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Effect of herbal medicine daikenchuto on gastrointestinal symptoms following laparoscopic colectomy in patients with colon cancer: A prospective randomized study

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ABSTRACT

We conducted a prospective randomized study to investigate the effect of daikenchuto (DKT) on abdominal symptoms following laparoscopic colectomy in patients with left-sided colon cancer. Patients who suffered from abdominal pain or distention on postoperative day 1 were randomized to either the DKT group or non-DKT group. The primary endpoints were the evaluation of abdominal pain, abdominal distention, and quality of life. The metabolome and gut microbiome analyses were conducted as secondary endpoints. A total of 17 patients were enrolled: 8 patients in the DKT group and 9 patients in the non-DKT group. There were no significant differences in the primary endpoints and postoperative adverse events between the two groups. The metabolome and gut microbiome analyses showed that the levels of plasma lipid mediators associated with the arachidonic acid cascade were lower in the DKT group than in the non-DKT group, and that the relative abundance of genera *Serratia* and *Bilophila* were lower in the DKT group than in the non-DKT group. DKT administration did not improve the abdominal symptoms following laparoscopic colectomy. The effects of DKT on metabolites and gut microbiome have to be further investigated.

1. Introduction

Postoperative ileus is a common complication. The exact pathogenesis of ileus has been debated for years, but it remains unresolved [1]. Additional treatments (e.g., fasting, decompression tube use, antibiotic use, and surgical intervention) are needed for alleviating postoperative ileus, which can increase hospital stay and medical costs [1,2]. In recent years, laparoscopic surgery has become a popular alternative to laparotomy. Although the incidence of postoperative ileus following laparoscopic surgery is lower compared with that following laparotomy, it is still reported to occur in approximately 10% of patients [3]. To date, alvimopan, cisapride, erythromycin, and daikenchuto (DKT), a Japanese traditional herbal medicine (Kampo), have been used to treat postoperative ileus in clinical practice [4–7].

Abbreviations: DKT, Daikenchuto; CGRP, Calcitonin gene-related peptide; ADM, Adrenomedullin; QOL, Quality of life; UMIN, University Hospital Medical Information Network; POD, Postoperative day; ECOG, European Cooperative Oncology Group; NRS, Numeric rating scale; GIQLI, Gastrointestinal quality life index; PNI, Onodera's prognostic nutritional index; CONUT, Controlling Nutritional Status; GC-MS/MS, Gas chromatography-tandem mass spectrometry; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; SD, Standard deviation; PLS-DA, Partial least-squares discriminant analysis; OUT, Operational taxonomic unit; MMRM, Mixed-Effects Model for Repeated Measures; CRP, C-reactive protein.

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DKT is a crude drug extract composed of processed ginger, ginseng, Japanese zanthoxylum peel and maltose powder. Regarding the pharmacological mechanisms, DKT has been reported to suppress inflammation, increase intestinal blood flow, and promote bowel movements [8–14]. Hydroxy-α-sanshool, a major component of DKT, has been reported to promote serotonin secretion and intestinal peristalsis via the TRPA1 channels [15–19]. Intestinal blood flow is increased via endogenous calcitonin family peptides, calcitonin gene-related peptide (CGRP), and adrenomedullin (ADM) [10,20]. DKT also exerts the anti-inflammatory effects via the release and production of endogenous ADM [21]. Recently, we reported that DKT administration could modulate the mRNA levels of several inflammation-related cytokines including TNF-α, TGF-β1, and VEGF-α in the intestinal tissues of a rat anastomotic healing model [13].

Several clinical trials have shown the usefulness of DKT [22–28]. In most of previous randomized controlled trials, the primary endpoints were objective outcomes such as the time to first flatus and incidence of postoperative ileus [22–28]. There are few reports to investigate the subjective outcomes (e.g., patient symptoms and quality of life (QOL)) as primary endpoints. Although the assessment of postoperative QOL has been performed as a secondary outcome in some studies, no significant difference was found between the DKT group and non-DKT control group [24–27]. The present study was a randomized controlled trial that examined the postoperative effects of DKT on subjective outcomes (i.e., grades of abdominal symptoms and QOL) as primary endpoints. In addition, we exploratively analyzed the plasma and fecal metabolites as well as the gut microbiome. DKT has been reported to affect fecal metabolites via gut microbiome metabolism in animal studies [29,30]. However, no study has investigated the effects of DKT on the gut microbiome and metabolites in humans. The clinical symptoms related to colonic movement (e.g., defecation) is mainly associated with the left-sided colon. In addition, the gut microbiota of patients with right-sided colon cancer have been reported to be quite different from those with left-sided colon cancer [31,32]. Considering these points, we focused on the patients with left-sided colon cancer in this study. Therefore, we investigated the postoperative effects of DKT on abdominal symptoms, metabolome, and gut microbiome following laparoscopic colectomy in patients with left-sided colon cancer.

2. Materials and methods

2.1. Randomized controlled trial

2.1.1. Study design and setting

This randomized controlled trial was conducted at Kyoto University Hospital in Japan. The study protocol was published previously [33]. This study protocol was conducted according to the Declaration of Helsinki, and was approved by the institutional review board of Kyoto University, Kyoto, Japan, and by the institutional review board of Tsumura & Co, Tokyo, Japan. This trial was registered in the University Hospital Medical Information Network (UMIN) Clinical Trial Registry as UMIN000023318. Written informed consent was obtained from all participants before enrollment and randomization by the investigator. All patients received mechanical bowel preparation (75 mg sodium picosulfate and 12 mg pursennid) and oral antibiotics (oral doses of 1 g kanamycin and 750 mg metronidazole), and surgical procedures were performed by board-certified laparoscopic colorectal surgeons at our institution.

2.1.2. Patient selection

The inclusion criteria of this trial were as follows: i) patients with left-sided colon cancer scheduled to undergo laparoscopic colectomy, ii) clinical stage I-III, iii) patients who suffered from abdominal pain or distention on postoperative day (POD) 1, iv) the European Cooperative Oncology Group (ECOG) performance status of 2 or less, v) aged 20 years or older at registration, vi) ability to take medications orally, and vii) provided written informed consent.

The exclusion criteria of this trial were as follows: i) a history of abdominal surgery or bowel obstruction, ii) concomitant inflammatory bowel disease, iii) concomitant endometriosis, iv) emergency surgery, v) patients who have been or will be treated by chemotherapy or radiotherapy, vi) severe comorbidities such as cardiac disease, liver disease, pulmonary disease, and renal disease, vii) patients who took Japanese herbal medicine (Kampo) up to 4 weeks before registration, viii) patients who took gastrointestinal prokinetic drugs, antipsychotic drugs or antidepressant drugs up to 4 weeks before registration, ix) patients with a history of allergy to a component in other Kampo formulations, x) hepatitis B or C, xi) inability to take medications orally on POD 1, and xii) patients unsuitable for study inclusion as determined by the investigator (e.g., patients with severe dementia).

