Clinical significance of redox effects of Kampo formulae, a traditional Japanese herbal medicine: comprehensive estimation of multiple antioxidative activities

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To clarify the clinical significance of the redox-controlling effects of Kampo, a traditional Japanese herbal medicine, we determined the scavenging activities of various reactive oxygen species in clinically used Kampo formulae using an electron spin resonance-based technique. Formulæ containing Rheum obtusum (i.e., mashinginan and daiobotanpito) showed high scavenging activity against the alkoxyl radical, and crude extract quantity was significantly correlated with scavenging activity. Hydroxyl radical scavenging activity was positively correlated with the quantity of Zingiberis Rhizoma. Strong hydroxyl radical scavenging activity was also found in formulæ containing both Bupleuri Radix and Scutellariae Radix, a widely used anti-inflammatory combination. Formulæ containing a clinically common combination of Scutellariae Radix, Coptidis Rhizoma, and Phellodendri Cortex induced high superoxide scavenging activity. Single oxygen scavenging activity was high in formulæ containing Bupleuri Radix and Glycyrrhiza Radix. In contrast, formulæ containing Rehmanniae Radix showed generally low reactive oxygen species scavenging activities, and the quantity of Rehmanniae Radix was negatively correlated with hydroxyl radical and singlet oxygen scavenging activities. These results indicate that the antioxidative effects of Kampo formulæ are not uniform but complexly varied against multiple reactive oxygen species. Some formulæ have almost no antioxidative effects but may act as pro-oxidants.

Key Words: Kampo, traditional Japanese herbal medicine, hydroxyl radical, multiple radical scavenging activity, Bupleuri Radix, Rehmanniae Radix

Traditional herbal medicines have attracted much attention as complementary treatments. Kampo, a traditional Japanese herbal medicine, is an established system of alternative or complementary medicine that has demonstrated beneficial treatment effects and reduced side effects.1–2 Multiple reports have revealed antioxidative effects for Kampo formulæ or other herbal extract prescriptions, and the antioxidative potencies of Kampo formulæ are now pharmacologically recognized as an important therapeutic mechanism.3–8 However, most studies have evaluated antioxidative mechanisms using a few substances or stable end products of oxidative reactions. In contrast to the substantial developments in the analysis of post-cellular reactions, the “upstream” aspect of oxidative stress [i.e., the identification of reactive oxygen species (ROS) that stimulate oxidative stress reactions, or interactions among ROS that generate oxidative stimulators] remains largely unclear.9,10 Because ROS are not uniform, and individual ROS show specific characteristics during in vivo reactions,11–13 analysis of multiple ROS is clearly necessary. To address this problem, we have developed an electron spin resonance (ESR)-based method to analyze multiple ROS scavenging activities (multiple free radical scavenging activities: MULTIS) in biological samples and have used it to analyze the upstream aspect of oxidative stress-related reactions.12,13

This study aimed to investigate the multidimensional antioxidative profiles of Kampo formulæ by analyzing their relations to clinical effects, and to provide explanations of their Kampo or oriental medical therapeutic effects using Western medical terms. We determined the multiple ROS scavenging activities of 48 clinically used Kampo prescription formulæ for prescription. The measured ROS were hydroxyl radical (·OH), alkoxyl radical (RO·, tert-BuO·), alkylperoxyl radical (ROO·, tert-BuOO·), superoxide (O2·−), and singlet oxygen (1O2). We also analyzed the effects of crude drugs on the antioxidative activities of Kampo formulæ. Our findings demonstrate previously unknown antioxidative effects in multiple Kampo formulæ.

Materials and Methods

Kampo extract preparation. We used 48 Kampo extract prescription formulæ widely used in Japan; these are shown in Table 1 and 2. The Kampo extract preparations were donated by Tsumura Co., Ltd (Tokyo, Japan). All preparations were clinical grade extract granules. In clinical use, these extract granules are easily dissolved in approximately 200 ml of hot water or orally administrated as they are. In this study, 2.5 g of the Kampo extract granules were dissolved in ultrapurified water at 90°C and subjected to ESR measurement in 10% aqueous solutions. The relations between ROS scavenging activity and quantity of crude drug components for Kampo preparations were statistically analyzed.

Measurements of multiple free radical scavenging activity. MULTIS were measured using a previously described ESR-based method with minor modifications.12,13 MULTIS measurements were performed three times per each ROS. The ESR spectrometer used was a RR-X1 equipped with 100 kHz field modulation and WIN-RAD operation software (Radical Research
Inc., Tokyo, Japan). The compound 5-(2,2-dimethyl-1,3-propano-cyclophosphoryl)-5-methyl-1-pyrrrole N-oxide (CYPMPO) was used as an ESR spin-trapping reagent. Typical spectrometer settings were field modulation width 0.1 mT; microwave power 10 mW; field scan width and rate ±7.5 mT/2 min; and time constant 0.1 s. Each ROS was produced via in situ illumination with UV/visible light from an illuminator (RUVF-203SR UV illuminator; Radical Research Inc., Tokyo, Japan) equipped with a 200 W medium-pressure mercury/xenon arc lamp and a quartz light-guide connected to the resonator cavity. Table 3 summarizes the light sources, illumination times, precursors, and photosensitizers used to produce ROS. ROS scavenging activities were calculated according to a previously described method and expressed as the unit equivalent to known pure scavengers: glutathione (GSH) for ‘OH and ‘O₂, superoxide dismutase (SOD) for O₂⁻, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) for RO, and α-lipoic acid for ROO.

