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ANTIOXIDANT PROPERTIES OF ANTIULCER KAMPO MEDICINES

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Kampo medicines, aqueous extracts of a mixture of natural crude drugs, have numerous ingredients. Recent pharmacologic studies on Kampo medicines have clarified their many and varied biological activities. In this study, based on recent research that has been directed toward the excellent antioxidant properties of Kampo medicines, we investigated antioxidant activities of three Kampo medicines (TJ-10, TJ-35, TJ-43), which are clinically used for gastritis or peptic ulcer, by the electron paramagnetic resonance (EPR) spin trapping method. These Kampo medicines, especially TJ-35 scavenged superoxide generated from the hypoxanthine-xanthine oxidase system, and slightly inhibited the superoxide generation from polymorphonuclear leukocytes stimulated by phorbol myristate acetate or opsonized zymosan. Three Kampo medicines, especially TJ-35 also inhibited the generation of hydroxyl radicals by the Fenton reaction. These results suggest that these antioxidant properties may be partly responsible for anti-ulcer actions of these three Kampo medicines, especially TJ-35.

KEY WORDS: Kampo medicines, electron paramagnetic resonance (EPR)-spin trapping method, antioxidant properties, crude drugs, superoxide, hydroxyl radical.

INTRODUCTION

Kampo medicines, aqueous extracts of a mixture of natural crude drugs, have been widely accepted in Japan, as well as in China, but traditional studies on the action mechanism of Kampo medicines have been devoid of scientific methodology. Many important aspects have been left unclarified. Recent renewed appreciation of the clinical efficacy of these medicines has inspired energetic pharmacological studies in various sectors. Active ingredients exerting various biological activity have been identified in many Kampo medicines. However, because a Kampo medicine is a composite system consisting of numerous ingredients, and because its pharmacological action involves diverse modification with additive and synergic effects, it is often very difficult to analyze the action mechanism from an investigation of a certain site of action. In addition, little knowledge has been obtained concerning the pharmacological significance of the combinations of crude drugs used in Kampo medicines.

As a recent approach to the analysis of the action mechanism of Kampo medicines, several efforts are being made from the viewpoint of oxygen-derived free radicals[1–4] which are attracting much attention because of their close involvement in various diseases, carcinogenesis, aging, and other conditions. We have been contributing to

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TABLE 1
Examined Kampo medicines and their constituent crude drugs

Kampo medicine	Constituent crude drugs
TJ-10 (Saiko-keishi-to)	Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Glycyrrhizae Radix, Cinnamomi Cortex, Paeoniae Radix, Zizyphi Fructus, Ginseng Radix, Zingiberis Rhizoma
TJ-35 (Shigyaku-san)	Bupleuri Radix, Paeoniae Radix, Aurantii Fructus Immaturus, Glycyrrhizae Radix
TJ-43 (Rikkunshi-to)	Atractylodis Lanceae Rhizoma, Ginseng Radix, Pinelliae Tuber, Hoelen, Zizyphi Fructus, Aurantii Nobilis Pericarpium, Glycyrrhizae Radix, Zingiberis Rhizoma

These three Kampo medicines are aqueous extracts of a mixture of constituent crude drugs.

the clarification of the fact that many Kampo medicines have excellent activities to scavenge free radicals and prevent oxidative stress[1,4]. On the other hand, some antioxidants including superoxide dismutase (SOD) have been established as effective in the treatment of acute gastric mucosal injuries using experimental models[5,6], and the antiulcer actions of some drugs are due to their antioxidant properties[4,7]. In the present study, as a part of the basic examination to provide better analysis of the action mechanism of Kampo medicines which are useful for gastritis or gastric ulcer, we investigated antioxidant properties of Kampo medicines and compared them with each other.

