

ORIGINAL**Impact of the use of Kampo medicine in patients with esophageal cancer during chemotherapy : a clinical trial for oral hygiene and oral condition**

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Abstract : Objective : The aim of this study was to investigate the impact of the use of two Kampo medicines on oral mucositis, tongue coating bacteria, and gingiva condition in patients with esophageal cancer undergoing chemotherapy. **Methods :** Twenty-three esophageal cancer patients who receive chemotherapy at Tokushima University Hospital, were included. The participants, who received professional oral healthcare, were randomly divided into three groups : 7 subjects received Daiokanzoto sherbets, 7 subjects received Hangeshashinto sherbets, and 9 subjects received nothing (control). The numbers of total bacteria and specific periodontopathogenic bacteria in tongue coating were determined in addition to clinical parameters. **Results :** No difference on the onset of oral mucositis was found among the three groups. However, tongue coating index, gingival index (GI), plaque index, the number of total bacteria, *Fusobacterium nucleatum* and *Campylobacter rectus* were decreased during chemotherapy. More specifically, GI as well as the number of *F. nucleatum* and *C. rectus* were decreased significantly in the Daiokanzoto group when compared to the control group ($p < 0.05$). No such differences were observed for the group receiving Hangeshashinto. **Conclusion :** This clinical trial showed that Daiokanzoto might be effective in attenuating gingival inflammation and reducing the levels of periodontopathogenic bacteria in patients with esophageal cancer. **J. Med. Invest. 65 : 184-190, August, 2018**

Keywords : Kampo medicine, esophageal cancer, periodontopathogenic bacteria, gingival inflammation

INTRODUCTION

Cancer is the most prevalent cause of death in developed countries. Although the advancement in cancer therapy improved survival rates, radiotherapy and chemotherapy treatments cause several side-effects including some in the oral cavity. Oral mucositis that manifests as inflamed erosive or ulcerative lesions of the oral mucosa, is currently considered as a severe complication of anti-cancer therapy, affecting 40-80% of patients undergoing chemotherapy (1). Pain associated with oral mucositis can restrain patients from eating, thus resulting in a major impact on the quality of life in such patients.

A number of Kampo medicines have been used clinically for the treatment of various disease ; interestingly, 148 kinds of Kampo medicinal formula are approved and reimbursed by Japan's National Health Insurance (2). However, few Kampo medicines have been proposed for diseases affecting the oral cavity. Rikkosan has been suggested for controlling pain after tooth extraction, while Hangeshashinto, Orento and Inchinkoto may be effective against stomatitis (3, 4). Among them, Hangeshashinto was used topically, in addition to an oral intake. Moreover, several *in vitro* studies on the beneficial effects of Kampo medicines against periodontal disease have been published. For instance, it has been reported that

specific Kampo medicines possess anti-inflammatory potential by reducing the secretion of pro-inflammatory cytokines by host cells (3-6), in addition to prevent biofilm formation (6) and to inhibit the growth and the adherence properties of important periodontopathogenic bacteria, including *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (7).

Historically, mucositis has been considered to result exclusively from damage to basal epithelial cells induced by chemotherapy or chemoradiotherapy. Nowadays, a five-stage model for the pathobiology of oral mucositis is widely accepted ; this model incorporates complex interactions among multiple components (8) These include direct damage to basal epithelial cells from cancer therapy as well as secondary insults to tissue caused by an upregulation of pro-inflammatory mediator production (9). Ulcerative oral mucositis exposed to the oral microbiota may be complicated by a localized infection, thus providing a potential route for systemic sepsis (9).

In order to minimize the harmful effects associated with oral mucositis, the search for novel potent molecules is of interest. Natural compounds exhibiting anti-inflammatory and antimicrobial properties may represent potentially interesting molecules for the prevention or treatment of ulcerative lesions of oral tissue. Very few clinical studies investigated the potential of Kampo medicines with regard to oral mucositis and mucosal inflammation (10, 11). Hangeshashinto was effective for the treatment of oral ulcer (4) and it is covered by the Japanese insurance. In addition, it demonstrated in our previous study that Daiokanzoto possessed the preventive effect on 5-FU induced human gingival cell death (12).

The aim of this study was to investigate the impact of the use of

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two Kampo medicines (Daiokanzoto and Hangeshashinto) on oral mucositis, tongue coating bacteria, and gingiva condition in patients with esophageal cancer undergoing chemotherapy.