To evaluate overall scavenging activity, scavenging activities of each ROS were expressed as relative values with the Kampo formulation containing large amounts of Rhei Rhizoma (4 g per daily dose in the extract), showed remarkably high RO scavenging activity. There was a significant strong positive correlation between the amount of Rhei Rhizoma and RO scavenging activity (r = 0.87, p < 0.05, Fig. 2). Other formulae containing Rhei Rhizoma, including mashiningan (MNG) and daiobotanpito (DBT), also showed high RO scavenging activity [the amount of Rhei Rhizoma was 4 g in mashiningan (MNG) and 2 g in daiobotanpito (DBT) per daily dose in the extract]. Formulae that included Coptidis Rhizoma, namely orangedokuto (OGT), unseiin (USI), and saikoseikanto (SSET), also showed high RO scavenging activity; however, the results were not significant owing to the small number of formulae that contained the component. A previous report found that Kampo formulae containing Rhei Rhizoma showed high oxygen radical absorbance capacity (ORAC) values; our results for RO scavenging activity generally agree with these previous findings.

Alkoxyl radical scavenging activity of Kampo formulae. Table 5 shows the RO scavenging activity of Kampo formulae extracts. Daiokanzoto (DKZT), which contains large amounts of Rhei Rhizoma (4 g per daily dose in the extract), showed remarkably high RO scavenging activity. There was a significant strong positive correlation between the amount of Rhei Rhizoma and RO scavenging activity (r = 0.87, p < 0.05, Fig. 2). Other formulae containing Rhei Rhizoma, including mashiningan (MNG) and daiobotanpito (DBT), also showed high RO scavenging activity [the amount of Rhei Rhizoma was 4 g in mashiningan (MNG) and 2 g in daiobotanpito (DBT) per daily dose in the extract]. Formulae that included Coptidis Rhizoma, namely orangedokuto (OGT), unseiin (USI), and saikoseikanto (SSET), also showed high RO scavenging activity; however, the results were not significant owing to the small number of formulae that contained the component. A previous report found that Kampo formulae containing Rhei Rhizoma showed high oxygen radical absorbance capacity (ORAC) values; our results for RO scavenging activity generally agree with these previous findings.

Alkylperoxyl radical scavenging activity of Kampo formulae. Table 6 shows the ROO scavenging activity of Kampo formulae extracts. The highest ROO scavenging activity was found in hakkontokasenkyushin’i (KTSS) and maoto (MT), both of which contain Ephedrae Herba. However, this component had no significant effect on ROO scavenging activity. The only crude element that significantly contributed to ROO scavenging activity was Rhei Rhizoma (r = 0.84, p < 0.05, Fig. 3). Instead, ROO scavenging activity was notable in saibokuto (SBT), hangekobokuto (HKT), and goreisan (GRS), whose antioxidative activities were previously unknown. Moreover, many of our results related to ROO scavenging activity could not be explained by the constitution of herbal ingredients.

**Table 1. List of Kampo formulae and their abbreviations**

| Name | Abbr. | Name | Abbr. | Name | Abbr. |
|------|-------|------|-------|------|-------|
| bakumondoto | BAK | kakkontokasenkyushin’i | KTSS | saikokeishito | SAKT |
| bofutushosan | BTS | kamikihito | KKT | saikoseikanto | SSET |
| boihoito | BOT | kamishoyosan | KSS | saireito | SRT |
| daikenchuto | DKT | keigairengyoto | KRT | shimbuto | SIT |
| daioobotanpito | DBT | keishibukuryogon | KBG | shimomtuso | SMT |
| daiokanzoto | DKZT | keishikajutsubuto | KSTJ | shokenchuto | SHKT |
| daiaikoto | DST | keishikaryokutsuboreito | KSTRB | shosaikoto | SSK |
| gosisan | GRS | keihito | KST | shoseiyuto | SST |
| goshajinkigan | GJG | maoto | MT | tokiihishi | TKI |
| goshuyuto | GSY | mashiningan | MNG | tokishakuyakusan | TSS |
| hachimijigon | HJG | ninjinto | NT | tokishiyakukagoshuyushokuyoto | TSGST |
| hangekobokuto | HKT | orangedokuto | OGT | tsudosan | TDS |
| hangeshoshiento | HST | rikkunshito | RKT | unseiin | USI |
| hochuekkito | HET | rokumigan | RJG | yokuninton | YT |
| jumihaidokuto | JHT | saibokuto | SBT | yokukansan | YKS |
| juzentaihoto | JTT | saikokaryokutsuboreito | SRB | |
| kakkonto | KT | saikokeishikanykoto | SAKK | |