MATERIALS AND METHODS

Reagents

The following three Kampo medicines examined in this study were gifts from Tsumura Co. Ltd. (Tokyo): TJ-10(Saiko-keishi-to), TJ-35(Shigyaku-san), TJ-43(Rikkunshi-to). These Kampo medicines are clinically useful for the treatment of gastritis or peptic ulcer. Kampo medicines were dissolved in distilled water and water-soluble components were examined. The constituent crude drugs of these Kampo medicines are shown in Table 1. Xanthine oxidase(XO), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), phorbol myristate acetate(PMA) and zymosan A were purchased from Sigma Chemical Co. (St. Louis, MO). Hypoxanthine(HX), diethylenetriamine-pentaacetic acid(DETAPAC), Hanks' balanced salt solution(HBSS), $FeSO_4$, and hydrogen peroxide were obtained from Wako Pure Chemical Industry(Osaka). Recombinant human superoxide dismutase(SOD) was a gift from Nippon Kayaku Co. Ltd. (Tokyo).

EPR Apparatus

EPR spectra were measured at room temperature (22°C) or at 37°C with a JEOL-JES-FR80, X-band EPR spectrometer (JEOL Co. Ltd., Tokyo). A standard quartz

flat cell (LABOTEC Co., Tokyo) was used, and the spectrometer settings were as follows: magnetic field 334.8 ± 5 mT, microwave power 8 mW (6 mW in case of hydroxyl radical scavenging activity), modulation frequency 100 kHz, modulation amplitude 0.1 mT, sweep time 5 mT/min, and response time 0.03 sec[8-10]. The intensity of the EPR signal was corrected as a ratio to the intensity of Mn^{2+} which was used as an internal standard[8-10].

Superoxide Scavenging Activity Assay

Superoxide scavenging activity(SOSA) was measured by the EPR spin trapping method previously reported[8]. The superoxide generated by the HX-XO system was trapped by DMPO, the rate of inhibition by a sample of the resulting DMPO-OOH signal intensity was determined, and compared with that obtained with a standard human SOD. For the actual measurement, 0.5 mM HX, 0.1 mM DETAPAC, 0.1 M DMPO, and the test sample or standard SOD were added to 20 mM phosphate buffer (pH 7.4). The reaction was started by the addition of XO, and the EPR signal measured 2 min later.

Assay for Superoxide Generation from Polymorphonuclear Leukocytes

Superoxide generated from human polymorphonuclear leukocytes (PMN) was also measured by the EPR spin trapping method previously reported[9]. PMN were isolated from the peripheral blood of normal volunteers by dextran sedimentation followed by Ficoll-Paque separation and hypotonic lysis of contaminating erythrocytes. PMN were suspended in HBSS at pH 7.4 and kept at 0°C until use. Cell viability was assessed by trypan blue exclusion and exceeded 96%.

The standard reaction mixture contained 4×10^6/mL PMN, 0.1 mM DETAPAC, 0.1 M DMPO and stimulus in a total volume of 0.2 mL HBSS. PMN in HBSS were preincubated with a tested sample at 37°C for 5 min; the reaction was initiated by the addition of PMA or opsonized zymosan(OZ), as a stimulus, at a final concentration of 400 ng/mL and 3 mg/mL, respectively. The effect of the sample on the DMPO-OOH signal was expressed as a percentage by comparing its signal intensity to 100, which was the measured value when distilled water was used as the control. In another experiment, PMN were preincubated with a sample for 5 min, followed by cell washing, and then a stimulus was added, in order to exclude the effect of the sample's superoxide scavenging activity.

Hydroxyl Radical Assay

Hydroxyl radical generated from the Fenton reaction was also measured by EPR spin trapping method[10]. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.4), 50 μM ferrous sulfate, 0.1 mM DETAPAC, 1 mM or 10 mM DMPO and 1 mM hydrogen peroxide in a total volume of 0.2 mL. The ferrous sulfate solution and then DMPO and a sample solution were added to the phosphate buffer containing DETAPAC. The reaction was started by the addition of hydrogen peroxide within 1 min after the addition of ferrous sulfate. To distinguish whether the inhibition of DMPO-OH signal is due to the competition of a substance with DMPO or the inhibition of the Fenton reaction, at least two DMPO concentrations were used[10]; i.e. if the inhibition by a substance is totally due to the inhibition of the Fenton reaction, the inhibition rate must be dependent on the absolute concentration

TABLE 2
Effect of Kampo medicines on the EPR signal of DMPO-OOH spin adduct generated from the hypoxanthine-xanthine oxidase system