METHODS

Study participants

Twenty-four patients aged from 52 to 81 years-old (21 males and 3 females, mean age, 64.7 ± 7.7 years), who received chemotherapy with weekly docetaxel, and low dose 5-FU and CDDP (DFP therapy) as the first regimen specified for esophageal cancer at the Tokushima University Hospital (13, 14) from June, 2012 to July, 2015 were initially enrolled in the study. The patients with severe infection, onset of severe complication and allergy against drug were excluded from this study. Prior to enroll the participants, they were informed about the methods and objectives of the study and they provided a written informed consent. This study was single-center randomized clinical trial. Eligible participants were randomly divided into three groups using the envelop method without a blind technique; seven subjects were given Daiokanzoto sherbets (TJ-84) (2), seven subjects were given Hangeshashinto sherbets (TJ-14) (2), and ten subjects were assigned to the control group. The placebo treatment was not conducted in this study. The Kampo sherbets were made by dissolving the Kampo formula in distilled H₂O, which were poured in a plastic dish and then frozen until used. The participants consumed sherbet containing 2.5 g of Kampo formula three times per day between meals during the chemotherapy.

Clinical parameters

The chemotherapeutic drug used, the treatment period, the systemic condition, including the presence of fever and blood test results, were obtained from the patient medical record. The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) for oral mucositis was used in this study. The highest grade of oral mucositis in each patient during the observation period was recorded from the medical record or the nursing record in addition to an oral examination according to NCI-CTCAE version 4 criteria as follows: Grade 1: asymptomatic or mild symptoms; intervention not indicated, Grade 2: moderate pain not interfering with oral intake; modified diet indicated, Grade 3: severe pain interfering with oral intake, Grade 4: life-threatening consequence; urgent intervention indicated, Grade 5: death. As clinical parameters of the oral cavity, the salivary flow rate, Plaque index (PII), Gingival index (GI) (15) and Tongue coating index (TCI) (16) were recorded. Collection of saliva samples were performed under resting condition in the afternoon at least 2 h after eating and drinking. Subject were asked to collect saliva in their mouths and to spit it into a 50 ml polypropylene conical tube with a diameter of 3 cm for 5 min (17). Oral mucosal humidity (%) was determined by using an oral moisture checker (Mucus™, Life Co., Koshigaya city, Saitama prefecture, Japan).

Assessment of clinical parameters, tongue coating sampling, and professional oral healthcare (POHC) intervention

Assessment of clinical parameters in subjects, including salivary flow rate, moisture measurement, GI, PII and TCI was performed by a dentist before and after chemotherapy, and every week during the observation period. The measurement and collection of tongue coating bacteria were also performed at the same day, at least 2 h after breakfast or lunch to avoid any influence related to eating. A dental hygienist performed the POHC weekly that includes oral prophylaxis with toothbrush and inter-dental brush followed by debridement with hand scaler and by cleaning with 0.1% H₂O₂ cotton ball. Oral healthcare guidance for self-care was also pro-

vided at every visit at patient bed-site.

The Dielectrophoretic impedance measurement apparatus for quantification of bacteria (Bacterial Counter™, Panasonic, Osaka, Japan) was used to assess tongue coating bacteria according to the manufacturer's instructions. Tongue coating samples were collected using sterile 5 mm-diameter cotton stick by swabbing the tongue dorsum 3 times from back to front (approx. 2-cm-long swabbing motions). Samples were suspended in 5 ml of distilled water in disposable cup and bacterial quantification with Bacterial Counter™ was performed. Thereafter, the samples were dispensed into vials and kept at -80°C until used.