2.1.3. Registration

Patients were enrolled on POD 1. The eligibility report form was sent to the registration center (APOPLUS STATION Co., Ltd, Tokyo, Japan). Eligible patients were centrally randomized to either the DKT group or non-DKT group at the registration center using an automatic random number generator with the minimization method for TNM stage, tumor location, and age. A flow diagram of this study is shown in Fig. 1A.

2.1.4. Endpoints

The following primary endpoints were evaluated: i) grade of abdominal pain determined using the numeric rating scale (NRS) [34], ii) grade of abdominal distention determined using the NRS, and (iii) QOL determined using the gastrointestinal quality life index (GIQLI) [35]. NRS measurements of abdominal pain and distention were performed before surgery (Pre) and on PODs 1, 4, 7, 14, and 28. The GIQLI was taken before surgery (Pre) and on PODs 14 and 28.

The following secondary endpoints were evaluated: i) postoperative nutritional status (Onodera's prognostic nutritional index (PNI) [36] and Controlling Nutritional Status (CONUT) score [37]), ii) time to initial flatus, iii) time to initial defecation, iv) bowel gas volume measured using analysis software, v) defecation frequency per day, vi) postoperative adverse events, vii) duration of postoperative hospital stay, viii) plasma and fecal metabolome analyses using gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), and ix) gut microbiome analysis (Fig. 1B).

2.1.5. Treatment methods

In the DKT group, DKT (5 g) was administered orally three times per day between PODs 2 and 28. DKT manufactured by Tsumura & Co. was used. In the non-DKT group, no additional medicine was administered as a comparator. Except for the administration of DKT, treatment protocols including perioperative management and surgical procedures were congruent between the DKT group and non-DKT group.

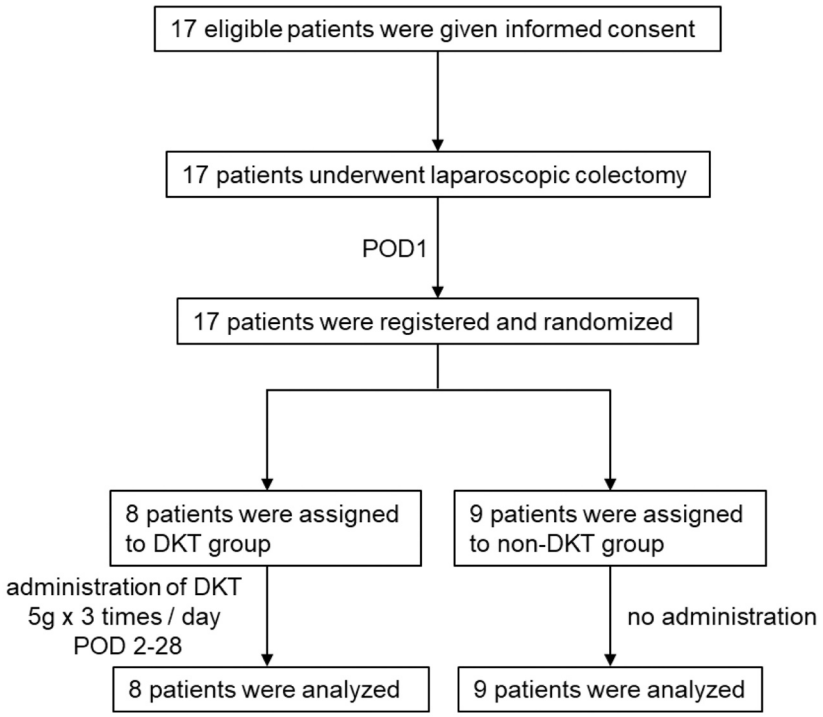
2.1.6. Prohibited or limited drugs

Drugs known to regulate intestinal movement were prohibited during the protocol treatment. Prohibited drugs were erythromycin, mosapride citrate, pantethine, panthenol, prostaglandin F2α, diastase, pancreatin, atropine sulfate, scopolamine butylbromide, sodium picosulfate, albumin tannate, aluminum silicate, dimethicone, diazepam, flunitrazepam, lactomin, lactic acid bacteriae, clostridium butyriam, anti-Parkinsonian drugs, and gastrografin. Limited drugs were antiemetic drugs including metoclopramide and domperidone. Rescue drugs were antidiarrheal drugs (magnesium oxides, sennoside), cathartic drugs (loperamide), and analgesic drugs (loxoprofen).

2.1.7. Data collection

Prospective data including physical examination, laboratory data, perioperative clinical information and complications were collected. Data collection, management, analysis, and interpretation of data were

A



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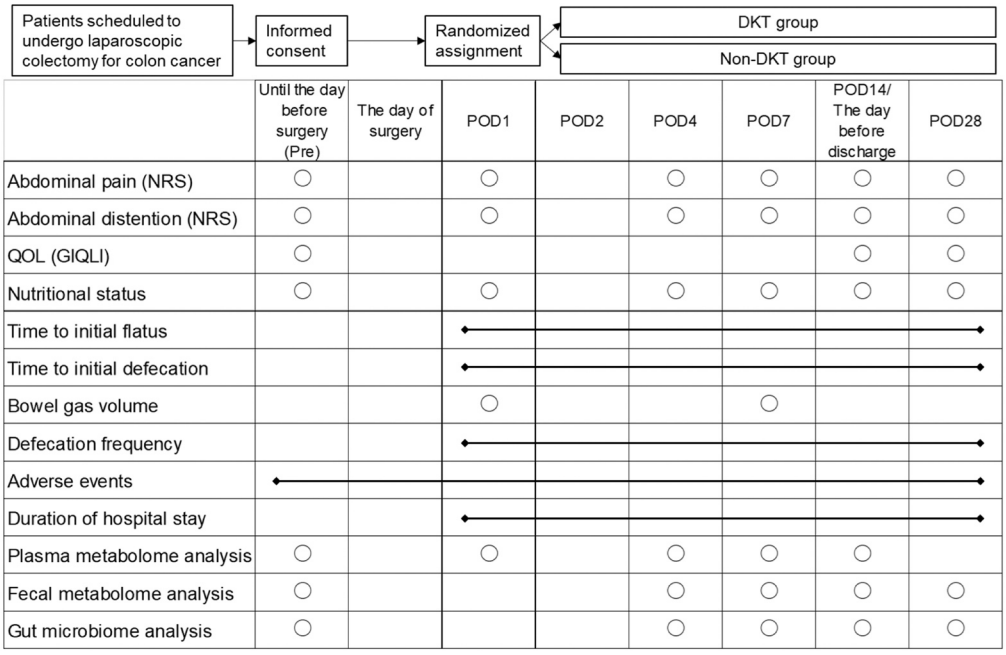