Kampo medicine	Relative intensity of DMPO-OOH signal (ratio to the value in the absence of sample; %)			$IC_{50}(10^{-5}$ g/ml)
	10^{-5} g/ml	10^{-4} g/ml	10^{-3} g/ml	
TJ-10 (Saiko-keishi-to)	73.3 ± 0.4#	34.3 ± 0.4#	9.7 ± 0.2#	3.8
TJ-35 (Shigyaku-san)	57.8 ± 0.6#	23.3 ± 0.3#	6.0 ± 0.1#	1.5
TJ-43 (Rikkunshi-to)	96.3 ± 1.1	76.0 ± 0.6#	34.7 ± 1.2#	51.0

Each value indicates the mean ± SEM of 3 experiments. # $p < 0.01$ when compared with the value in the absence of sample.

of the substance, and independent of the DMPO concentration. On the contrary, if the inhibition is totally due to competition between DMPO and a sample, the inhibition rate should be dependent on the relative concentration of the substance vs. DMPO.

Data were given as mean ± SEM. Comparisons between groups were made with the Dunnet test. With all statistical analyses, an associated probability (p value) of <1% was considered significant.

RESULTS

Superoxide Scavenging Activity of Three Kampo Medicines

The DMPO-OOH signal was observed when XO was added to the complete system containing HX and DMPO in phosphate buffer. The addition of each Kampo medicine in concentration of 0.01–1.0 mg/mL to the system reduced the DMPO-OOH signal intensity in a dose-dependent manner (Table 2). TJ-35 exhibited the highest inhibition of the DMPO-OOH signal (Table 2). SOSA of the water soluble components of 1 mg of TJ-10, TJ-35 or TJ-43 was, if converted to SOD unit, equivalent to 2.02, 2.28, or 0.14 units, respectively.

Effect of Each Kampo Medicine on Superoxide Production by Stimulated Human PMN

The intensity of the DMPO-OOH spin adduct generated from human PMN stimulated with PMA or OZ significantly decreased in proportion to the concentration of each Kampo medicine. TJ-35 exhibited the highest inhibition rate. The marked inhibition of DMPO-OOH, however, could be observed only when the medicines were present in the reaction mixture. The inhibition rate by the medicines markedly decreased when the medicines were preincubated with PMN, followed by cell washing (Table 3). This inhibition after cell washing is due to the suppression of PMN superoxide production by the medicines. TJ-35 also exhibited the highest inhibition.

TABLE 3
Effects of Kampo medicines at the concentration of 0.1 mg/ml on the relative intensity of DMPO-OOH spin adduct generated from human polymorphonuclear leukocytes in the presence of DMPO

Kampo medicines	Relative intensity of DMPO-OOH signal (ratio to the value in the absence of sample; %)	
	Without cell-washing	With cell-washing
(1) when stimulated with phorbol myristate acetate		
TJ-10 (Saiko-keishi-to)	61.7 ± 2.7#	87.0 ± 4.2
TJ-35 (Shigyaku-san)	53.3 ± 1.2#	76.3 ± 0.9#
TJ-43 (Rikkunshi-to)	73.7 ± 2.2#	82.3 ± 4.5
(2) when stimulated with opsonized zymosan		
TJ-10 (Saiko-keishi-to)	49.3 ± 3.5#	89.0 ± 3.8
TJ-35 (Shigyaku-san)	42.3 ± 2.0#	80.3 ± 0.9#
TJ-43 (Rikkunshi-to)	67.7 ± 1.8#	92.3 ± 3.6

Each value indicates the mean ± SEM of 3 experiments. # $p < 0.01$ when compared with the value in the absence of sample.

Effect of Each Kampo Medicine on Hydroxyl Radical Generation by Fenton Reaction

All of three Kampo medicines reduced the DMPO-OH signal in the Fenton system. TJ-35 also exhibited the highest inhibition. The inhibition was almost dependent on the relative concentration of the tested sample vs. DMPO, but independent of the absolute concentration of the sample, which indicates that the signal inhibition was mainly due to the inhibition based on competition with DMPO, and that some part of the inhibition was due to the inhibition of the Fenton reaction itself in case of TJ-35 (Table 4).