Tongue coating samples were also used to quantify three periodontopathogenic bacteria (*Porphyromonas gingivalis*, *Fusobacterium nucleatum* and *Campylobacter rectus*) by quantitative PCR as previously reported by Amou *et al* (18). with slight modifications. The MiniOpticon system (Bio-Rad Laboratories, Hercules, CA, USA) with SYBR Green I dye was used for the quantitative PCR analysis. One hundred eighty microliter of InstaGene Matrix (Bio-Rad Laboratories) was added to 20 µl of each tongue coating sample. The mixtures were incubated at 56°C for 30 min, vortexed for 30 s, incubated at 100°C for 8 min, and then stored at -20°C until used for the quantitative PCR analysis. Prior to analysis, the mixtures were thawed and centrifuged at 10,000 g for 10 min at 4°C. The supernatant of the samples was used for DNA template and was added (2 µl) to the PCR reaction mixture (18 µl) made of 10 µl of SsoFast™ EvaGreen® Supermix (Bio-Rad Laboratories), 0.04 µl of 100 µM of primers (Forward, Reverse) and 7.92 µl of diethylpyrocarbonate-treated water. The liquid mixtures were heat-treated as follows: initial denaturation step (3 min at 95°C), followed by denaturation (5 s at 95°C), annealing (10 s at 60°C) and extension (10 s at 60°C). The number of cycles for *P. gingivalis*, *F. nucleatum* and *C. rectus* were 45, 38 and 38, respectively. The primers used for the quantitative PCR have been previously described (19).

Three endpoints were measured in this study. The primary endpoint was to determine the efficacy of Kampo medicine for reducing the incidence of severe oral mucositis when compared to the control. The other endpoints were to evaluate oral hygiene and oral condition, especially, alternation of the number of oral bacteria and gingival inflammation.

Statistical analysis

The statistics package of IBM SPSS Statistics ver. 20.0 software (IBM SPSS Japan Inc, Tokyo, Japan) was used to analyze the data. One-way analysis of variance (ANOVA) was used to compare the clinical value from the three groups at baseline. A two-way repeated measures ANOVA was used to assess the effect of Kampo treatment on the number of bacteria, moisture measurement, GI, PII and TCI. Tukey's post hoc test were performed for comparison within groups after a significant Kampo group x observation period interaction. Values of $p < 0.05$ were considered as statistically significant.

Ethics

The Ethics Committee of Tokushima University Hospital approved this study (Approval #: 1920).

RESULTS

Baseline characteristics

One patient in the control group was excluded from the study because of the onset of an aspiration pneumonia prior the first week of examination following the chemotherapy period. Table 1 summarizes the demographic variables and clinical parameters at baseline for each patient group. No significant difference of items at baseline was found between patients among the three groups

Table 1 Items and parameters associated to patient groups at baseline.

Item or parameter	Control group	Daiokanzoto group	Hangeshashinto group
Number of subjects (male : female)	9 (8 : 1)	7 (5 : 2)	7 (7 : 0)
Age	61.8 ± 7.9	67.1 ± 8.3	66.6 ± 6.2
Salivary flow rate (ml/5 min)	2.66 ± 1.69	3.09 ± 1.63	1.83 ± 0.81
Tongue Coating Index (TCI : %)	43.3 ± 19.3	42.9 ± 19.7	45.3 ± 28.2
Moisture measurement	27.2 ± 4.7	25.2 ± 3.2	26.7 ± 3.4
Gingival Index (GI)	1.37 ± 0.66	1.45 ± 1.05	1.44 ± 0.48
Plaque Index (PII)	1.13 ± 0.73	1.12 ± 1.05	1.07 ± 0.48
Total bacteria (log [cells/ml])	6.37 ± 0.57	6.83 ± 0.36	6.55 ± 0.75
<i>P. gingivalis</i> (log [cells/ml])	1.96 ± 2.35	1.01 ± 1.74	0.63 ± 1.67
<i>F. nucleatum</i> (log [cells/ml])	4.90 ± 1.87	5.76 ± 0.90	5.71 ± 0.76
<i>C. rectus</i> (log [cells/ml])	3.41 ± 2.05	4.70 ± 0.48	3.36 ± 2.43

(control group, n = 9 ; Daiokanzoto group, n = 7 ; Hangeshashinto group, n = 7) by the analysis of one-way ANOVA.

Onset of oral mucositis associated with chemotherapy

The onset of oral mucositis in the three patient groups during the chemotherapy is reported in Table 2. Six patients did not complete the chemotherapy treatments. Five subjects showed mucositis at grade 2 or more, and oral mucositis lesions were observed on the lower labial mucous, angulus oris, palate and tongue (data no shown). A subject with severe pain (grade 3) that interfered with oral intake was observed in the Hangeshashinto group. No difference in the onset of slightly and/or severe mucositis was observed among the three groups.

Table 2 Highest grade of oral mucositis observed in patients.