	Until the day before surgery (Pre)	The day of surgery	POD1	POD2	POD4	POD7	POD14/ The day before discharge	POD28
Abdominal pain (NRS)	○		○		○	○	○	○
Abdominal distention (NRS)	○		○		○	○	○	○
QOL (GIQLI)	○						○	○
Nutritional status	○		○		○	○	○	○
Time to initial flatus			◆—	—	—	—	—	—◆
Time to initial defecation			◆—	—	—	—	—	—◆
Bowel gas volume			○			○		
Defecation frequency			◆—	—	—	—	—	—◆
Adverse events	◆—	—	—	—	—	—	—	—◆
Duration of hospital stay			◆—	—	—	—	—	—◆
Plasma metabolome analysis	○		○		○	○	○	
Fecal metabolome analysis	○				○	○	○	○
Gut microbiome analysis	○				○	○	○	○

Fig. 1. Details of the study. A. Study profile. Investigators obtained informed consent from eligible patients before surgery. Patients were enrolled in the study on postoperative day (POD) 1, if they can take medicine orally and had an abdominal pain and/or distention. Enrolled patients were randomly assigned to either the daikenchuto (DKT) or non-DKT group. B. Schedule of enrollment, intervention, and assessments. NRS, numeric rating scale; QOL, quality of life; GIQLI, gastrointestinal quality life index.

performed by the registration center.

2.1.8. Sample size determination

The sample size was calculated based on a primary outcome, the GIQLI. The responses to questions are summed to assign a numerical score (0–128 points). A previous report indicated that the mean-± standard deviation (SD) change in the GIQLI score between two measurements before and 2 weeks after laparoscopic cholecystectomy in 194 patients was 17 ± 20.7 points [35]. Therefore, the clinically significant mean difference in the GIQLI score was set at 20 with a SD of 20. Using a power level of 80% and a two-sided significance level of 5%, 40 patients (20 patients in each group) were considered adequate. However, the trial was stopped on December 31, 2018, because of slow recruitment. Finally, 17 patients were enrolled and randomly assigned to the two groups: 8 patients in the DKT group and 9 patients in the non-DKT group.

2.2. Plasma and fecal metabolome analysis

Plasma metabolome analysis was performed as previously reported [38,39]. Hydrophilic metabolites and lipid mediators were measured using GC-MS/MS and LC-MS/MS. The GC-MS/MS system consisted of a

GCMS-TQ8040 (Shimadzu, Kyoto, Japan) device with the automated derivatization system SGI-M100 (AiSTI SCIENCE, Wakayama, Japan). The LC-MS/MS system consisted of two LC-30 AD pumps, a SIL-30AC auto-sampler, a CTO-20A column oven, a CBM-20A system controller, and a triple quadrupole mass spectrometer LCMS-8050 (Shimadzu). The detailed protocol is described in the Supplementary material.

Fecal metabolome analysis was performed using the SGI-M100 derivatization system and GC-MS/MS. The lyophilized feces samples were weighed about 10–20 mg and added 1.2 mL of 80% acetonitrile containing crotonic acid and 2-isopropylmalic acid as internal standards. Then, the samples were homogenized using zirconia beads in an automill (Tokken, Chiba, Japan). After centrifugation, the supernatant was transferred to a vial and subjected to metabolome analysis. The detailed protocol is described in the Supplementary material.

The peak intensity of each quantified ion was calculated and normalized to internal standards, and feces weight. Metabolites with normalized area variation in pooled samples > 30% were removed from the detected metabolites because they were unstable. Regarding the redundant targets between LC-MS/MS and GC-MS/MS, the targets with smaller CV value in the pooled samples were adopted.

2.3. Gut microbiome analysis; 16S rRNA gene metagenome sequencing in fecal samples

The bacterial genomic DNA was isolated using standard method with some modifications [40]. In brief, the fecal samples were freeze dried and weighed 10–30 mg in Lysing matrix E tube (MP Biomedicals, LLC., Santa Ana, USA). The fecal samples were homogenized with elution buffer using the FastPrep-24 automated cell disruptor (MP Biomedicals) at a speed setting of 6 m/s for 40 s which process was repeated twice. Fecal DNA was extracted by a phenol/chloroform/isoamyl alcohol method. The concentration of fecal DNA was measured by Quant-iT PicoGreen dsDNA assay kit (Thermo Fisher Scientific, Inc., Waltham, USA). The preparation of 16S rRNA gene metagenome library for MiSeq (illumina, Inc., San Diego, USA) was prepared following manufacturer's protocol. The library was applied to MiSeq Reagent Kit v3 (illumina, Inc.) and sequenced following manufacturer's standard protocol. The sequence data was processed as follows using 16S rRNA sequence analysis pipeline, QIIME 1.8.0 [41]. At first, the both side of sequences were joined and the sequences whose phred quality score was below 20 were removed as poor-quality data. Chimella elimination by U-search was performed and the contaminated sequences were removed from the dataset. The open reference operational taxonomic unit (OTU)□picking was performed against green gene 97 13_8 as reference dataset. The summary of taxonomy in each sample was obtained using the script 'summarize_taxonomy_through_plots.py' in QIIME 1.8.0.

2.4. Statistical analysis

All analyses were performed under the intention-to treat principle. Both primary and secondary endpoints were compared between the DKT group and non-DKT group. A two-tailed P value less than 0.05 was considered statistically significant. Primary endpoints were analyzed using Mixed-Effects Model for Repeated Measures (MMRM). As secondary endpoints, *t*-test was used when continuous variables followed a normal distribution, whereas the Mann-Whitney *U* test was used when continuous variables followed a non-normal distribution. Kaplan-Meier plots were analyzed using log-rank test. Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Metabolome and gut microbiome analyses were conducted as follows. The plots, charts, and heatmap were prepared using the R software (version 4.0 or newer, http://r-project.org) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Univariate analysis between two groups were performed with the Mann-Whitney *U* test using the R. Since the microbiome and metabolome analysis are exploratory outcomes, we did not adjust P-values for multiple comparisons. PLS-DA was performed using the SIMCA 16 software (Umetrics, Umeå, Sweden).

3. Results

3.1. Patient characteristics

Fig. 1 A shows the study profile. The originally-planned sample size of 20 patients per group was not achieved during the study period. From March 2017 to December 2018, 17 patients were randomly assigned to the two groups on POD 1: 8 patients in the DKT group and 9 patients in the non-DKT group. All patients with left-sided colon cancer underwent laparoscopic colectomy. Fig. 1B shows the schedule of the study. Table 1 shows the clinicopathological characteristics of the study participants. There were no significant differences in the baseline characteristics of the patients between the groups, suggesting that the two groups were balanced at baseline.

3.2. Primary endpoints

As primary endpoints, the following subjective outcomes were evaluated: i) abdominal pain using the NRS [34], ii) abdominal distension using the NRS, and iii) QOL using the GIQLI [35]. At all assessment time points, the grade of abdominal pain in the DKT group was not significantly different from that in the non-DKT group (Fig. 2A). Regarding the grade of abdominal distension, no significant differences were observed between the two groups (Fig. 2B). In addition, the grade of QOL was similar on PODs 14 and 28 between the two groups (Fig. 2C). Taken together, administration of DKT did not improve the postoperative gastrointestinal symptoms following laparoscopic colectomy in patients with left-sided colon cancer.