Abbr.: abbreviation

**Results**

**Hydroxyl radical scavenging activity of Kampo formulae.** Table 4 shows the ‘OH scavenging activity of Kampo extract preparations. The formulae shosaikoto (SSK), saikokaryokutsuboreito (SRB), unseiin (USI), and daisaikoto (DST) showed remarkably high ‘OH scavenging activity. Among them, shosaikoto (SSK), saikokaryokutsuboreito (SRB), and daisaikoto (DST) contain both Bupleuri Radix and Scutellariae Radix as components, which may relate to antioxidative activity [the amount of Bupleuri Radix and Scutellariae Radix were, respectively, 7 g and 3 g in shosaikoto (SSK), 5 g and 2.5 g in saikokaryokutsuboreito (SRB), and 6 g and 3 g in daisaikoto (DST), per daily dose]. Zingibers Rhiiza showed a significant positive correlation and Rehmanniae Radix showed a significant strong negative correlation between ‘OH scavenging activity and quantity (r = 0.54, p < 0.05 for Zingibers Rhiiza and r = −0.75, p < 0.05 for Rehmanniae Radix, Fig. 1).

**Alkoxyl radical scavenging activity of Kampo formulae.** Table 5 shows the RO scavenging activity of Kampo formulae extracts. Daiokanzoto (DKZT), which contains large amounts of Rhei Rhizoma (4 g per daily dose in the extract), showed remarkably high RO scavenging activity. There was a significant strong positive correlation between the amount of Rhei Rhizoma and RO scavenging activity (r = 0.87, p < 0.05, Fig. 2). Other formulae containing Rhei Rhizoma, including mashiningan (MNG) and daiobotanpito (DBT), also showed high RO scavenging activity [the amount of Rhei Rhizoma was 4 g in mashiningan (MNG) and 2 g in daiobotanpito (DBT) per daily dose in the extract]. Formulae that included Coptidis Rhizoma, namely orangedokuto (OGT), unseiin (USI), and saikoseikanto (SSET), also showed high RO scavenging activity; however, the results were not significant owing to the small number of formulae that contained the component. A previous report found that Kampo formulae containing Rhei Rhizoma showed high oxygen radical absorbance capacity (ORAC) values; our results for RO scavenging activity generally agree with these previous findings.

**Alkylperoxyl radical scavenging activity of Kampo formulae.** Table 6 shows the ROO scavenging activity of Kampo formulae extracts. The highest ROO scavenging activity was found in hakkontokasenkyushin’i (KTSS) and maoto (MT), both of which contain Ephedrae Herba. However, this component had no significant effect on ROO scavenging activity. The only crude element that significantly contributed to ROO scavenging activity was Rhei Rhizoma (r = 0.84, p < 0.05, Fig. 3). Instead, ROO scavenging activity was notable in saibokuto (SBT), hangekobokuto (HKT), and goreisan (GRS), whose antioxidative activities were previously unknown. Moreover, many of our results related to ROO scavenging activity could not be explained by the constitution of herbal ingredients.
Table 2. List of Kampo formulae and their composition of crude extracts. The crude amounts are indicated as daily dose

| Name                  | Major components (gram per daily dose) |  |
|-----------------------|----------------------------------------|---|
| bakumondoto           | BAK 2                                   |  |
| bofutsushosan         | BTS 2 2 1.2 1.5 1.2                    |  |
| boiohitot             | BOT 1.5 1 3                            |  |
| daikenchuto           | DKT 5                                   |  |
| daiohertapnto         | DBT 2                                   |  |
| daikanzoto            | DKZT 2 4                               |  |
| daikaiokito           | DST 3 6 3 1 1                         |  |
| gorykisuran           | GRS 3                                   |  |
| goshajinkigan         | GIG 5                                   |  |
| goshuyuto             | GSY 1.5                                 |  |
| hachimijopan          | HJG 6                                   |  |
| hangekobokuto         | HKT 1                                   |  |
| hangeshoshinhito      | HST 2.5 1 2.5 2.5                      |  |
| hochuikimoto          | HET 1.5 2 0.5 4                        |  |
| jumihaidokuto         | JHT 1 3                                |  |
| juzentaihoto          | JTT 1.5 3 3 3 3                       |  |
| kakkonto              | KT 2 2 2 3                             |  |
| kakkontokasenkyushin'i| KTSS 2 2 2 3                         |  |
| kamikihito            | KKT 1 3 1 3                            |  |
| kamishikotan          | KSS 1.5 3 3 3                         |  |
| keigaiyengyo          | KRT 1.5 1.5 1.5 1.5                   |  |
| keishibukuryogan      | KBG 3                                   |  |
| keishikujutsuboito    | KJSTB 2 4 1 4                         |  |
| keishikyotoksuboreito | KRSTB 2 4 1.5                         |  |
| keishito              | KST 2 4 1.5                            |  |
| maoto                 | MT 1.5                                 |  |
| mashiningan           | MNG 2 4                                |  |
| ninjinto              | NT 3 3 3                              |  |
| orangedokuto          | OGT 3 1.5 2                            |  |
| rikkunshito           | RKT 1 0.5 4                            |  |
| rokumigan             | RJG 5                                   |  |
| saibokuto             | SBT 3 2 7                              |  |
| saikokaryotoksuboreito| SRB 2.5 5 1 1                         |  |
| saikokeishikankyo     | SAKK 3 2 2 6                           |  |
| saikokeishito         | SAKT 2 2 5 2 1                       |  |
| saikoseikanto         | SSET 1.5 1.5 1.5 1.5 1.5 1.5 1.5      |  |
| sairito               | SRT 3 2 7 1                            |  |
| shibuto               | SIT 3 1.5 3 0.5                         |  |
| shimotokutu           | SMT 3 3                                |  |
| shochenobuto          | SHKT 2 6 1                             |  |
| shosaikoto            | SSK 2 7                                |  |
| shoseiruyito          | SST 3 3 3                               |  |
| tokiinshi             | TK1 1 4 3                              |  |
| tokishakuyakusan      | TSS 4 4                                |  |
| tokishiyakagoshuyushokyo| TSGST 2 3 1                          |  |
| tsudonan              | TDS 2 3 1                              |  |
| unseii                | USI 1.5 1.5 1.5 3 3                   |  |
| yokuinshito           | YT 2 3 4 4                             |  |
| yokuokansan           | YKS 1.5 2 4                            |  |

