Effect of Kampo medicine on doxorubicin-induced lipid peroxidation in mice

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Abstract

To evaluate the effects of Kampo medicine on free-radical-induced tissue injury, we examined the effect of four well-known Kampo medicines, Sho-saiko-to (SST), Dai-saiko-to (DST), Oren-gedoku-to (OGT), and Juzen-taiho-to (JTT) on doxorubicin-induced tissue lipid peroxide (LPO) formation in the liver, kidney, heart, and brain of mice and examined their effects on the activities of two antioxidative enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Intraperitoneally injected doxorubicin (20 mg/kg) significantly increased tissue LPO, measured as thiobarbituric acid reactive substances, in liver, kidney, heart, and brain of mice ; however, SOD and GSH-Px activity were not affected by this treatment. Daily administration of Kampo medicine after doxorubicin injection significantly reduced LPO levels ; SST, DST, and OGT suppressed LPO formation in the liver and kidney, and all 4 Kampo medicines suppressed LPO formation in the heart and brain. Of the four medicines, only SST reduced LPO formation in all four organs, a finding that suggests SST has broad antioxidative ability. In contrast to the significant decrease of tissue LPO levels, only small changes were observed in SOD and GSH-Px activities, suggesting Kampo reduces tissue LPO not by enhancing the enzymatic antiradical capacities, but rather by directly reducing the effect of radicals or the radicals itself. These results demonstrate that Kampo medicines have significant antioxidative effects and suggest that they might reduce radical-induced injury in various disease states.

Key words doxorubicin, lipid peroxide, superoxide dismutase, glutathione peroxidase, Kampo medicine.

Abbreviations DOX, doxorubicin ; DST, Dai-saiko-to (Da-Chai-Hu-Tang), 大柴胡湯 ; HWE, hot water extract ; JTT, Juzen-taiho-to (Shi-Quan-Da-Bu-Tang), 十全大補湯 ; GSH-Px, glutathione peroxidase ; LPO, lipid peroxide ; MDA, malondialdehyde ; OGT, Oren-gedoku-to (Huan-Lian-Jie-Du-Tang), 黄連解毒湯 ; SOD, superoxide dismutase ; SST, Sho-saiko-to (Xiao-Chai-Hu-Tang), 小柴胡湯 ; TBARS, thiobarbituric acid reacting substance ; XOD, xanthine oxidase.

Introduction

A substantial volume of research has indicated that free radicals are involved in tissue injury and the development of clinically significant disease.[1] Oxidative damage produced by free radicals is thought to be a basic mechanism underlying many diverse pathologic conditions.[2,3]

Lipid peroxide (LPO) is a product of chemical damage to the lipid component of cell membranes by free radicals and is regarded as a measure of free-radical damage. LPO is thought to be involved in various pathologic processes of disease.[4]

Many herbal ingredients of Kampo, or Japanese herbal medicine, have been found to have antioxidative effects. We have evaluated the effects of 32 herbs on the formation of LPO, and found that 25 suppressed LPO formation *in vitro*,[5-7] a finding result that agrees with those of other reports on antiox-

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idative effects of medicinal herbs.[8,9]

In vitro examination has confirmed the presence of many antioxidative principles in these herbs including phenolics (flavonoids, lignans and tannins), carotenoids, and saponins.[10] Although the effects of these natural compounds could be investigated by numerous methods, the same results are not always obtained in *in vivo* examination.

Today, more than 100 Kampo formulations are used in clinical practice in Japan and many are suspected to have antioxidative effects. However, the antioxidative effects of only a small number of the Kampo formulations has been investigated in *in vivo*. The actual *in vivo* effects and their relation to the clinical indications of each Kampo medicine remain to be proved.

Doxorubicin, an anthracycline antibiotic agent, increases tissue LPO levels in several organs of mice.[11] Because doxorubicin generates oxygen radicals,[12] this doxorubicin-induced increase in LPO level is regarded to be a result of oxygen radical stress. In this study, we evaluated the effects of four frequently used Kampo formulations on LPO formation in four organs using this doxorubicin-induced mice model and also examined their effects on the activities of the antioxidative enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px).