3.3. Secondary endpoints

There were no significant differences between the two groups in the secondary endpoints, that is, postoperative nutritional status (PNI [36] and CONUT score [37]), time to initial flatus, time to initial defecation, bowel gas volume, and defecation frequency (Fig. S1A–S1F). Regarding the incidence of postoperative adverse events and duration of hospital stay, there were no significant differences between the two groups

Table 1
Baseline characteristics of the patients.

	DKT group (n = 8)	Non-DKT group (n = 9)
Age, years	63.0 (55.0–73.8)	64.0 (58.0–67.0)
Gender		
Male	4 (50.0%)	6 (66.7%)
Female	4 (50.0%)	3 (33.3%)
Height, cm	162.0 (157.0–163.6)	159.0 (157.1–176.7)
Body weight, kg	60.0 (51.6–68.5)	69.0 (56.1–81.0)
Performance status (ECOG)		
0	7 (87.5%)	9 (100%)
1	1 (12.5%)	0 (0%)
Past medical history[a]		
Yes	5 (62.5%)	5 (55.6%)
No	3 (37.5%)	4 (44.4%)
Surgery type		
Sigmoid colectomy	4 (50%)	8 (88.9%)
Left hemicolectomy	2 (25%)	1 (11.1%)
High anterior resection	2 (25%)	0 (0%)
Operation time, min	208 (146–238)	155 (140–171)
Blood loss, mL	0 (0–20)	0 (0–0)
Pathological stage		
I	6 (75%)	3 (33.3%)
II	1 (12.5%)	0 (0.0%)
III	1 (12.5%)	2 (22.2%)
IV	0 (0.0%)	4 (44.4%)

Data are n (%) or median (interquartile range).
DKT, Daikenchuto; ECOG, Eastern Cooperative Oncology Group.
[a] hypertension, hyperlipidemia, diabetes mellitus, and ureteric stone.

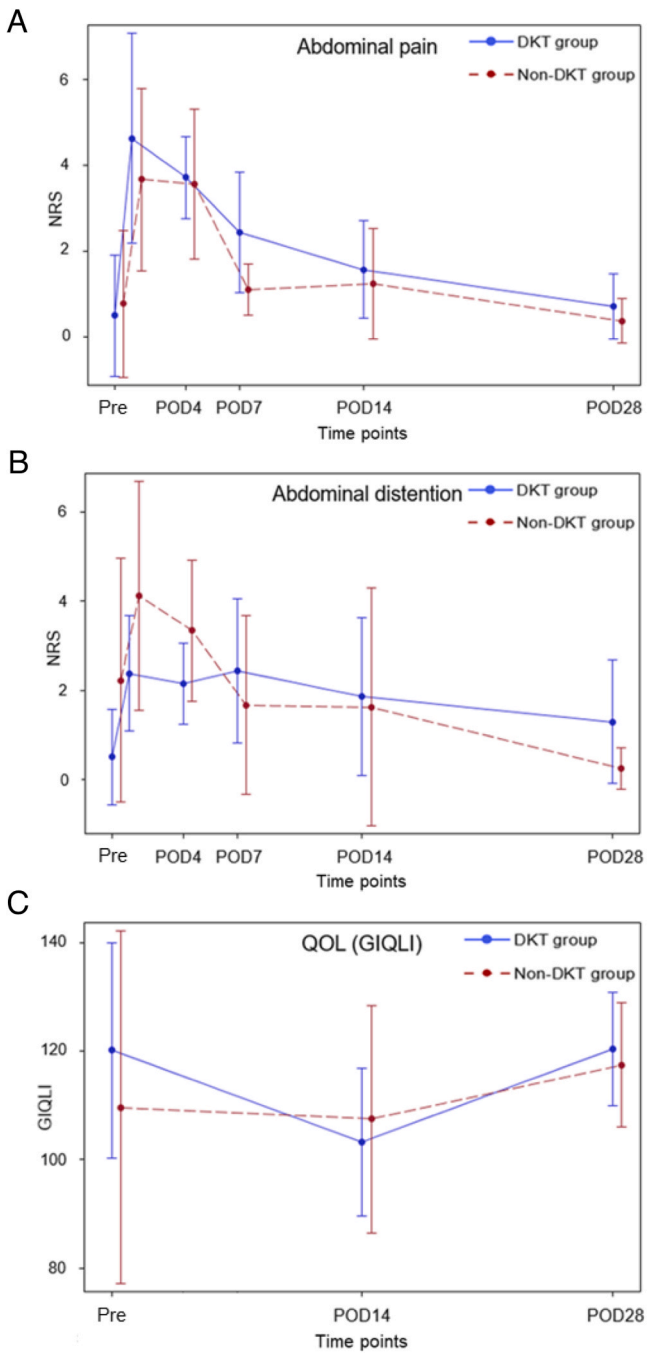


Fig. 2. Primary endpoints. A. Grades of abdominal pain determined using the NRS in perioperative patients with or without DKT treatment. The data are expressed as mean ± standard deviation (SD). Red dotted line, non-DKT group (n = 8–9); blue line, DKT group (n = 7–8). B. Grades of Abdominal distention determined using the NRS in perioperative patients with or without DKT treatment. The data are expressed as mean ± SD. Red dotted line, non-DKT group (n = 8–9); blue line, DKT group (n = 7–8). C. QOL determined using the GIQLI in perioperative patients with or without DKT treatment. The data are expressed as mean ± SD. Red dotted line, non-DKT group (n = 8–9); blue line, DKT group (n = 6–7).

(Fig. S2A and S2B). The details of the adverse events are shown in Table 2.

In the plasma, 216 metabolites were evaluated. Fig. 3A shows the distribution of all detected metabolites using partial least-squares discriminant analysis (PLS-DA). We found that the plasma metabolites substantially changed before and after surgery, and that the distribution

Table 2
Details of adverse events.

	DKT group (n = 8)	Non-DKT group (n = 9)
Any adverse events	6 (75.0%)	6 (66.7%)
Elevated serum AST,ALT, ALP,γ-GTP and T-Bil	3 (37.5%)	4 (44.4%)
Infectious colitis	1 (12.5%)	0 (0.0%)
Insomnia	1 (12.5%)	1 (11.1%)
Hypertension	1 (12.5%)	0 (0.0%)
Sore throat	1 (12.5%)	0 (0.0%)
Pruritus	1 (12.5%)	1 (11.1%)
Gastroesophageal reflux disease	1 (12.5%)	1 (11.1%)
Intra abdominal hemorrhage	1 (12.5%)	0 (0.0%)

Data are n (%). DKT, Daikenchuto; AST, Aspartate transaminase; ALT, alanine aminotransferase; ALP, Alkaline Phosphatase; γ-GTP, γ-glutamyl transpeptidase; T-Bil, Total Bilirubin.

of samples on POD 14 returned close to the preoperative state. As shown in the heat map of each metabolite, the levels of lipid mediators associated with the arachidonic acid cascade were overall lower in the DKT group than in the non-DKT group (Fig. 3B). In particular, 18-HETE level was significantly lower in the DKT group on POD 7 than in the non-DKT group (Fig. 3C).