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Table 7 shows the \( \text{O}_2^- \) scavenging activity of Kampo formulae extracts. Formulae containing *Scutellariae Radix*, *Phellodendri Cortex*, and *Coptidis Rhizoma*, namely orengedokuto (OGT), unseiin (USI), and keigairengyoto (KRT), showed high scavenging activity against \( \text{O}_2^- \) [the amounts of *Scutellariae Radix*, *Phellodendri Cortex* and *Coptidis Rhizoma* were, respectively, 3 g, 1.5 g and 2 g in orengedokuto (OGT); 1.5 g, 1.5 g and 1.5 g in unseiin (USI); and 1.5 g, 1.5 g and 1.5 g in keigairengyoto (KRT) per daily dose]. High \( \text{O}_2^- \) scavenging activity was also found in formulae containing *Rhei Rhizoma* [i.e., daiokanzoto (DKZT) and tsudosan (TDS)] and *Ephedrae Herba* [i.e., kakkontokasenkyushin’i (KTSS) and maoto (MT)]. Again, no significant results were observed owing to the small number of formulae that contained the component.

Table 8 lists the Kampo formulae that showed high \( ^1\text{O}_2 \) scavenging activity of extracts. Formulae containing *Bupleuri Radix*, namely saikokeishikankyoto (SAKK), daisaikoto (DST), shosaikoto...
(Sakiseikanto (SSET), Saikokeishikankyo (SAKK), 6 g in Daisaikoto (DST), 5.9 g in saibokuto (SBT) and 5.8 g in Saikokeishikankyo (SAKK), showing high antioxidant activity.

Table 5. Alkoxyl radical scavenging activity of Kampo formulae

| Kampo formula   | RO_1 scavenging activity (mM-TROLOXeq) per daily dose per gram |
|-----------------|---------------------------------------------------------------|
| Daiokanzoto     | 200.7                                                        |
| Mashiningan     | 47.3                                                         |
| Orengedokuto    | 32.3                                                         |
| Daibotanboto    | 32.2                                                         |
| Maoto           | 25.7                                                         |
| Saikokeishikankyo | 17.0                                                         |
| Unsein         | 14.9                                                         |
| Yokuininto      | 12.5                                                         |
| Saikoseikanto   | 11.9                                                         |
| Bo fistsusohan  | 9.2                                                          |
| Kakkontokasenkyushin’i | 9.2                                                          |
| Tsudosan        | 8.7                                                          |
| Keigairengyoto  | 8.4                                                          |
| Hangeshashinto  | 6.8                                                          |
| Keishibukuryogan | 6.5                                                          |
| Saibokuto       | 5.9                                                          |
| Daisaikoto      | 5.3                                                          |
| Hochuekkito     | 4.8                                                          |
| Hangekobukuto   | 4.8                                                          |
| Saikokaryukotsuboreito | 4.8                                                      |
| Ninjinto        | 4.5                                                          |
| Kakkonto        | 4.2                                                          |
| Daikenchuto     | 4.2                                                          |
| Shosaikoto      | 4.2                                                          |
| Shokenchuto     | 4.1                                                          |
| Yokkansan       | 4.1                                                          |
| Saikokeishito   | 3.9                                                          |
| Keishi          | 3.8                                                          |
| Saireito        | 3.7                                                          |
| Keishikaryukotsuboreito | 3.5                                                        |
| Keishikajutsubuto | 3.1                                                        |
| Biobito         | 3.1                                                          |
| Goshuyuto       | 3.1                                                          |
| Rokumigan       | 3.0                                                          |
| Tokiinshi       | 2.8                                                          |
| Jyuzendaishito  | 2.7                                                          |
| Rikunshito      | 2.6                                                          |
| Kamishoyosan    | 2.5                                                          |
| Kamikihito      | 2.4                                                          |
| Shimbuto        | 2.4                                                          |
| Goshajinkigan   | 2.3                                                          |
| Shinotsuto      | 2.3                                                          |
| Shoseiyuto      | 2.2                                                          |
| Jumihaidokuto   | 1.8                                                          |
| Bakumondoto     | 1.7                                                          |
| Tokishigayakugoshuyushokyo | 1.7                                                        |
| Tokishakuyakusan | 1.7                                                        |
| Goreisan        | 1.4                                                          |
| Hachimijiogon   | 0.9                                                          |

Fig. 2. Correlation between alkoxyl radical scavenging activity and the amount of Rhein Rhizoma (r = 0.87, p<0.05).