	Maximum grade of oral mucositis [#]		
	No more than Grade 1	Grade 2	Grade 3
Control group	8(1)	1	0
Daiokanzoto group	6(3)	1(1)	0
Hangeshashinto group	4	2(1)	1
Total	18	4	1

[#] : Evaluated by NCI-CTCAE Ver. 4.0

Parenthesis means the number of patient who was not completed on a course of chemotherapy

Comparison of the numbers of bacteria

Fourteen subjects completed the 2-week period of Kampo sherbet intake following chemotherapy. Tongue coating samples were collected at baseline, 1 week and 2 weeks after chemotherapy. Figure 1 and Table 3 reports the variations in the numbers of total bacteria during the observation period for patients consuming or not the Daiokanzoto sherbets. Table 3 shows a statistically significant decrease in the number of total bacteria ($p < 0.05$), *F. nucleatum* ($p < 0.01$) and *C. rectus* ($p < 0.05$) in both the control group and the Daiokanzoto group during the full observation period. There were significant decreases in those value at 1 week and 2 weeks within the Daiokanzoto group by a Tukey's post hoc test (Fig. 1). Furthermore, a two-way repeated measures ANOVA

showed that the numbers of *F. nucleatum* and *C. rectus* in the control and Daiokanzoto groups were not similar across the observation period (Kampo group x observation period interaction, $p < 0.05$). These results suggest that the consumption of Daiokanzoto causes a significant decrease in the numbers of *F. nucleatum* and *C. rectus* compared with the control group. Although the numbers of *P. gingivalis* tended to decrease following Daiokanzoto intake, the difference was not significant (Fig. 1). A statistically significant decrease in the numbers of *F. nucleatum* ($p < 0.05$) in both the control and Hangeshashinto groups across the observation period was also observed (Table 3). However, no significant difference was observed between the control and Hangeshashinto groups.

Comparison of clinical parameters

Analysis of variance of the index in intervention groups (Daiokanzoto or Hangeshashinto sherbet intake) compared to the control group with regard to the clinical parameters during the observation period is shown in Table 4. While no significant differences were observed for values of salivary rate and moisture (data no shown), a statistically significant decrease of TCI ($p < 0.01$), GI ($p < 0.01$) and PII ($p < 0.05$) in both the control and Daiokanzoto groups across the observation period was recorded (Table 4). There were significant decreases in the value of TCI and GI within both Daiokanzoto group and the control group by post hoc test (Fig. 2). Furthermore, a two-way repeated measures ANOVA showed that GI was reduced significantly more in the Daiokanzoto group compared with the control group during the observation period (Table 4). A statistically significant decrease of TCI ($p < 0.01$) and GI ($p < 0.05$) values in both the control group and the Hangeshashinto group was observed during the observation period. However, the values between the control group and the Hangeshashinto group were not significantly different (Table 4).

DISCUSSION

This study clearly showed that the numbers of *F. nucleatum* and *C. rectus* were decreased significantly in patients consuming Daiokanzoto sherbets when compared with those in the control group during the early stage of chemotherapy. This result was similar with Fukamachi *et al.* who reported the antimicrobial activity of Hangeshashinto against 19 oral bacteria including *F. nucleatum* and *P. gingivalis* (20). Moreover, the Kampo medicine Rokumigan prevented biofilm formation by *F. nucleatum* (6). Recently, our group showed that Kampo medicines containing rhubarb have the ability to inhibit the growth and virulence properties of *P. gingivalis* (7). Daiokanzoto contains rhubarb-derived