In the feces, 132 metabolites were evaluated. Fig. 4A shows the distribution of all fecal samples using PLS-DA score plot. Similar to the plasma metabolome analysis, the distribution of fecal metabolites substantially changed before and after surgery. On PODs 14 and 28, the distribution returned close to the preoperative state. The evaluation of each metabolites using the volcano plot revealed that several short-chain fatty acids were significantly lower in the DKT group than in the non-DKT group on POD 28 (Fig. 4B). For example, the levels of succinic acid, propanoic acid, and acetic acid were significantly lower in the DKT group than in the non-DKT group on POD 28 (Fig. 4C).

Finally, we performed the gut microbiome analysis. The concentration of fecal DNA decreased before and after surgery (Fig. 5A), which can be attributed to the preoperative bowel preparation (mechanical preparation and antibiotic prophylaxis) received in all participants. The concentration of fecal DNA on POD 4 was significantly lower than that before surgery in the non-DKT group, and such a difference was not observed in the DKT group. Of note, the postoperative recovery of the concentration of fecal DNA tended to be faster in the DKT group than in the non-DKT group. Fig. 5B shows the alteration of gut microbiome using the non-metric multidimensional scaling method at each assessment time point. Similar to the fecal metabolome analysis results, the gut microbiome substantially changed before and after surgery, and the gut microbiome on POD 28 recovered close to the preoperative state. Fig. 5C shows the time-dependent changes in the gut microbiome classified by genus; similar to the results presented in Fig. 5B, the composition of gut microbiota substantially changed before and after surgery, and then gradually returned to the preoperative state on POD 28. On POD 4, the abundance of obligate anaerobes, the major constituents of gut microbiota, decreased compared with the preoperative state, while that of facultative anaerobes increased (Table S1). This alteration is considered to be due to preoperative bowel preparation (mechanical preparation and antibiotic prophylaxis) and the opening of the bowel during surgery. As shown in Fig. 5B and C, there was no clear difference in the postoperative gut microbiota between the DKT group and non-DKT group. By focusing on each genus, we found that the abundance of genera *Serratia* and *Bilophila* (belonging to the phylum Proteobacteria) decreased postoperatively in the DKT group (Fig. 6A). In the DKT group, the relative abundance of genus *Serratia* was significantly lower than in the non-DKT group on POD 7, whereas that of genus *Bilophila* was significantly lower on POD 28 (Fig. 6B).

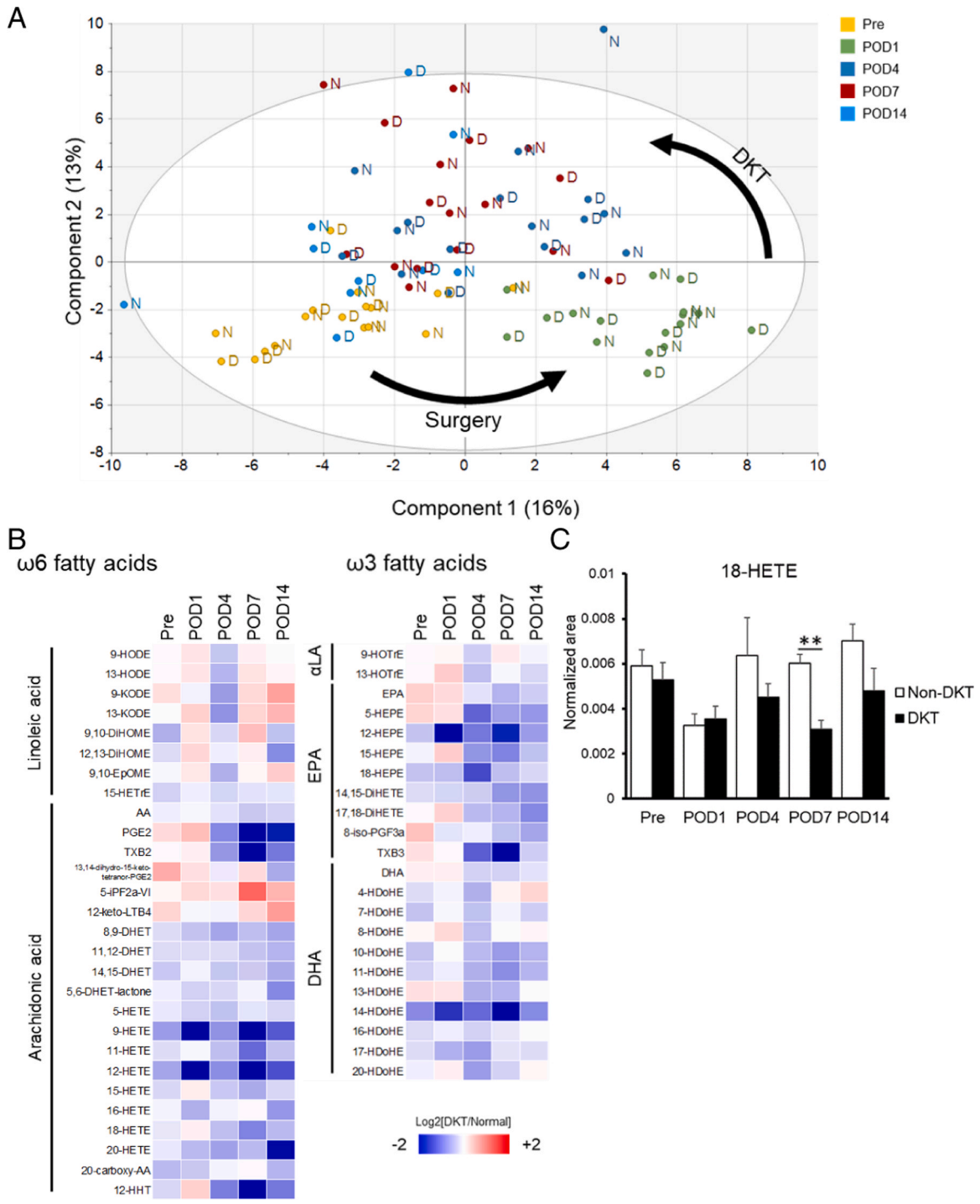


Fig. 3. Plasma metabolome analysis in perioperative patients with or without DKT treatment. A. PLS-DA score plot of the first two components. Each component explains 16% and 13% of the variance. Samples were classified based on the sampling point. The treatments are represented as N (non-DKT group; n = 5–9) and D (DKT group; n = 5–8), respectively. The eclipse represents 95% confidence interval. B. Heat map of plasma lipid mediator levels. The fold change (FC) of each lipid mediator in the plasma at each sampling point was calculated as log2 FC (DKT group/non-DKT group). C. Plasma 18-HETE levels. The data are expressed as mean ± standard error of the mean (SEM). Open column, non-DKT group (n = 5–9); closed column, DKT group (n = 5–8). **; $P < 0.01$ by Mann-Whitney *U* test. AA, arachidonic acid; DHA, docosahexaenoic acid; DHET, dihydroxy-eicosatrienoic acid; DiHETE, dihydroxy-eicosatetraenoic acid; DiHOME, dihydroxy-octadecenoic acid; EPA, eicosapentaenoic acid; EpOME, epoxy-octadecenoic acid; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydroxy-eicosapentaenoic acid; HETE, hydroxy-eicosatetraenoic acid; HETrE, hydroxy-eicosatrienoic acid; HHT, hydroxyheptadecatrienoic acid; HODE, hydroxy-octadecadienoic acid; KODE, keto-octadecadienoic acid; LT, leukotriene; OEA, oleoylethanolamide; PG, prostaglandin; TX, thromboxane.