Fig. 3. Overall scavenging activities against multiple ROS.

Discussion

Because Kampo or other traditional herbal prescriptions contain various active ingredients, the evaluation of multiple ROS scavenging activity, although these results were not statistically significant (data not shown).

Overall scavenging activities against multiple ROS.

Figure 5 summarizes the total ROS scavenging profile of five representative Kampo formulae that exhibited high scavenging activity against each ROS compared with tsudosan (TDS), the formula reported to possess the highest ORAC value. Formulae containing high amounts of Rhein Rhizoma, daiokanzoto (DKZT) and mashiningan (MNG) showed solid RO_1 and ROO_2 scavenging activities. The RO_1 scavenging activity of these two formulae was remarkably strong even when they were compared with tsudosan (TDS). The ROS scavenging activities of orengedokuto (OGT), which contains Scutellariae Radix, Phellodendri Cortex and Coptidis Rhizoma, were remarkable against O_2^-.

Discussion

Because ROS possess high reactivity and their reactions are varied and complicated, it is difficult to describe the details of these pathways. This uncertainty is an obstacle for clinical anti-oxidative therapy and an analysis of multiple ROS pathways is strongly needed. Typically, an oxidative stress-related reaction is initiated by an excess production of primary ROS leading to further radical chain reactions, which then evoke cellular anti-oxidative responses. It is mainly superoxide that has this initiative role in biological systems. Thereafter, radical chain reactions involving ‘OH are evoked, leading to lipid hydroperoxide (LOOH) production. The presence of iron, copper, or heme protein, LOOH is converted to lipid alkoxyl radical (LO) or lipid alkylperoxyl radical (LOO), leading to further radical chain reactions. O_2^- also plays an important role in LOOH production. Thus, each ROS shows specific characteristics during in vivo reactions. Because Kampo or other traditional herbal prescriptions contain various active ingredients, the evaluation of multiple ROS dynamics is necessary for detailed analysis of their redox activity.

Oxidative stress is a critical and universal pathological factor that impacts various diseases. Antioxidative effects are recognized as the most important therapeutic mechanism of Kampo formulae. Many studies have demonstrated antioxidative activities for Kampo formulae and other herbal medicines, including their crude...
components,\(^3\)–\(^7\) but many details about the mechanisms of these activities remain to be clarified. Our results revealed that Kampo formulae known to present high ORAC values,\(^9\) such as mashiningan (MNG) and daiokanzoto (DKZT), possess high RO\(^\cdot\) scavenging activity. We also found that Rhei Rhizoma, a key crude drug that shows high ORAC values, showed a positive correlation between its quantity in the formulae and RO\(^\cdot\) scavenging activity. Therefore, much of the previously demonstrated antioxidative activity of Kampo formulae reflects RO\(^\cdot\) scavenging activity.

‘OH is considered the most toxic oxygen radical because of its extremely high reactivity against biological substances, leading to tissue/organ damage. No specific substances eliminate ‘OH \textit{in vivo}; however, ‘OH scavenging activity is strongly related to the pathophysiology of multiple diseases.\(^3\)–\(^5\)\(^,\)\(^16\) Kampo formulae containing \textit{Zingiberis Rhizoma} showed high scavenging activity against ‘OH. \textit{Zingiberis Rhizoma} contains multiple pharmacologically active components, including zingiberol and shogaol, and exhibits various pharmacological effects.\(^7\) Although \textit{Zingiberis Rhizoma} shows low antioxidative activity against AAPH-induced oxidative stress,\(^16\) our findings revealed remarkable ‘OH scavenging activity in formulae containing \textit{Zingiberis Rhizoma}. Some of its pharmacological effects may be induced by high ‘OH scavenging activity. In addition, formulae containing both \textit{Bupleuri Radix} and \textit{Scutellariae Radix}, namely shosaikoto (SSK), saikokaryukotsuboreito (SRB), and daisaikoto (DST), showed strong ‘OH scavenging activity [but saibokuto (SBT) did not]. Clinically, \textit{Bupleuri Radix} and \textit{Scutellariae Radix} are frequently used in combination and this combination shows stronger anti-inflammatory effects than single usage.\(^19\) Among the formulae used in this study, 69% of formulae containing \textit{Scutellariae Radix} also contain \textit{Bupleuri Radix} and their high ‘OH scavenging activity provides a pharmacological explanation for the traditional use of the crude composition.

We measured the scavenging activity of ROO\(^\cdot\) using tert-BuOOH; thus, this activity might reflect the lipid peroxidation process. Again, details of the activities of ROO\(^\cdot\) remain to be clarified, but current research indicates its importance in an animal model of hepatic carcinoma.\(^20\) Unlike the other ROS, ROO\(^\cdot\) scavenging activity was notable in formulae whose antioxidative activities were previously unknown, such as saibokuto (SBT), hangkekobokuto (HKT) and goreisan (GRS). Because saibokuto (SBT) is a blended formula comprising hangkekobokuto (HKT) and goreisan (GRS), the components of hangkekobokuto (HKT) and saibokuto (SSK) may relate to ROO\(^\cdot\) scavenging activity. As there were a small number of formulations and many combinations of crude drug ingredients, it was difficult to identify specific important responsive substances. Many results related to ROO\(^\cdot\) scavenging activity could not be explained by the constitution of herbal ingredients.