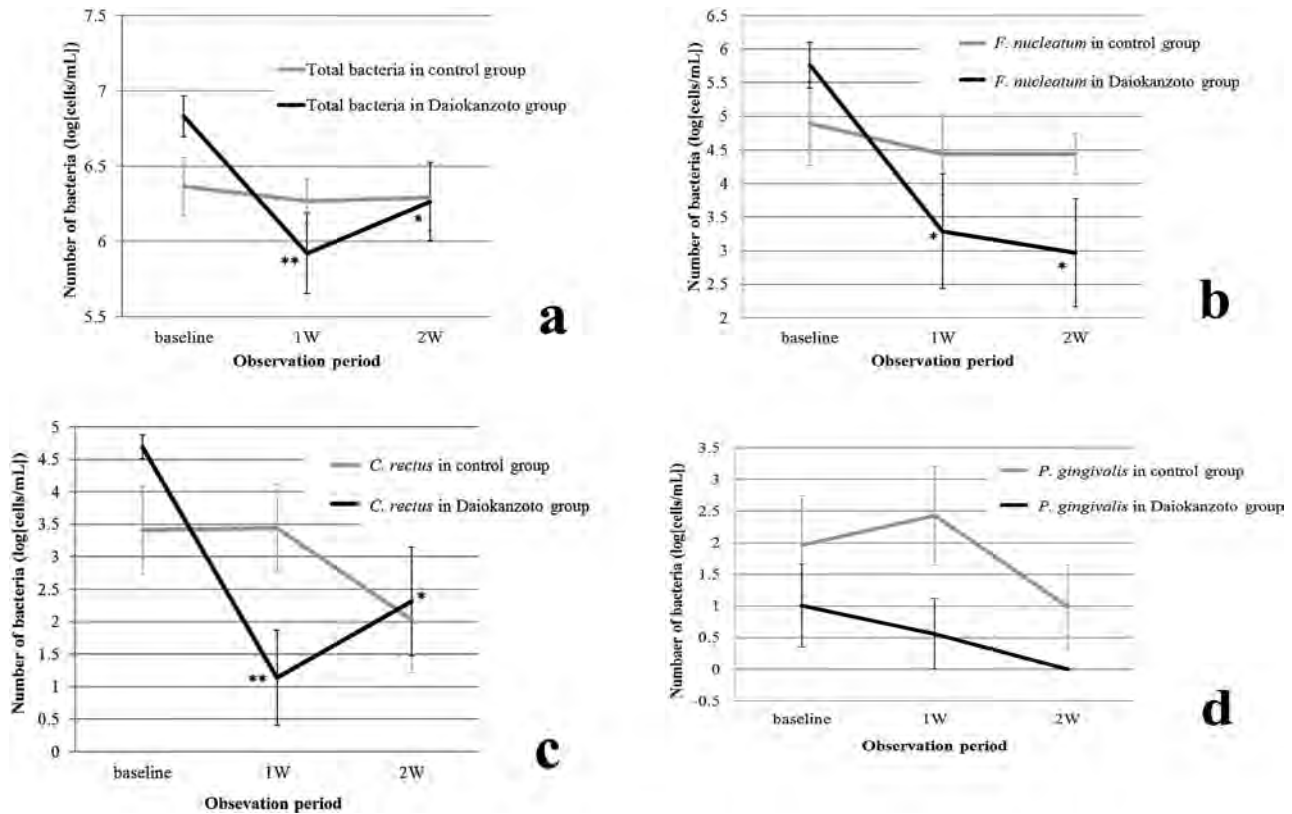


Fig. 1 Numbers of bacteria in tongue coating from patients with or without intake of Daiokanzoto sherbets. Data are expressed as mean \pm standard error. Asterisk indicates a significant difference of values within group compared with those at baseline, * : p < 0.05, ** : p < 0.01. a : total bacteria, b : *F. nucleatum*, c : *C. rectus*, d : *P. gingivalis*

Table 3 Analysis of variance of the numbers of bacteria in Kampo intervention groups compared to control group.

Index		Daiokanzoto group			Hangeshashinto group		
		F-value	Degree of freedom	P-value	F-value	Degree of freedom	P-value
Total bacteria	Kampo group	0.31	1	0.58	0.01	1	0.91
	Observation period	4.84	1.43	0.03*	1.11	1.41	0.3
	Kampo group \times Observation period	1.72	1.43	0.21	0.47	1.41	0.85
<i>P. gingivalis</i>	Kampo group	2.62	1	0.13	0.71	1	0.41
	Observation period	2.98	2	0.07	1.37	2	0.27
	Kampo group \times Observation period	0.61	2	0.55	1.87	2	0.17
<i>F. nucleatum</i>	Kampo group	0.88	1	0.36	0.04	1	0.85
	Observation period	7.28	2	<0.01**	4.57	1.25	0.04*
	Kampo group \times Observation period	4.26	2	0.02*	3.46	1.25	0.07
<i>C. rectus</i>	Kampo group	0.12	1	0.73	0.12	1	0.74
	Observation period	5.68	2	0.01*	1.54	2	0.23
	Kampo group \times Observation period	4.38	2	0.02*	0.75	2	0.48

Two-way repeated measures ANOVA, * : p < 0.05, ** : p < 0.01

molecules, including anthraquinones, which exhibit antibacterial activity towards several oral bacterial pathogens (7, 21, 22) as well as synergistic effects against methicillin-resistant *Staphylococcus aureus* when combined with antibiotics (23, 24). Therefore, anthraquinone in Daiokanzoto may contribute to decrease the numbers of *F. nucleatum* and *C. rectus*.