Materials and Methods

Chemicals : Doxorubicin was purchased from Kyowa Hakko (Tokyo, Japan). Bovine erythrocyte

Table I Prescription of Kampo formulations

Formulation	Name	Herbs	Daily dose (g)
Sho-saiko-to (SST)	saiko	Bupleuri Radix	7.0
	ninjin	Ginseng Radix	3.0
	kanzo	Glycyrrhizae Radix	2.0
	hange	Pinelliae Tuber	5.0
	ogon	Scutellariae Radix	3.0
	syokyo	Zingiberis Rhizoma	0.5
	taiso	Zizyphi Fructus	3.0
Dai-saiko-to (DST)	kijitsu	Aurantii Fructus Immaturus	2.0
	syakuyaku	Paeoniae Radix	3.0
	saiko	Bupleuri Radix	6.0
	hange	Pinelliae Tuber	4.0
	daio	Rhei Rhizoma	1.0
	ogon	Scutellariae Radix	3.0
	taiso	Zizyphi Fructus	3.0
	syokyo	Zingiberis Rhizoma	0.5
Oren-gedoku-to (OGT)	oren	Coptidis Rhizoma	2.0
	sansisi	Gardeniae Fructus	2.0
	obaku	Phellodendri Cortex	2.0
	ogon	Scutellariae Radix	3.0
Juzen-taiho-to (JTT)	toki	Angelicae Radix	4.0
	ougi	Astragali Radix	3.0
	byakujutsu	Atractyloidis Rhizoma	4.0
	jiou	Rehmanniae Radix	4.0
	keihi	Cinnamomi Cortex	3.0
	senkyu	Cnidii Rhizoma	3.0
	ninjin	Ginseng Radix	3.0
	kanzo	Glycyrrhizae Radix	2.0
	bukuryo	Hoelen	4.0
	syakuyaku	Paeoniae Radix	3.0

SOD (EC 1.15, 1.1) and xanthine oxidase (XOD, EC 1.2.3.2) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Oriental Yeast Co., Ltd. (Osaka, Japan), respectively. The other chemicals used in this study were of the highest purity available.

Preparation and administration of Kampo medicines : We selected four representative Kampo formulations, Sho-saiko-to (SST, Xiao-Chai-Hu-Tang ; 小柴胡湯), Dai-saiko-to (DST, Da-Chai-Hu-Tang ; 大柴胡湯), Oren-gedoku-to (OGT, Huan-Lian-Jie-Du-Tang ; 黃連解毒湯), and Juzen-taiho-to (JTT, Shi-Quan-Da-Du-Tang ; 十全大補湯). They are widely used in clinical medicine, and regarded as 'basic' formulation because they make common subsets for many other important formulations.

Formulations for human adults are listed in Table I. The crude drugs were purchased from Uchida Wakan-Yaku Co., Ltd. (Tokyo, Japan), Tsumura Co., Ltd. (Tokyo, Japan), and Tochimoto-Tenkaido Co. (Osaka, Japan). Hot water extracts (HWEs) were prepared as follows. The crude drugs were immersed in a 20-fold (w/w) amount of distilled water and was boiled for 40 minutes, and then immediately passed through filter paper (Advantec 2, Toyo Roshi Co., Tokyo Japan). The filtrate was evaporated under reduced pressure, then lyophilized and stored. The yields of lyophilized HWEs were 24.1 %, 25.2 %, 27.6 %, and 32.1 % for SST, DST, OGT, and JTT, respectively. With these HWEs, the appropriate daily doses for a human adult were 94, 94, 41, and 176 mg/kg for SST, DST, OGT, and JTT, respectively.

Animal experiments : Six-week-old male CDF_1-mice (Nihon SLC Co., Shizuoka, Japan) were randomly divided into groups of six to seven and kept in a room with controlled temperature of 24±1°C and relative humidity (55±10%) and a 12-hour light-dark cycle. Mice were given a normal diet (MF ; Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*.

The HWEs were dissolved in distilled water and administered orally once a day from the day before doxorubicin injection for 4 days at doses of 20, 100, and 500 mg/kg body weight. For control mice, the same volume of distilled water was administered.

LPO, SOD, GSH-Px : After the mice had been anesthetized with pentobarbital (30 mg/kg), the liver, kidney, heart, spleen, and brains were removed after systemic perfusion with ice-cold saline via the inferior vena cava. The organs were homogenized with saline in a Potter Elvehjem homogenizer. For LPO assay, 2 % homogenate was used and for protein content, GSH-Px activity, and SOD activity assay, 1 % homogenate was used. The LPO in tissue was detected as thiobarbituric acid-reacting substances (TBARS)[13] with the method of Tanizawa *et al.*[14] and the amount was expressed as mol equivalents of malondialdehyde (MDA).