The superoxide scavenging activity of Kampo formulae has been widely reported.\(^21\)–\(^26\) A series of studies by Kohno and colleagues report an extensive screening of O\(_2\)\(^\cdot\) scavenging activity of herbal extracts.\(^43\) They found that extracts such as \textit{Rheum palmatum}, \textit{Ephedra sinica}, \textit{Panica granatum} and \textit{Caesalpinia sappan} show high O\(_2\)\(^\cdot\) scavenging activity. In our analysis of the formulations, we could not obtain a single crude component that enhanced O\(_2\)\(^\cdot\) scavenging activity. However, formulae containing the three crude extracts \textit{Scutellariae Radix}, \textit{Coptidis Rhizoma} and \textit{Phellodendri Cortex} \[i.e.,\] orangedokuto (OGT), unseiin (USI) and keigairengyoto (KRT) showed remarkably high O\(_2\)\(^\cdot\) scavenging activity. Consequently, in contrast to

### Table 6. Alkylperoxyl radical scavenging activity of Kampo formulae

| Kampo formula                  | ROO\(^\cdot\) scavenging activity (mM-aLaEq.) per daily dose per gram |
|-------------------------------|---------------------------------------------------------------|
| kaikokontokasenkyushin’i     | 148.4 19.8                                                   |
| maoto                         | 117.4 15.7                                                   |
| keishikaryukotsuboreito       | 90.8 12.1                                                    |
| saibokuto                     | 89.6 12.0                                                    |
| hangekobokuto                 | 86.3 11.5                                                    |
| mashiningan                   | 86.0 11.5                                                    |
| hochuekkito                   | 85.6 11.4                                                    |
| goreisan                      | 81.1 10.8                                                    |
| keishikajutsubuto             | 79.0 10.5                                                    |
| saikokeishito                 | 76.9 10.2                                                    |
| yokkansan                     | 74.1 9.9                                                     |
| jyuzendaihoto                 | 72.5 9.7                                                     |
| unseiin                       | 71.8 9.6                                                     |
| ninjinto                      | 71.7 9.6                                                     |
| yokuinintou                   | 70.6 9.4                                                     |
| keigairengyoto                | 69.8 9.3                                                     |
| saikokeishikankyouotto        | 68.8 9.2                                                     |
| daikokanzoto                  | 63.5 8.5                                                     |
| keishito                      | 62.8 8.4                                                     |
| orangedokuto                  | 59.1 7.9                                                     |
| daibotanbuto                  | 58.2 7.8                                                     |
| saikoseikanto                 | 54.7 7.3                                                     |
| rokumigan                     | 53.5 7.1                                                     |
| tsudosan                      | 53.4 7.1                                                     |
| kamikihito                    | 53.4 7.1                                                     |
| shosaikoto                    | 52.8 7.0                                                     |
| saireito                      | 52.8 5.9                                                     |
| saikokaryukotsuboreito        | 52.4 7.0                                                     |
| jumihaidokuto                 | 51.3 6.8                                                     |
| gosuyuto                      | 50.3 6.7                                                     |
| daisaikoto                    | 49.7 6.6                                                     |
| shimbuto                      | 49.7 6.6                                                     |
| kamishoyosan                  | 49.3 6.6                                                     |
| keishibukuryogyan             | 49.0 6.5                                                     |
| goshajinkigan                 | 48.7 6.5                                                     |
| hachimijigohan                | 48.0 6.4                                                     |
| tokishakuyakusun              | 47.9 6.4                                                     |
| tokinshii                     | 47.5 6.3                                                     |
| bofutsushosan                 | 47.1 6.3                                                     |
| boihoito                      | 46.6 6.2                                                     |
| rikkunshito                   | 44.1 5.9                                                     |
| tokishigayukagoshuyushokyoto  | 42.9 5.7                                                     |
| daikenchuto                   | 42.6 2.8                                                     |
| shimotsuto                    | 42.4 5.7                                                     |
| shokenchuto                   | 41.8 2.8                                                     |
| hangeshashinto                | 41.0 5.5                                                     |
| kakkontokou                   | 41.0 5.5                                                     |
| bakumondo                     | 36.5 4.1                                                     |
| shoseiyuuto                   | 34.6 4.6                                                     |

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\[\text{Fig. 3. Correlation between alkylperoxyl radical scavenging activity and the amount of Rhei Rhizoma (r=0.84, p<0.05).}\]
Table 7. Superoxide scavenging activity of Kampo formulae