In this study, TCI, GI and PII values decreased during the observation period. These results suggested that POHC was effective for improving oral hygiene and gingival condition in patients with esophageal cancer during chemotherapy. Especially, GI was decreased significantly in the Daiokanzoto group when compared with that in the control group. Interestingly, it has been previously

Table 4 Analysis of variance of the clinical parameters in Kampo intervention groups compared to control group.

Index		Daiokanzoto group			Hangesyashinto group		
		F-value	Degree of freedom	P-value	F-value	Degree of freedom	P-value
TCI	Kampo group	0.01	1	0.92	0.60	1	0.45
	Observation period	33.00	2	<0.01**	18.00	2	<0.01*
	Kampo group × Observation period	1.72	2	0.26	0.93	2	0.27
GI	Kampo group	0.04	1	0.84	0.47	1	0.51
	Observation period	22.20	2	<0.01**	7.35	1.19	0.02*
	Kampo group × Observation period	3.74	2	0.04*	0.93	1.19	0.61
PII	Kampo group	0.18	1	0.68	1.39	1	0.27
	Observation period	4.29	2	0.03*	1.32	1.34	0.29
	Kampo group × Observation period	0.95	2	0.40	0.21	1.34	0.73

Two-way repeated measures ANOVA, * : $p < 0.05$, ** : $p < 0.01$

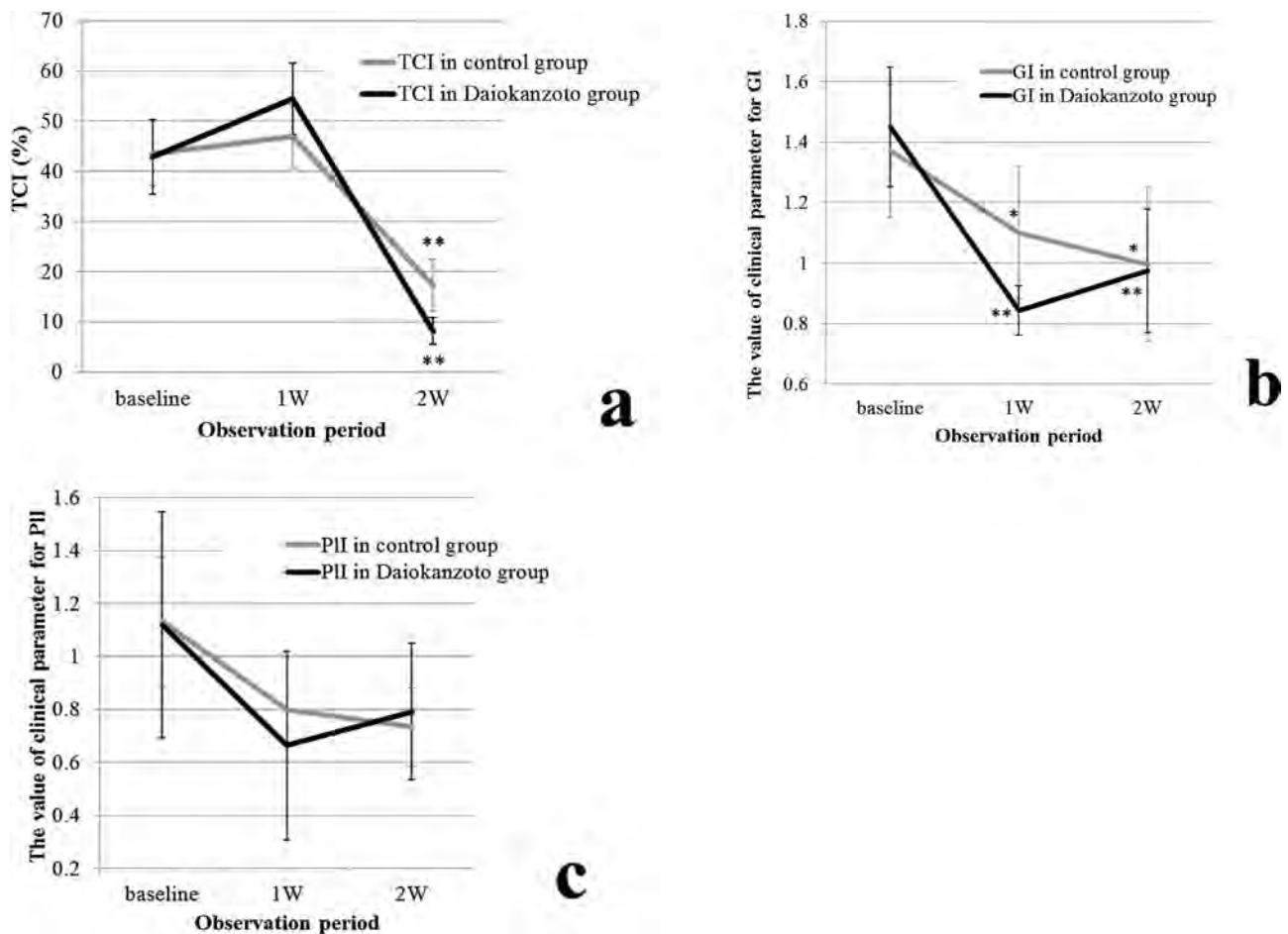


Fig. 2 Clinical parameters of patients with or without intake of Daiokanzoto sherbets. Data are expressed as mean \pm standard error. Asterisk indicates a significant difference of values within group compared with those at baseline, * : $p < 0.05$, ** : $p < 0.01$. a : TCI, b : GI, c : PII

demonstrated that Daiokanzoto reduces the secretion of pro-inflammatory cytokines (IL-6 and CXCL8) by LPS-stimulated oral epithelial cells and gingival fibroblasts, and inhibits the catalytic activity of matrix metalloproteinases (25). This ability of Daiokanzoto to attenuate the host inflammatory response may be involved in the reduction of gingival inflammation observed in this study.

Regarding the anti-inflammatory property of Kampo formulas, Rikkosan has been reported to inhibit both IL-1 β and PGE₂ production (26). Moreover, Shosaikoto decreases LPS-induced PGE₂ production through inhibition of both cyclooxygenase-2 expression and activity (5), while Juzentaihoto effectively inhibits restraint stress and osteoclastogenesis involved in periodontal tissue destruction

