chinese medicine, Shengmaisan and their relation to in vivo protective effect on cerebral oxidative damage in rats. *Biol. Pharm. Bull.*, **25**, 898–903.
- 12) Finkelstein, E., Rose, G. M. and Rauckman, E. J. (1980) Spin trapping of superoxide and hydroxyl radical. *Pract. Aspects*, **200**, 1–16.
- 13) Yoshida, T., Mori, K., Hatano, T., Okumura, T., Uehara, I., Komagoe, K., Fujita, Y. and Okuda, T. (1989) Studies on inhibition mechanism of autoxidation by tannis and flavonoids, V. radical scavenging effects on tannins and related polyphenols on 1,1-diphenyl-2-piclylhydroxyl radi-

- cal. *Chem. Pharm. Bull. (Tokyo)*, **37**, 1919–1921.
- 14) Ichikawa, H., Wang, X. J. and Konishi, T. (1999) Role of component herbs in antioxidant activity of Shengmaisan—a traditional Chinese medicine formula preventing cerebral oxidative damage in rat. *Am. J. Chin. Med.*, **31**, 509–521.
 - 15) Chen, P., Xu, D. M. and Lei, J. (2004) Separation and identification of effective constituents in *Ohio-pogen japonicus*. *J. Changchun Colledge TCM*, **20**, 35–36.
 - 16) Li, L. Q. and Zhang, L. J. (2000) Study on chemical constituents and pharmacological effects of *Ohio-pogen japonicus*. *J. Henan TCM Pharmacol.*, **15**, 34–35.
 - 17) Ying, G. Q., Yu, Z. M., Shan, J. F. and Yi, Y. (2005) Study on effective constituents of *Schisandra chinensis*. *J. Henan TCM*, **25**, 84–87.
 - 18) Guan, Y. L., Cao, P., Yu, K. B. and Laio, X. (2006) Study on chemical constituents of *Schisandra chinensis* (Turczl) Baill. *Chin. Tradit. Herbal Drugs*, **37**, 185–187.
 - 19) Yue, L., Zhu, D. N. and Yan, Y. Q. (2006) Relationship between component changes and efficacy of Shengmaisan VII, chemical dynamic change of schisandrin in Shengmaisan. *China J. Chin. Materia Medica*, **31**, 1010–1012.
 - 20) Yue, L., Zhu, D. N. and Yan, Y. Q. (2004) Determination of total saponins in Shengmai powders preparation. *Chin. Tradit. Patent Med.*, **26**, 897–900.
 - 21) Abraham, N. G., Asija, A., Drummond, G. and Peterson, S. (2007) Heme oxygenase-1 gene therapy: recent advances and therapeutic applications. *Curr. Gene Ther.*, **7**, 89–108.
 - 22) Dziadkowiec, D., Krasowska, A., Liebner, A. and Sigler, K. (2007) Protective role of mitochondrial superoxide dismutase against high osmolarity, heat and metalloid stress in *Saccharomyces cerevisiae*. *Folia Microbiol. (Praha)*, **52**, 120–126.
 - 23) Chattopadhyay, S., Sahoo, D. K., Subudhi, U. and Chainy, G. B. (2007) Differential expression profiles of antioxidant enzymes and glutathione redox status in hyperthyroid rats: A temporal analysis. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.*, **146**, 383–391.
 - 24) Kou, Z. P., Pei, X. C., Yu, B. Y., Zhu, D. N. and Yan, Y. Q. (2004) Protective effect of total saponin from Shengmaisan (TSS) on myocardial anoxia by oral and intravenous administration. *Study J. TCM*, **22**, 822–824.
 - 25) Wang, H. Y., Yu, B. Y., Liu, J. and Men, X. Y. (2001) Effects of the crude saponins in Sheng-maisan, Radix Geseng and Radix Ophiopogonis on the respiratory burst induced by different stimulants in rat polymorphonuclear leukocytes. *J. Chin. Pharm. Univ.*, **32**, 384–387.