| Kampo formula               | O$_2^-$ scavenging activity (μM-SOD eq) per daily dose | per gram |
|----------------------------|--------------------------------------------------------|----------|
| orangedokuto               | 3,522.3                                                 | 469.6    |
| saikokeishikanykoto        | 2,499.8                                                 | 333.3    |
| unsein                    | 2,321.0                                                 | 305.9    |
| keigaiyokoto               | 1,482.9                                                 | 197.7    |
| yokkansan                 | 1,400.1                                                 | 186.7    |
| kakkontokasenkyushin'i    | 1,107.3                                                 | 147.6    |
| saireito                  | 1,036.4                                                 | 115.2    |
| daiokanzoto               | 1,023.3                                                 | 136.4    |
| maoto                     | 991.3                                                   | 132.2    |
| tokiinshi                 | 834.1                                                   | 111.2    |
| tsudosan                  | 833.5                                                   | 111.1    |
| saibokuto                 | 751.0                                                   | 100.1    |
| goshajinkigan             | 704.1                                                   | 93.9     |
| shokenchuto               | 698.5                                                   | 46.6     |
| daiokanzoto               | 661.6                                                   | 88.2     |
| kamikihito                | 617.4                                                   | 82.0     |
| rokumigan                 | 604.1                                                   | 80.5     |
| hangeshashinto            | 580.5                                                   | 77.4     |
| hochuekkitto              | 540.2                                                   | 72.0     |
| kakkonto                   | 538.9                                                   | 71.9     |
| yokuinintou               | 525.4                                                   | 70.1     |
| daikenchuto               | 469.0                                                   | 31.3     |
| mashiningan               | 468.5                                                   | 62.5     |
| keishibukuryogan           | 454.4                                                   | 60.6     |
| daiobotanbito             | 444.4                                                   | 59.3     |
| shoseiryuto               | 423.4                                                   | 56.5     |
| saikokeishikanykoto       | 406.6                                                   | 54.2     |
| goshuyuto                 | 394.8                                                   | 52.6     |
| keishito                  | 382.9                                                   | 51.0     |
| hangekobokuto             | 359.8                                                   | 48.0     |
| shosaiyoto                | 343.1                                                   | 45.7     |
| ninjinto                  | 333.2                                                   | 44.4     |
| bofutsushosan             | 331.8                                                   | 44.2     |
| shimotsuto                | 313.5                                                   | 41.8     |
| boihoito                  | 304.5                                                   | 40.6     |
| keishikyokutsobureito      | 299.5                                                   | 39.9     |
| tokishigyakukagoshuyokyo   | 287.8                                                   | 38.4     |
| hachimijigoku             | 277.0                                                   | 36.9     |
| saikoikeishikanto         | 269.0                                                   | 35.9     |
| jumihaidokuto             | 260.0                                                   | 34.7     |
| keishikajutsubuto         | 258.1                                                   | 34.4     |
| goriisan                  | 251.1                                                   | 33.5     |
| kamishoyosan              | 240.0                                                   | 32.0     |
| saikoikeishito            | 230.9                                                   | 30.8     |
| rikunshito                | 198.4                                                   | 26.5     |
| jyuzaidaihito             | 187.4                                                   | 25.0     |
| shimbuto                  | 179.7                                                   | 24.0     |
| bakumondoto               | 161.6                                                   | 18.0     |
| tokishakuyakusen          | 143.7                                                   | 19.2     |

Table 8. Singlet oxygen scavenging activity of Kampo formulae

| Kampo formula               | O$_2$ scavenging activity (μM-GSH eq) per daily dose | per gram |
|----------------------------|-------------------------------------------------------|----------|
| saikokeishikanykoto        | 2,265.4                                                | 302.1    |
| daiokanzoto               | 1,410.9                                                | 188.1    |
| kokonito                   | 1,356.2                                                | 180.8    |
| shosaiyoto                | 1,071.4                                                | 142.9    |
| goshuyuto                 | 977.9                                                   | 130.4    |
| maoto                     | 742.3                                                   | 99.0     |
| hangeshashinto            | 732.4                                                   | 97.6     |
| saibokuto                 | 705.7                                                   | 94.1     |
| unsein                    | 654.6                                                   | 87.3     |
| saikoikeishikanykanto      | 584.5                                                   | 77.9     |
| daiobotanbito             | 515.0                                                   | 68.7     |
| bofutsushosan             | 485.7                                                   | 64.8     |
| keishibukuryogan           | 475.1                                                   | 63.4     |
| orangedokuto              | 467.3                                                   | 62.3     |
| kakkontokasenkyushin'i    | 454.0                                                   | 60.5     |
| hangekobokuto             | 451.6                                                   | 60.2     |
| shokenchuto               | 408.1                                                   | 27.2     |
| daiokanzoto               | 386.4                                                   | 51.5     |
| tsudosan                  | 350.5                                                   | 46.7     |
| tokishigyakukagoshuyokyo   | 338.5                                                   | 45.1     |
| saikokeishikanykoto       | 332.5                                                   | 44.3     |
| keigaiyokoto              | 319.9                                                   | 42.6     |
| yokuinintou               | 307.9                                                   | 41.0     |
| saireito                  | 295.9                                                   | 32.9     |
| keishito                  | 288.1                                                   | 38.4     |
| ninjinto                  | 269.0                                                   | 35.9     |
| daikenchuto               | 243.8                                                   | 16.3     |
| boihoito                  | 228.9                                                   | 30.5     |
| rikunshito                | 205.7                                                   | 27.4     |
| shoseiryuto               | 172.2                                                   | 23.0     |
| saikoikeishito            | 171.3                                                   | 22.8     |
| jumihaidokuto             | 163.1                                                   | 21.7     |
| rokumigan                 | 131.6                                                   | 17.5     |
| keishikyokutsobureito      | 125.6                                                   | 16.8     |
| keishikajutsuboto         | 124.4                                                   | 16.6     |
| yuzaidaihito              | 95.9                                                    | 12.8     |
| hochuekkitto              | 91.1                                                    | 12.1     |
| shimbuto                  | 82.6                                                    | 11.0     |
| yokkansan                 | 68.6                                                    | 9.1      |
| goriisan                  | 66.8                                                    | 8.9      |
| shimotsuto                | 66.0                                                    | 8.8      |
| tokiinshi                 | 8.0                                                     | 1.1      |
| kamikihito                | 6.6                                                     | 0.9      |
| goshajinkigan             | 6.0                                                     | 0.8      |