4. Discussion

In the present study, there was no significant difference between the DKT group and non-DKT group in the primary endpoints (i.e., abdominal pain, abdominal distension, and QOL). We also verified the safety of DKT in patients with left-sided colorectal cancer who underwent laparoscopic colectomy. On the other hand, the metabolome and gut microbiome analyses showed substantial changes before and after

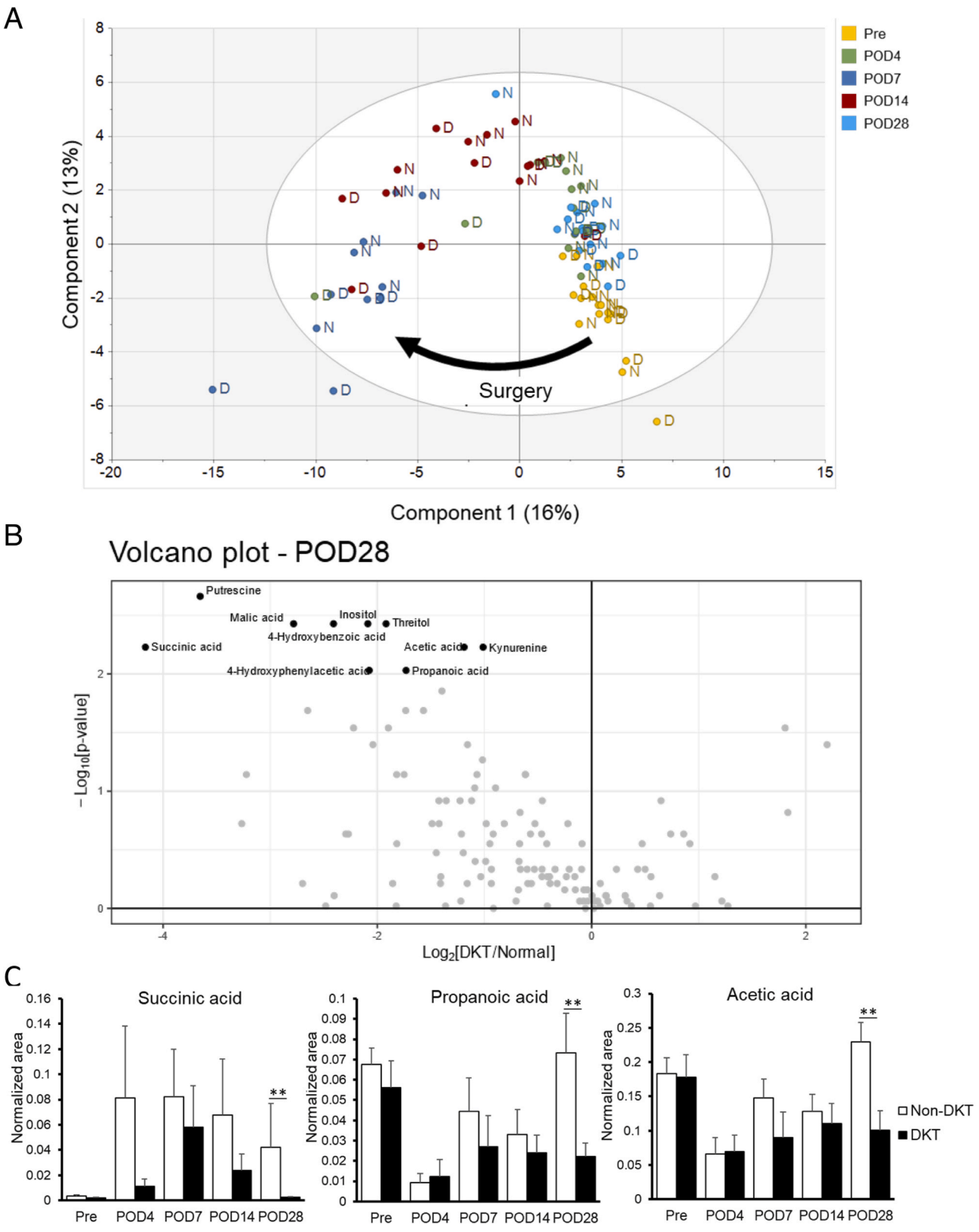


Fig. 4. Fecal metabolome analysis in perioperative patients with or without DKT treatment. A. PLS-DA score plot. Each component explains 16% and 13% of the variance. The other information is the same as that in Fig. 3A. B. Volcano plot of the fecal metabolome analysis on POD 28. The plots mapped by the log2 fold-change value for the DKT group (n = 8) /non-DKT group (n = 7) versus log10 P-value. Black points represent the metabolites whose p-value was less than 0.01 by Mann-Whitney *U* test. C. The representative fecal metabolites changed in the DKT group. The data are expressed as mean ± SEM. Open column, non-DKT group (n = 6–9); closed column, DKT group (n = 5–8). **; P < 0.01 by Mann-Whitney *U* test.