The tissue SOD activity was determined with the nitrite method.[15] Briefly, 100 μl of sample was mixed with a reagent consisting of hydroxyl amine, xanthine, and KH_2PO_4-$Na_2Ba_4O_7$ buffer (pH 8.2) with 0.05 mM EDTA. After XOD (400 mU/ml) was added, the reaction mixture was incubated for 30 minutes at 37°C. The reaction was stopped by adding coloring reagents, consisting of 300 μg/ml sulfanilic acid, 5 μg/ml *N*-1-naphthylethylenediamine, and 16.7 % acetic acid. One hundred microliters of distilled water was added for control. For the blank, the same volume of distilled water was added instead of XOD. The optical absorption was measured at 550 nm, and the SOD activity of the sample was expressed as the percent inhibition of the control group. With bovine erythrocyte SOD as standard, 50 % inhibition was 39.9 nitrate units (NU) per organ weight (g) equivalent.

GSH-Px activity was assayed according to the method of Hafeman *et al.*[16] In this method, the rate of oxidation of reduced glutathione (GSH) by hydroperoxide is calculated from the change of concentration of GSH as catalyzed by GSH-Px present in the tissue sample. The reduced GSH was measured by optical absorption at 412 nm as the concentration of 2-nitro-5-thiobenzoic acid generated from the reaction of GSH with 5',5'-dithiobis-2-nitrobenzoic acid. One unit of GSH-Px (GSH-PxU) is the amount that transforms 1 μmol of GSH per minute. For tissue samples, the activity was expressed as GSH-PxU per protein content (mg).

The protein content of samples was analyzed with the method of Lowry *et al.*[17]

Administration of doxorubicin : Doxorubicin was thawed and diluted with sterile isotonic saline to obtain a 1.0 mg/ml solution, which was injected

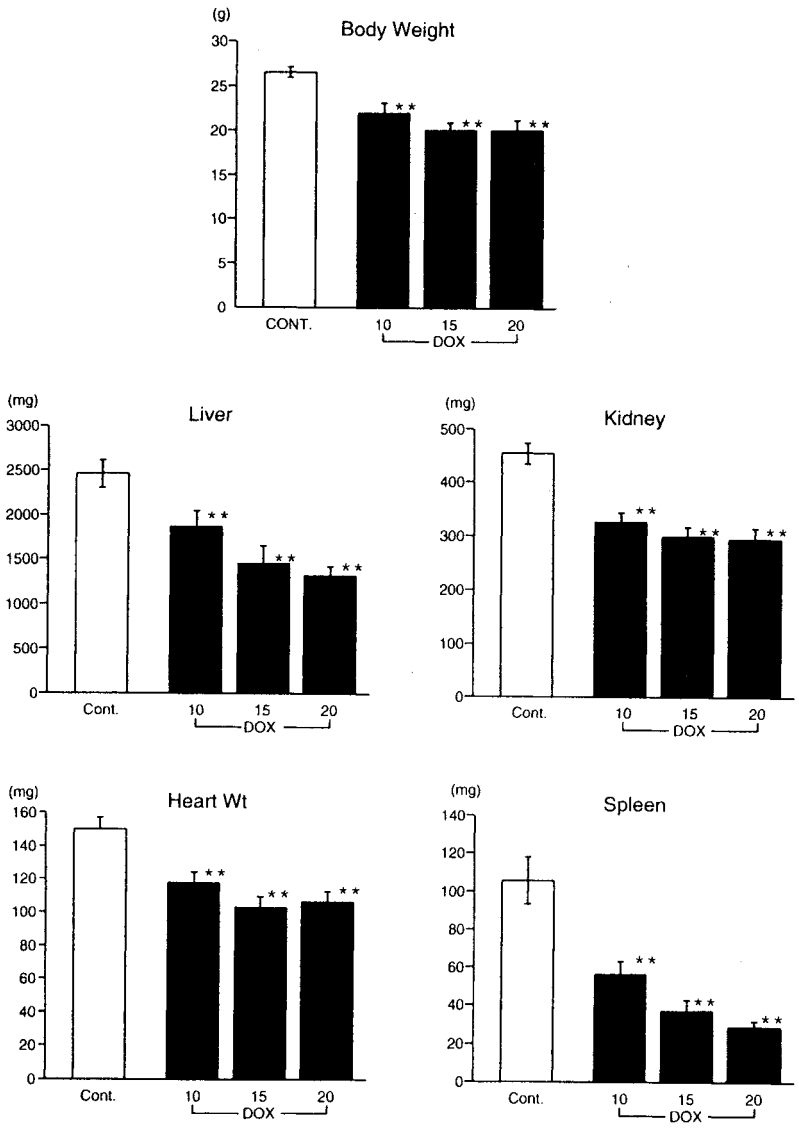

Fig. 1 Effect of doxorubicin on body weights and organ weight. Doxorubicin (DOX) was administered intraperitoneally at doses of 10, 15, and 20 mg/kg. Each value represents the mean±S.D., and is expressed as box and error bar. $^{**}p<0.01$, significant difference from control group (Cont.)

intraperitoneally. Control animals received injections of the same volume of sterile isotonic saline.