the 'OH scavenging activity of Scutellariae Radix, the combination of these three crude extracts may be crucial for O$_2^-$ control. Moreover, though unsein (USI) contains Rehmanniae Radix, which decreases multiple ROS scavenging activity as described below, O$_2^-$ scavenging activity was still high. Therefore, the combination of Scutellariae Radix, Coptidis Rhizoma and Phellodendri Cortex may recover the pro-oxidative effect of Rehmanniae Radix.

1O$_2$ shows high reactivity and toxicity in vivo and leads to lipid peroxidation, inducing further RO$^-$ and ROO$^-$ production.14 1O$_2$ is produced through myeloperoxidase and prostaglandin hydroperoxidase activity, and by the interaction between O$_2^-$ and H$_2$O$_2$ during the Haber-Weiss reaction.12,28 The importance of O$_2$ has been shown in several important pathophysiological processes, including ischemia-reperfusion injury and diabetes.29 We previously reported a crucial change of in vivo 1O$_2$ scavenging activity after an administration of kagen-karyu, a Kampo formula; however, the importance of this activity in Kampo or other herbal medicine remains to be clarified. High 1O$_2$ scavenging activity was detected in the formulae containing Bupleuri Radix; that is, saikoikeishikanykanto (SAKK), daiokanzoto (DST) and shosaikoto (SSK). The quantity of Bupleuri Radix showed a significant positive correlation with 1O$_2$ scavenging activity. Thus, Bupleuri...
Radix is responsible for both ‘OH and ‘O₂ scavenging activity. In contrast, it is notable that some formulae showed remarkably low ‘O₂ scavenging activity. In particular, the ‘O₂ scavenging activity of tokiinshi (TKI), kamikihito (KT) and goshajinkigan (GJG) was less than 1/200 of that of saikokeishikankyoto (SAKK).

Interestingly, the quantity of Rehmanniae Radix showed a significant negative correlation with ‘OH and ‘O₂ scavenging activities. Furthermore, formulae containing Rehmanniae Radix [i.e., hachimijiogan (HJG), goshajinkigan (GJG), rokumigan (RUG) and tokiinshi (TKI)] generally revealed low ROS scavenging activities against ‘OH, RO’, ROO’ and ‘O, but unseiin (USI) did not. Thus, Rehmanniae Radix may act as a pro-oxidant, rather than an antioxidant. Clinically, formulae that include Rehmanniae Radix are used in "hohou." Hohou is a revitalizing treatment that stimulates or compensates functions that are lacking and is used to treat chronic diseases, exhaustion, or aging, conditions in which pathophysiology is strongly related to oxidative stress. Kampo emphasizes the concept of equilibrium, which is similar to homeostasis in Western medicine; the concept of oxidative stress is based on the assumption of a pro-oxidant–antioxidant equilibrium. Thus, treatment of oxidative stress by Kampo medicine aims to rebalance the oxidative–antioxidative equilibrium (which is assumed to be tilted toward oxidation) by supplementing anti-oxidative crude extracts or elements. However, our findings of the low ROS scavenging activities of Rehmanniae Radix, which is a representative crude extract in hohou formulae, totally contradicts this assumption. Therefore, we hypothesize that Kampo oxidative stress treatments do not simply complement antioxidants, but induce internal antioxidative activity by stimulating pro-oxidative elements.

As the “antioxidant paradox” concept indicates, it remains unclear whether treatment of oxidative stress by administration of antioxidants benefits the whole body. Rather, it is highly likely that weak pro-oxidants have better effects on living bodies than antioxidants, through a radiation hormesis-like beneficial effect. However, it is currently difficult to obtain approval for clinical trials using pro-oxidants owing to ethical problems. Kampo is the most systematically organized complementary and alternative medical treatment. Its safety has been established and it is widely used. Therefore, elucidation of the pro-oxidative effects of Kampo might be a promising yet realistic research pathway toward the therapeutic application of pro-oxidants. Further investigations that include oxidative stress evaluations may lead to a new concept of oxidative stress treatment using pro-oxidants.

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Abbreviations

Table 1 shows abbreviations of the Kampo formulae. Because Kampo formulae have complex names, we have included both the formal name of Kampo formulae and their abbreviations throughout the manuscript.

Conflict of Interest

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Fig. 5. Total ROS scavenging profile of representative Kampo formulae, namely daiokanzoto (DKZT) (a), orengedokuto (OGT) (b), saikokeishikanyakuto (SAKK) (c), mashinginan (MNG) (d), maoto (MT) (e), and tsudosan (TDS) (f). The scavenging activities against each ROS are expressed as relative values in comparison with tsudosan.

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