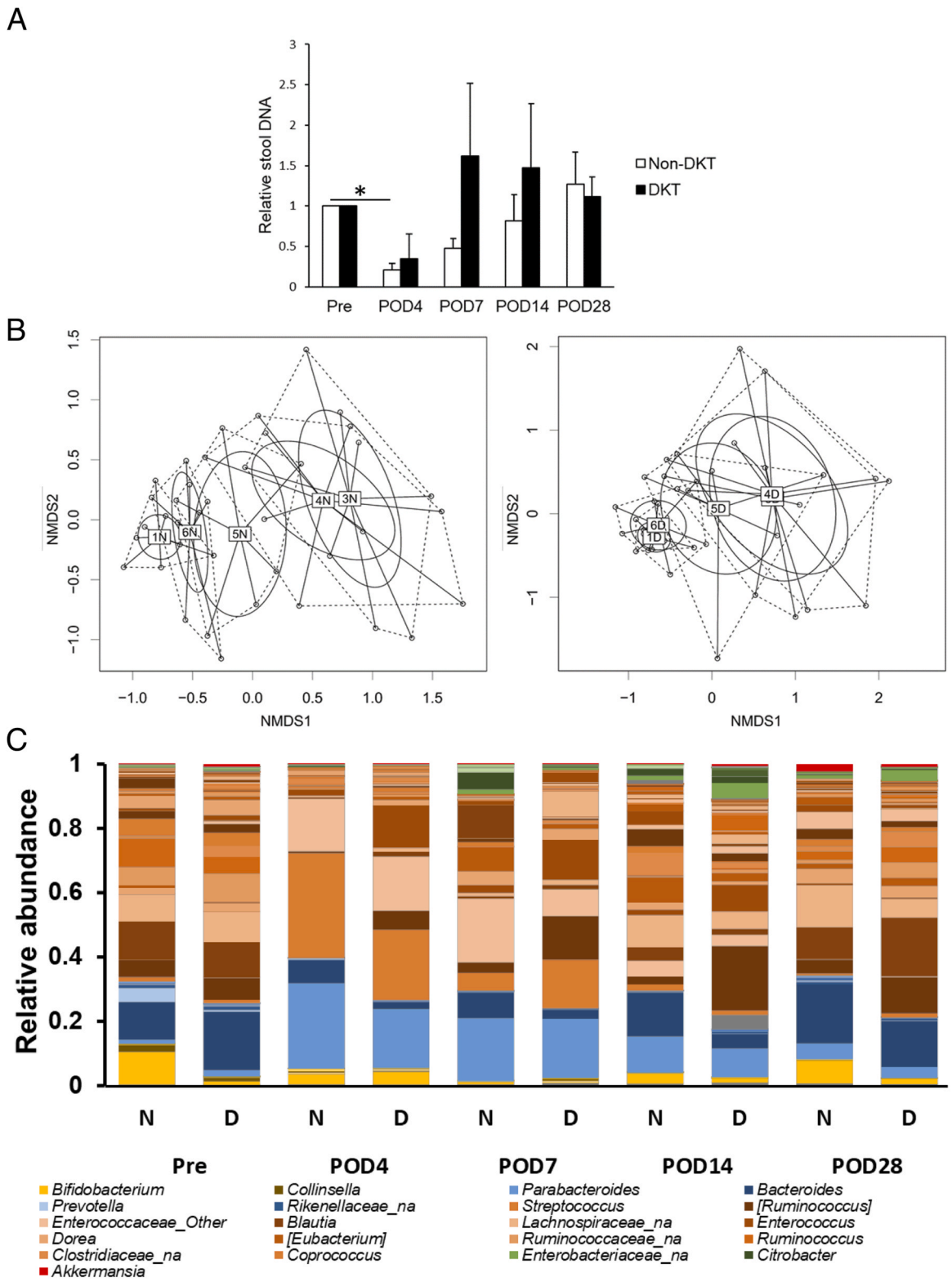
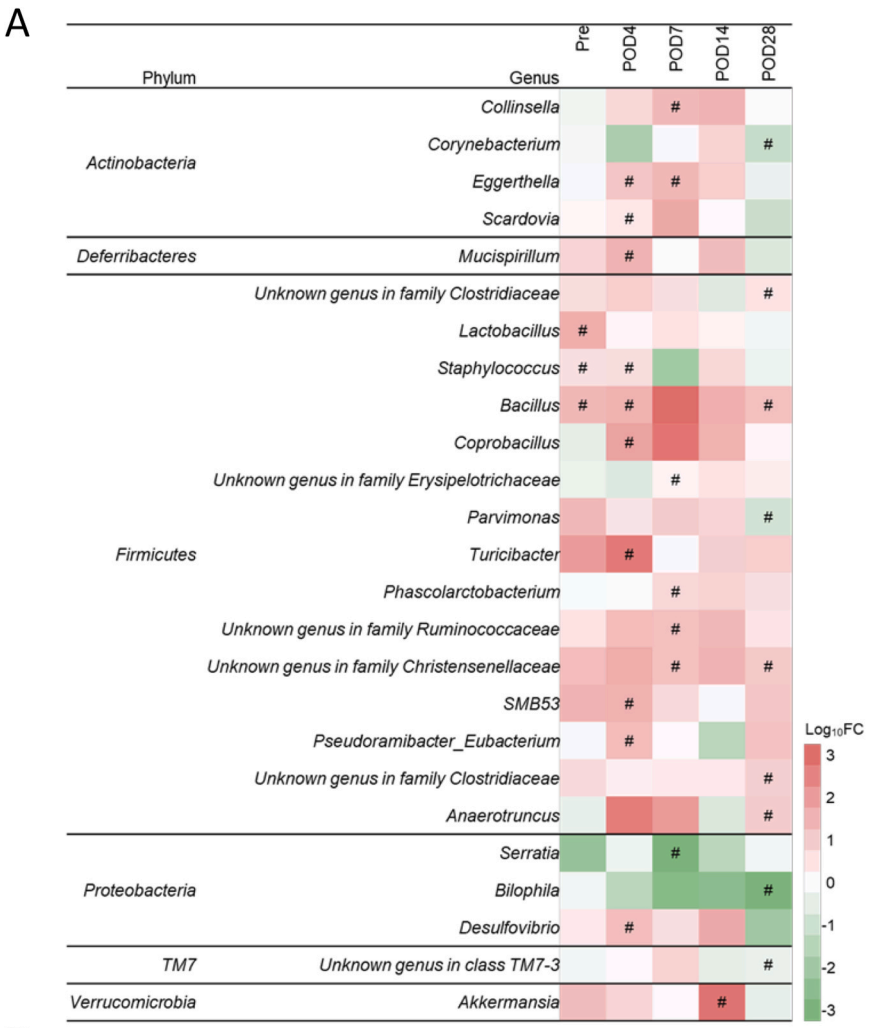


Fig. 5. Microbiome analysis in perioperative patients with or without DKT treatment. A. The relative fecal DNA concentration was calculated based on Pre (until the day before surgery). The data are expressed as mean ± SEM. Open column, non-DKT group (n = 6–9); closed column, DKT group (n = 7–8). *; $P < 0.05$ by Dunnett's test. B. A longitudinal profile of microbiome in perioperative patients was analyzed by non-metric multi dimension scaling using R 4.0.2. The fecal samples were collected on Pre (1), POD 4 (3), POD 7 (4), POD 14 (5), and POD 28 (6). The limb of each group is shown with a dotted line and 95% confidence interval is expressed as a circle line. Left, non-DKT group (n = 6–9) (N); right, DKT group (n = 7–8) (D). C. Relative abundance of the gut microbiota at the genus level was determined using the 16S metagenome sequence analysis. Average relative abundance at each sampling point is shown as a bar chart. N, non-DKT group (n = 6–9); D, DKT group (n = 7–8).