Before the experiments with Kampo medicines, we investigated the effect of doxorubicin on LPO formation in four organs (liver, kidney, heart, and spleen) in mice.

Although no mice died through the 4th day after intraperitoneal doxorubicin injection, significant body weight loss was observed. In each organ, weight loss was more severe (Fig. 1).

The effect on tissue LPO is shown in Fig. 2. At all three doses, marked elevations of LPO were observed, except in the spleen, where LPO levels were reduced by doxorubicin treatment. In liver, LPO levels were

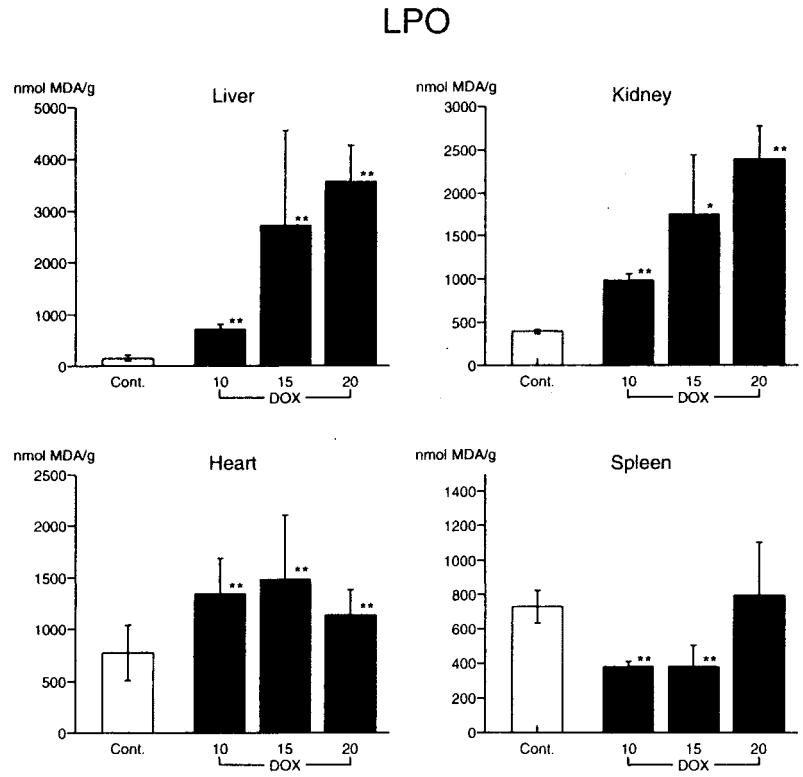

Fig. 2 Effect of doxorubicin on LPO formation in mice organs. Doxorubicin (DOX) was administered intraperitoneally at doses of 10, 15, and 20 mg/kg. LPO was calculated as mol equivalent of malondialdehyde (MDA) per tissue weight. Each value represents the mean±S.D. $*p<0.05$, $**p<0.01$: significant difference from control group (Cont.)

elevated 5 to 25 fold compared with those in the control group in a dose-dependent manner ; in kidney, 2.5 to 6.0 fold and in heart, 1.7 to 1.9 fold.

The effects of doxorubicin on levels of SOD and GSH-Px are shown in Table II. The SOD activity was reduced by doses of 15 and 20 mg/kg in the liver and kidney ; GSH-Px activity was reduced by 20 mg/kg doxorubicin in the liver, but increased by 20 mg/kg doxorubicin in the heart. No other significant changes were observed.

From these results, 20 mg/kg of doxorubicin was administered in the experiments for Kampo medicine.

Statistical analysis : Statistical comparisons were performed with Student's *t*-test. All results are expressed as mean±S.D. In all comparisons, differences with $p<0.05$ were considered significant.

Results

Effect of Kampo medicine on tissue LPO levels of doxorubicin-treated mice

In body weights and organ weights, no significant difference was obsereved between Kampo-treated groups and the control group.

In the liver, tissue levels of LPO were increased significantly with administration of doxorubicin at a dose of 20 mg/kg. However LPO levels were reduced with three Kampo medicines : SST at 20, 100, and 500 mg/kg ; DST at 500 mg/kg ; and OGT at 20 mg/kg significantly decreased LPO levels as compared with those in the water group (Fig. 3).

In kidney tissue, SST at 500 mg/kg, DST at 500

Table II Effect of doxorubicin on SOD and GSH-Px activities in mice.