(27). Lastly, we also showed in a previous study that Rokumigan inhibits the IL-6 secretion by LPS-stimulated gingival epithelial cells and fibroblast, in addition to promote wound healing in a fibroblast model (6).

In a previous *in vitro* study, we showed that Daiokanzoto attenuates 5-FU-induced cell death through the inhibition of mitochondrial ROS production (12). However, in this study we could not show any preventive effect of Daiokanzoto on the onset of oral mucositis. Similarly, Hangeshashinto which has been proposed to treat oral mucositis (11) and found to suppress the mechanical pain in ulcerative oral mucosa in a rat model (28), did not provide benefits in this clinical trial. It may be hypothesized that POHC provided to all subjects has a preventive effect against the onset of severe oral mucositis. This is supported by the previously reported efficacy of regular POHC in reducing the risk to develop oral mucositis in breast cancer patients undergoing chemotherapy in comparison with a self-oral care group (29).

It has been reported by Oyama *et al.* (30) that authors developed the ice ball or freeze cotton swabs containing kampo medicine and found that they were useful for reducing oral pain in cancer patients. We designed the sherbet form to simulate the effect for topical use of Kampo medicine by taking slowly in addition to intake per orally. This clinical trial suggests that Daiokanzoto provided as sherbets has the potential to prevent gingival inflammation and reduce the numbers of tongue coating bacteria, including important periodontopathogenic bacteria. The major limitation of this study relates to the small number of subject in each group. In order to clarify the preventive effects of Kampo medicines for oral mucositis, it may be relevant to include an additional control group with self-oral care.

CONCLUSION

This clinical trial did not demonstrate a preventive effect of Kampo medicine on the onset of mucositis in patients receiving POHC during chemotherapy for esophageal cancer. However, it suggests that the use of Daiokanzoto may provide a beneficial effect for the oral health of patients under chemotherapy for esophageal cancer, by attenuating the gingival inflammation and reducing the numbers of periodontopathogenic bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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