DISCUSSION

Recent research (11–16) has clarified that oxygen-derived free radicals are closely involved in the pathology of experimental and clinical gastric mucosal injury, as with the diseases of other organs. Ever since the reports of Itoh[12] and Perry[13] *et al.*, there have many reports pertaining to gastric mucosal injury induced by ischemia-reperfusion. These reports were almost all inhibition experiments, and indicated the efficacy of SOD, catalase or allopurinol. We have also already reported[4] that gastric mucosal injury induced by ischemia-reperfusion is significantly inhibited by pretreatment with TJ-35, but not with TJ-10, or TJ-43, and that this inhibition is perhaps due to free radical-scavenging activity of TJ-35. Based on this study, antioxidant properties of these three Kampo medicines were examined and compared with each other in the present study.

These three Kampo medicines, especially TJ-35 decreased dose-dependently the EPR signal intensity of DMPO-OOH spin adduct produced by the reaction between superoxide and DMPO. In general, a Kampo medicine contains several crude drugs, and a simple crude drug contains at least several hundred types of biologically meaningful substances, and it is hardly easy to identify which substance is responsible to the main action of the crude drug. In our previous study[17], however, we have examined SOSA of various crude drugs and demonstrated that higher SOSA was found in Rhei Rhizoma, Moutan Cortex, Paeoniae Radix, and other crude drugs

TABLE 4
Effect of Kampo medicines on the EPR signal of DMPO-OH spin adduct produced by the Fenton reaction

Kampo medicines	Relative intensity of DMPO-OH signal (%) (ratio to the value in the absence of sample; 10 mM DMPO/1 mM DMPO)		
	10^{-5} g/ml	10^{-4} g/ml	10^{-3} g/ml
TJ-10 (Saiko-keishi-to)	98.3 ± 0.1/95.7 ± 0.1	96.7 ± 0.2 /84.3 ± 0.1#	84.0 ± 0.2#/29.0 ± 0.3#
TJ-35 (Shigyaku-san)	95.7 ± 0.3/96.0 ± 0.2	90.7 ± 0.3#/70.7 ± 0.4#	64.0 ± 0.3#/13.7 ± 0.6#
TJ-43 (Rikkunshi-to)	102.0 ± 0.3/96.0 ± 0.2	92.3 ± 0.2 /74.0 ± 0.1#	75.0 ± 0.2#/15.7 ± 0.1#

Each value indicates the mean ± SEM of 3 experiments. #$p < 0.01$ when compared with the value in the absence of sample

containing tannins, as well as Scutellariae Radix and others containing baicalein, suggesting that these substances are the main factors exerting SOSA of crude drugs. Some authors have also reported[18-20] that many of the tannins and flavonoids are strong free radical scavengers and antioxidants. TJ-35 is a combination of four crude drugs, i.e., Bupleuri Radix, Glycyrrhizae Radix, Paeoniae Radix, and Aurantii Fructus Immaturus. Paeoniae Radix contains tannins. Bupleuri Radix and Aurantii Fructus Immaturus contain flavonoids. We speculate these ingredients are responsible for excellent SOSA of TJ-35.

PMN have been considered the main source of oxygen-derived free radicals *in vivo*. An increasing number of reports[21,22] demonstrate the suppression of experimental gastric mucosal injury in neutropenic rats by the administration of an anti-PMN antibody, which suggests that resident and inflammatory PMN may produce oxygen-derived free radicals during the formation of gastric mucosal injury. In the present *in vitro* study, superoxide production from PMN was significantly inhibited by TJ-35 at the concentration of 0.1 mg/ml in isolated human PMN stimulated by PMA or OZ. This result suggests that the NADPH oxidase may be affected by supplementation with some ingredients of Kampo medicine, and that membrane receptors such as C_{3b} receptor or intracellular activating system may be affected. However, this result may not be applied directly to the oral administration of Kampo medicines, in which the problem must be much more complicated. That is, some ingredients may be inactivated by gastric acid. On the contrary, the activity of some ingredients may be enhanced in such a manner that the *in vitro* activity is originally masked because of the polymerization of a low molecular-weight active substance and the degradation by gastric acid removes the masking. For example, glycyrrhizin in Glycyrrhizae Radix and saikosaponins in Bupleuri Radix have an inhibitory effect on superoxide production by PMN *in vitro*[23]. The inhibition by these three Kampo medicines which contain such ingredients was not marked in our *in vitro* study, but when orally administered, these ingredients may have some beneficial effects *in vivo* because of the reasons mentioned above, or some interactions among ingredients. Inversely these may have no significant effects *in vivo*.