Organ	Dose of doxorubicin (mg/kg)	SOD activity[1] (inhibition%)	GSH-Px activity[2] (GSH-PxU/protein mg)
Liver	10	80.4±4.2	101.9±17.1
	15	73.0±7.2**	92.4±22.5
	20	71.5±2.7**	70.4±17.0*
	control	83.2±1.7	98.0±16.4
Kidney	10	74.8±3.4	60.8±8.9
	15	72.2±2.2*	65.2±13.0
	20	70.8±3.6**	69.2±11.1
	control	78.0±4.5	65.0±12.8
Heart	10	57.7±2.5**	46.5±7.4
	15	56.6±2.7**	47.1±6.8
	20	60.7±3.9	50.5±5.3*
	control	63.0±2.8	40.7±7.5

1) The tissue SOD activity was determined with the nitrite method[15] and is expressed as inhibition of blank (H_2O) sample. Using boving erythrocyte SOD as standard, 50% inhibition was 39.9 nitrate units (NU) per organ weight (g) equivalent. 2) GSH-Px activity was assayed according to the method of Hafeman *et al.*[16] One unit of GST-Px (GSH-PxU) is amount that transforms 1 μmol of GSH/min. Each datum is expressed as the mean±S.D. $^*p<0.05$, $^{**}p<0.01$: significant differece from control.

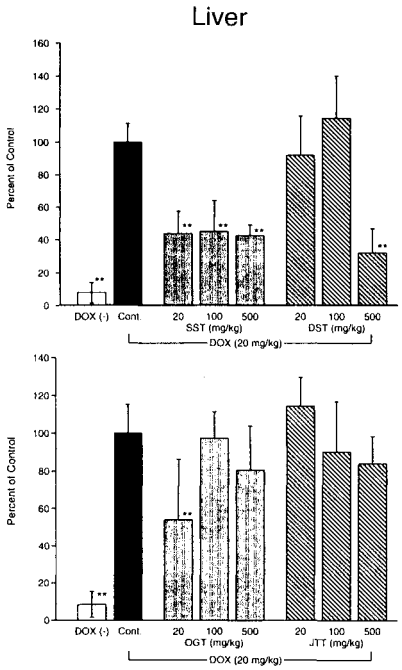

Fig. 3 Effects of Kampo medicine on doxorubicin-induced LPO formation in liver.
DOX (-) : Saline was administered instead of doxorubicin (DOX). In the other groups doxorubicin was injected intraperitoneally at a dose of 20 mg/kg. SST : Sho-saiko-to, DST : Daisaiko-to, OGT : Oren-gedoku-to, and JTT : Juzen-taiho-to (See Table I for ingredients). Kampo medicine was administered orally at doses of 20, 100, and 500 mg/kg after doxorubicin injection for 4 days. Each datum was calculated as the percent of control group ; mean value and S.D. are expressed as box and error bar, respectively. $^{**}p<0.01$, significant difference from control group (Cont.)

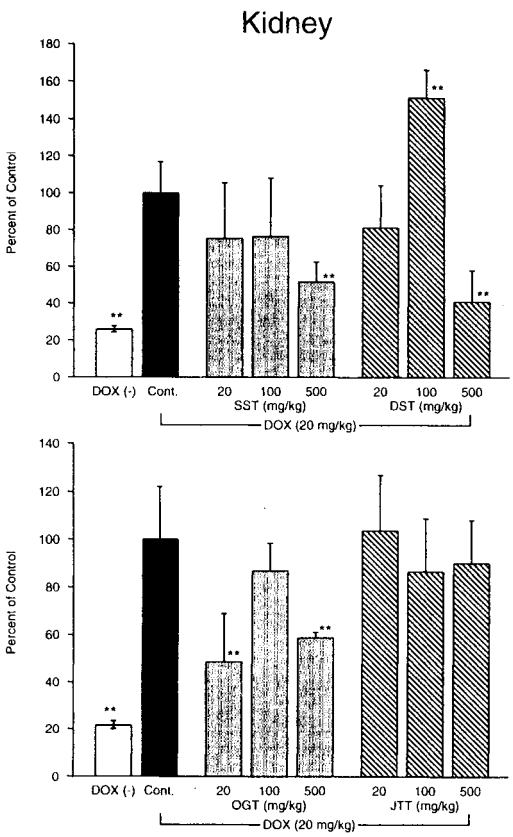

Fig. 4 Effects of Kampo medicine on doxorubicin-induced LPO formation in kidney. See the legend for Fig. 3. Each datum was calculated as the percent of control group ; mean value and S.D. are expressed as box and error bar, respectively. $^{**}p<0.01$, significant difference from control group (Cont.)

mg/kg, and OGT at 20 and 500 mg/kg significantly reduced LPO levels ; however, increased levels of LPO were observed with DST at a dose of 100 mg/kg (Fig. 4).

In heart tissue, elevations in tissue levels of LPO were suppressed by all three dosages of SST and DST, by OGT at 20 and 500 mg/kg, and by JTT at 500 mg/kg (Fig. 5).

In brain tissue, significantly elevated LPO levels were observed after doxorubicin injection. However, after each of the four Kampo medicines were given, reductions in tissue LPO levels were observed : SST, DST, and OGT at 100 and 500 mg/kg and JTT at 20 and 500 mg/kg significantly reduced LPO levels (Fig. 6).

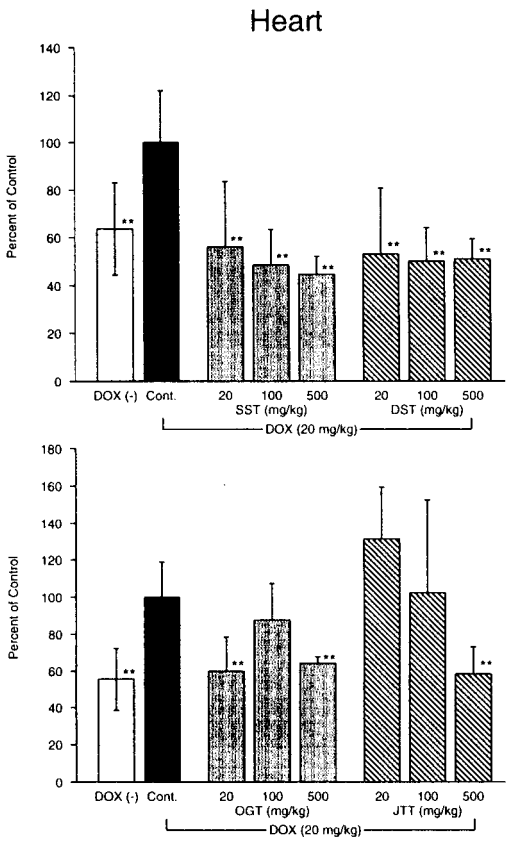

Fig. 5 Effects of Kampo medicine on doxorubicin-induced LPO formation in heart. See the legend for Fig. 3. Each datum was calculated as the percent of control group ; mean value and S.D. were expressed as box and error bar, respectively. $^{**}p<0.01$, significant difference from control group (Cont.)

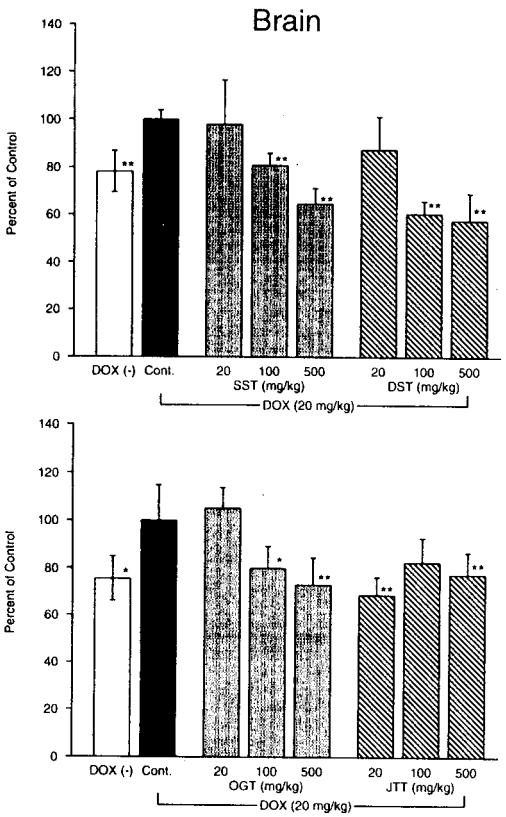

Fig. 6 Effects of Kampo medicine on doxorubicin-induced LPO formation in brain. See the legend for Fig. 3. Each datum was calulated as the percent of control group ; mean value and S.D. are expressed as box and error bar, respectively. $^{*}p<0.05$, $^{**}p<0.01$, significant difference from control group (Cont.)

Effect of Kampo medicine on SOD and GSH-Px activities of doxorubicin-treated mice

Reductions in SOD activity were observed after administration of Kampo medicine ; however, the reductions were less than 10 % in most groups (Table III).