Each Kampo medicine inhibited the EPR signal of DMPO-OH in the Fenton reaction, and the inhibition was almost dependent on the relative concentration of the medicine vs. DMPO. That is, a large part of the inhibition was due to the inhibition based on competition between DMPO and a sample[10]. In case of TJ-35, some parts of the inhibition were due to the inhibition of Fenton reaction[10]. The hydroxyl radical, an extremely highly reactive oxygen species, reacts rapidly with biological substances, resulting in oxidative damage. Some reports have demonstrated

that dimethyl sulfoxide (DMSO), a hydroxyl radical scavenger, attenuated gastric mucosal injury induced by ischemia[13]. The hydroxyl radical-scavenging activity of Kampo medicines *in vitro* might have some beneficial effect *in vivo*.

These findings indicate that TJ-35 is superior to TJ-10 or TJ-43 in antioxidant properties *in vitro*. This is consistent with the result previously reported that TJ-35 was superior to TJ-10 or TJ-43 in inhibitory effect on ischemia-reperfusion-induced gastric mucosal injury *in vivo*. A combination of these antioxidant actions *in vitro* might be one of main actions of TJ-35. However, it is considered necessary to clarify the active ingredients and to conduct detailed *in vitro* examination of how the interaction among ingredients influences the manifestation of antioxidant actions of Kampo medicines. As well *in vivo* confirmation of the presence of antioxidant activity and the analysis of the action mechanism of such activity, if present, is necessary.

References

1. T. Yoshikawa, S. Takahashi, Y. Naito, T. Tanigawa, S. Ueda, H. Oyamada, S. Sugino and M. Kondo (1990) Influences of traditional Chinese medicines on oxygen-derived free radicals. *J. Clin. Exp. Med.*, **152**, 741–742 (in Japanese).
2. Y. Niwa and Y. Miyachi (1986) Antioxidant action of natural health products and Chinese herbs. *Inflammation*, **10**, 79–91.
3. T. Okuda, T Yoshida and T. Hatano (1989) Ellagitannins as active constituents of medical plants. *Planta Medica*, **55**, 117–122.
4. T. Yoshikawa, S. Takahashi, H. Ichikawa, H. Takano, N. Tasaki, M. Yasuda, Y. Naito, T. Tanigawa and M. Kondo (1991) Effects of TJ-35 (Shigyaku-san) on gastric mucosal injury induced by ischemia-reperfusion and its oxygen-derived free radical-scavenging activities. *J. Clin. Biochem. Nutr.*, **10**, 189–196.
5. T. Yoshikawa, Y. Naito, S. Ueda, H. Oyamada, T. Takemura, N. Yoshida, S. Sugino and M. Kondo (1990) Role of oxygen-derived free radicals in the pathogenesis of gastric mucosal lesions in rats. *J. Clin. Gastroenterol.*, **12**, S65–S71.
6. S.M. Smith, M.B. Grisham, D.N. Granger and J.M. Russell (1987) Gastric mucosal injury in the cat. Role of iron and xanthine oxidase. *Gastroenterol.*, **92**, 950–956.
7. T. Yoshikawa, Y. Naito, T. Tanigawa, T. Yoneta and M. Kondo (1991) The antioxidant properties of a novel zinc-carnosine chelate compound, N-(3-aminopropionyl)-L-histidinato zinc. *Biochim. Biophys. Acta.*, **1115**, 15–22.
8. H. Miyagawa, T. Yoshikawa, T. Tanigawa, N. Yoshida, S. Sugino and M. Kondo (1988) Measurement of serum superoxide dismutase activity by electron spin resonance. *J. Clin. Biochem. Nutr.*, **5**, 1–7.
9. T. Tanigawa, T. Yoshikawa, H. Oyamada, T. Takemura, Y. Morita, K. Tainaka, H. Miyagawa, N. Yoshida, S. Sugino and M. Kondo (1988) Determination of superoxide generation by human polytorphonuclear leukocytes by electron spin resonance and chemiluminescence. In Free Radicals in Digestive Diseases, edited by M. Tsuchiya, K. Kawai, M. Kondo, and T. Yoshikawa, pp. 37–42. Amsterdam: Excerpta Medica.
10. T. Tanigawa (1990) Determination of hydroxyl radical scavenging activity by electron spin resonance. *J. Kyoto Pref. Univ. Med.*, **99**, 133–143.
11. T. Yoshikawa, S. Ueda, Y. Naito, S. Takahashi, H. Oyamada, Y. Morita, T. Yoneta and M. Kondo (1989) Role of oxygen-derived free radicals in gastric mucosal injury induced by ischemia or ischemia-reperfusion in rats. *Free Rad. Res. Comm.*, **7**, 3–6.
12. M. Itoh and P.H. Guth (1985) Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesion in rats. *Gastroenterol.*, **88**, 1162–1167.
13. M.A. Perry, S. Waddhwa, D.A. Parks, W. Rickard and D.N. Granger (1986) Role of oxygen radicals in ischemia-induced lesions in the cat stomach. *Gastroenterol.*, **90**, 362–367.
14. T. Yoshikawa, N. Yoshida, Y. Naito, T. Takemura, H. Miyagawa, T. Tanigawa and M. Kondo (1990) Role of oxygen radicals in the pathogenesis of gastric mucosal lesions induced by water-immersion restraint stress and burn stress in rats. *J. Clin. Biochem. Nutr.*, **8**, 227–234.
15. T. Yoshikawa, N. Yoshida, H. Miyagawa, T. Takemura, T. Tanigawa, S. Sugino and M. Kondo (1987) Role of lipid peroxidation in gastric mucosal lesions induced by burn shock in rats. *J. Clin. Biochem. Nutr.*, **2**, 163–170.