The activity of GSH-Px was merely affected by Kampo treatment. Significant differences in this activity were observed as follows : in kidney, DST (20 mg/kg) increased GSH-Px activity to 119.8±13.9 % of control, and in heart, DST (500 mg/kg) increased GSH-Px activity to 130.5±23.9 % of control levels. In brain, DST at 20 and 100 mg/kg increased GSH-Px activity to 135.5±20.0 % and 113.7±17.8 %, respectively, of control levels. In the other groups, no significant differences were observed (data not shown).

Table III Effect of Kampo medicine on SOD activity of doxorubicin-treated mice.

Kampo (mg/kg)		Liver	Kidney	Heart	Brain
doxorubicin(-)		101.5±2.1	94.0±5.4*	90.5±4.0**	93.0±3.5**
Control		100.0±2.5	100.0±3.8	100.0±3.8	100.0±2.8
SST	20	102.3±6.1	97.0±3.6	102.5±9.5	103.1±6.1
	100	101.1±3.8	99.3±3.6	99.9±5.7	102.9±3.7
	500	101.2±5.8	97.4±2.1*	96.1±6.6	102.4±4.8
DST	20	93.4±7.3	94.3±5.3	97.7±3.5	100.5±2.9
	100	95.6±3.4*	87.4±10.7*	93.9±8.9	100.2±15.6
	500	99.4±5.9	89.6±6.7**	98.0±4.1	97.2±8.0
JTT	20	99.8±5.9	94.8±2.8*	99.6±6.7	95.1±2.2**
	100	99.9±1.5	93.7±3.4*	96.4±4.6	96.0±3.5*
	500	95.4±5.7	92.3±3.6*	97.4±7.3	96.5±4.7
OGT	20	102.8±4.7	99.4±2.5	101.1±6.5	101.2±2.4
	100	101.9±4.8	101.0±2.7	100.8±2.7	98.4±3.8
	500	103.4±3.5	101.8±3.3	99.7±4.6	98.2±2.3

doxorubicin (-) : saline was injected instead of doxorubicin. Control : same volume of water was administered p.o. Each datum was calculated as the percent value of control group, and is expressed as mean±S.D. $*p<0.05$, $**p<0.01$: significant difference from control.

Discussion

Doxorubicin enhances LPO formation in the heart, liver, and kidney in mice.[11,18] This LPO induction is thought to be initiated by oxyradicals generated through the redox cycling of doxorubicin *in vivo* as has already been demonstrated in studies of cultured heart cells[18] and mitochondrial membranes of hepatocytes.[12] Both the superoxide anion radical and the hydroxyl radical have been demonstrated in rat myocardial sarcosome after treatment with doxorubicin.[19]

Our results clearly shows that Kampo medicines at near-clinical doses have the ability to suppress LPO formation after doxorubicin treatment *in vivo*. However, the effects of the formulations in the tested organs were not uniform. For example, DST suppressed LPO at all three doses in the heart, but only at the highest dose in the liver and kidney. Furthermore, JTT suppressed LPO formation in the heart and brain but not in the liver or kidney. These results show that Kampo medicines behave differently in different organs despite similar antioxidative effects. In clinical practice, different Kampo formulations are belived to be indicated in different disease states. The behavior of Kampo medicines observed in our study could partly explain the clinical effects of each formulation and indications.

The cardiotoxicity of doxorubicin, unique side-effect that limits doxorubicin's clinical use,[22] is believed to be an oxidative pathology due mainly to hydroxyl radicals.[19] Thus, the important roles of SOD and GSH-Px were expected to be detoxifying doxorubicin.[21] However, in our examination, administration of Kampo medicines at clinical doses significantly suppressed LPO formation in the heart without enhancing the activity of SOD or GSH-Px. In other organs as well, only small changes were observed in the activities of SOD and GSH-Px although LPO production was significantly suppressed. These findings suggest that Kampo medicines directly reduce the effect of radicals, rather than enzymatically enhancing the capacity for radicals. In particular, Kampo medicines may reduce doxorubicin's cardiotoxicity and may enhance its clinical usefulness.

In this study, LPO levels in the liver were suppressed by SST, DST, and OGT. SST and DST are thought to be similar medicines. SST has five ingredients in common with DST (see Table I). Both medicines were indicated in liver disease in traditional medicine and are still widely used for the treatment of liver disorders. Their benefits have been confirmed in clinical trials and experiments : SST lowers serum levels of transaminases in patients with chronic active hepatitis patients[23] and prevents the development of

hepatocellular carcinoma in patients with chronic hepatitis.[24] The anti-inflammatory effects of SST have been confirmed in various models of liver dysfunction.[25 28] Yamano *et al.* reported that DST lowers elevated serum levels of LPO in patients with hyperlipidemia after 3 months' administration.[29] In rats, DST suppresses LPO formation induced by a corn-oil diet.[30] The antioxidative effects of DST were also observed after hepatic injury induced by methoxyflurane inhalation in the rat.[31] The most well known antioxidative herb in SST and DST is Scutellariae Radix (ogon in Japanese), which is also present in OGT. Oral administration of the water extracts of Scutellariae Radix and its flavonoid components, such as wogonin, baicalein, and baicalin, are known to suppress the $FeCl_2$-ascorbic acid-ADP-stimulated lipid peroxidation of rat liver.[32] In the liver, however, we found that DST suppressed LPO formation only at the highest dose, 500 mg/kg, whereas SST suppressed it in all doses. In kidney, SST and DST at doses 500 mg/kg suppressed LPO, but DST at a dose of 100 mg/kg enhanced LPO formation. In the heart, SST and DST at all doses restored LPO levels. In the brain, they also restored LPO at doses of 100 and 500 mg/kg. These results suggest that SST and DST have similar antioxidative effects in the heart and brain, but that SST has stronger preventive effect than does DST in the liver and kidney.

Two herbs, Glycyrrhizae Radix and Ginseng Radix are included in SST but not in DST ; these two herbs are widely used and are regarded as extremely important in Kampo medicine. Glycyrrhizin[33] in Glycyrrhizae Radix and various ginsenosides[34] in Ginseng Radix are known to have antioxidative effects. It has been suggested that these two herbs play important roles in suppressing LPO formation in the liver in the doxorubicin-treated mice model.

OGT, the simplest formulation in the present study, consists of four herbs and is regarded as one of the basic formulations in Kampo medicine. Today, it is widely used to treat cardiovascular disorders (arterial hypertension, cerebrovascular disorders, and cerebral infarction), skin diseases, in psychiatric diseases. Its antioxidative effects have already been demonstrated *in vitro* by Uchida *et al.*[35] and Ohta *et al.*[36] and *in vivo* by Ohta *et al.* in rats stressed with water immersion and restraint.[37] Uchida *et al.* also reported that OGT prevents stroke and pathologic changes in the renal artery and increases the survival of stroke-prone spontaneously hypertensive rats. In our experiment, suppression of LPO formation by OGT was observed in all four organs. Oxidative stress and the formation of LPO are regarded as important factors in the pathogenesis of atherosclerosis.[1] It has been suggested that OGT's prevention of stroke and vascular pathology in arterial hypertension are partly explained by OGT's suppression of LPO formation under condition of oxidative stress.

JTT is also an important Kampo medicine. In traditional medicine, it is indicated for the treatment of fatigue, fever, anemia, anxiety, and other symptoms in various disease states. In modern medicine, JTT is frequently used to treat patients with malignant tumors, or used in combination with chemotherapy and radiation therapy. In clinical studies, JTT restores physical strength, and decreases various symptoms or side effects in patients undergoing chemotherapy or radiation therapy. In our experiments, the suppressive effect of JTT on radical induced injury was confirmed in the heart and brain. This effect may partly be a good explanation for these clinical effectiveness.

In summary, we have shown that doxorubicin-induced LPO formation in organs of mice is suppressed to various degrees by treatment with four representative Kampo medicines. This effect of Kampo medicine was not accompanied by enhancement of SOD or GSH-Px activity in each organ examined, suggesting Kampo reduces tissue LPO not by enhancing the enzymatic antiradical capacities, but rather by directly reducing the effect of radicals or the radicals itself. However, the supressive effects of the formulations were not equal in all organs. This difference might partly explain the actual indications of each Kampo formulation. Further investigation of these relationships is needed.

The findings of the present study are consistent with the idea that Kampo medicines have antioxidative effects and can reduce radical-induced injury in various disease states.

和文抄録

漢方薬の抗酸化活性は薬効の一部と考えられているが，in vivo ではごく一部の方剤において確かめられているにすぎない。抗腫瘍薬ドキソルビシンはマウスにおいて各臓器の脂質過酸化物（LPO）を増加させるが，今回このモデルにおいて小柴胡湯，大柴胡湯，黄連解毒湯，十全大補湯は臓器 LPO を様々に抑制した。しかし，ラジカル消去系の酵素である SOD，グルタチオンペルオキシダーゼの活性の変化は少なかったことから，漢方薬の抑制作用は直接的なものであると考えられた。今回得られた結果から，漢方薬は比較的強い抗酸化作用を in vivo においても保持していると考えられた。

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