16. N. Yoshida, T. Yoshikawa, T. Ando, Y. Naito, H. Oyamada, T. Takemura, T. Tanigawa, S. Sugino and M. Kondo (1989) Pathogenesis of platelet-activating factor-induced gastric mucosal damage in rats. *Scand. J. Gastroenterol.*, **24** (Suppl. 162), 210–214.
17. S. Takahashi, T. Yoshikawa, S. Nakamura, Y. Naito, T. Tanigawa and M. Kondo. Studies on superoxide scavenging activity of various crude drugs. In Oxygen Radicals, edited by K. Yagi, M. Kondo, E. Niki, T. Yoshikawa, pp. 679–682. Amsterdam: Elsevier Science Publishers.
18. S. Uchida, R. Edamatsu, M. Hiramatsu, A. Mori, G. Nonaka, I. Nishioka, M. Niwa and M. Ozaki (1987) Condensed tannins scavenge active oxygen free radicals. *Med. Sci. Res.*, **15**, 831–832.
19. M. Erben-Russ, W. Bors and M. Saran (1987) Reactions of linoleic acid peroxyl radicals with phenolic antioxidants – A pulse radiolysis study. *Int. J. Radiat. Biol.*, **52**, 393–412.
20. W. Bors and M. Saran (1987) Radical scavenging by flavonoid antioxidants. *Free Rad. Res. Comm.*, **2**, 289–294.
21. S.M. Smith, L.H. Rutili, M.A. Perry, M.B. Grisham, K. Arfors, D.N. Granger and P.R. Kvietys (1987) Role of neutrophils in hemorrhagic shock-induced gastric mucosal injury in the rat. *Gastroenterol.*, 93, 466–471.
22. M. Suzuki, M. Suematsu, S. Miura, H. Nagata, T. Morishita, M. Oda and M. Tsuchiya (1988) Acute gastric mucosal lesions induced by vasomotor derangement – Participation of xanthine oxidase and neutrophil-mediated oxidative stress –. *Jpn. J. Gastroenterol.*, **85**, 835–842 (in Japanese).
23. H. Abe (1985) Effect of saikosaponins-d on aminonucleoside nephrosis. *Jpn. J. Pharmacol.*, **38**, 221–225.