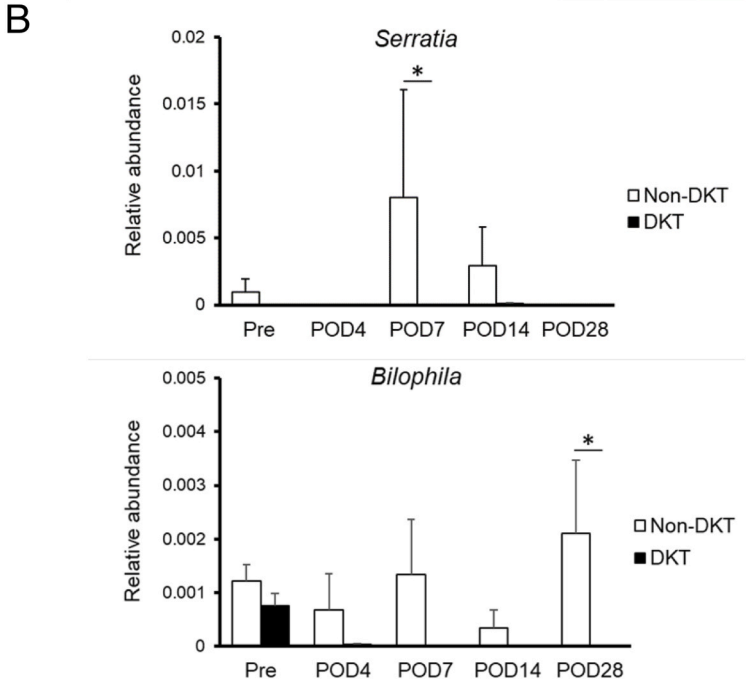


Fig. 6. Comparison of microbes between patients with or without DKT. A. The microbes at the genus levels at each sampling point were compared by Mann-Whitney *U* test and significantly different microbes in at least one sampling point are listed. The FC at each sampling point was calculated as; DKT group (n = 7–8) / non-DKT group (n = 6–9), and log10 FC is expressed as a heat map. #; $P < 0.05$ by Mann-Whitney *U* test. B. The relative abundance of genera *Serratia* (top) and *Bilophila* (bottom). The data are expressed as mean ± SEM. Open column, non-DKT group (n = 6–9); closed column, DKT group (n = 7–8). *; $P < 0.05$ by Mann-Whitney *U* test.

surgery, and this may be due to the effects of preoperative bowel preparation and surgery. Of note, these changes gradually returned to the preoperative state on POD 28. The metabolome analysis indicated that the levels of lipid mediators associated with the arachidonic acid cascade were overall lower in the DKT group than in the non-DKT group. In addition, the gut microbiome analysis indicated that the abundance of genera *Serratia* and *Bilophila* decreased postoperatively in the DKT group compared with in the non-DKT group. This study is valuable because there has been no long-term study to investigate the effects of DKT on the metabolome and gut microbiome in patients undergoing surgery.

Previous clinical studies on DKT have reported significant differences between the DKT group and control group in terms of some objective outcomes (e.g., time to first bowel movement and incidence of ileus), whereas some subjective outcomes (abdominal symptoms and QOL) were almost similar between the two groups [24–28]. Recently, Wakasugi et al. investigated the postoperative effect of DKT on subjective symptoms (i.e., abdominal pain and distention) in patients following laparoscopic colectomy, and found that abdominal pain and distention were similar between the two groups, while the number of bowel movement per day and the sensation of incomplete bowel evacuation were significantly lower in the DKT group than in the non-DKT group [28]. Katsuno et al. investigated the postoperative effect of DKT on gastrointestinal tract transit in patients following open colectomy, and found that DKT had a positive effect on delayed gastric emptying, while the time to first flatus was similar between the two groups [25]. Regarding the postoperative defecation control by DKT, no definite conclusion can be drawn from the findings of previous studies and the present study, and further research is needed in this regard.

Most of previous studies on the efficacy of DKT for postoperative ileus were based on the patients who underwent laparotomy and/or more invasive surgeries with more operation time and blood loss than colectomy (e.g., total gastrectomy, hepatectomy, and pancreatoduodenectomy [23–27]). We did not find any effect of DKT on abdominal symptoms probably because laparoscopic colectomy is minimally invasive and therefore has less effect on postoperative intestinal movement. In fact, the incidence of ileus following laparoscopic colectomy in our group was very low (6/579 patients: 1.03%) [42]. To investigate the postoperative effect of DKT on ileus, abdominal pain, and distention, patient selection based on surgical procedure and invasiveness may be important.

Regarding the serum C-reactive protein (CRP) level, Yoshikawa et al. previously reported that DKT administration reduced the CRP level on POD 3 after laparoscopic colectomy [22]. In the present study, we found no significant difference in postoperative white blood cell count and CRP level between the two groups (data not shown). Compared with the findings of Yoshikawa et al., the operation time and blood loss in the present study were similar. However, the study of Yoshikawa et al. included rectal resection, which might have been slightly more invasive than the procedure in the present study. Furthermore, Shimada et al. reported that DKT administration reduced the CRP level in patients with liver damage following hepatectomy [23]. The lower invasiveness of laparoscopic colectomy may have obscured the CRP-suppressive effect of DKT.

DKT exerts anti-inflammatory effect via the release of ADM [21]. It has been reported that ginger contained within DKT inhibits prostaglandin secretion in vitro, and that DKT administration inhibits the COX-2 activity in rats and mice [8]. Consistent with these previous study findings on the anti-inflammatory effects of DKT, the metabolome analysis in the present study showed that the levels of lipid mediators associated with the arachidonic acid cascade were overall lower in the DKT group than in the non-DKT group (Fig. 3B). Further validation is necessary because of the large variability and small sample size of this study.

In the present study, we found that the abundance of genera *Serratia* and *Bilophila* decreased postoperatively in the DKT group compared with that in the non-DKT group (Fig. 6A). Genus *Serratia* has been reported to be associated with postoperative anastomotic leakage by producing collagenase in a mouse model [43]. We previously reported that DKT administration reduced anastomotic leakage by increasing intestinal blood flow and decreasing inflammation in a rat anastomotic healing model [13]. We also found an increase in collagen density in the DKT-treated rats, which may be due to the effect of DKT on collagenase-producing bacteria, such as genus *Serratia*. Therefore, we suppose that the effect of DKT on the gut microbiome may decrease postoperative anastomotic leakage in humans. However, in the present study, there was no significant difference in the abundance of genera *Bifidobacterium*, *Pseudomonas*, *Enterococcus*, and *Escherichia* between the two groups; these bacteria have been reported to be associated with anastomotic leakage [44,45]. Genus *Bilophila*, a hydrogen sulfide-producing bacterium, has been reported to induce intestinal inflammation [46]. The effects of DKT on genus *Bilophila* abundance may contribute to the anti-inflammatory effects of DKT as well as plasma lipid mediators. Although this study suggests that DKT may have an effect on some intestinal bacteria, further investigation is needed.

This study had some limitations. The sample size was small. Recruiting patients was difficult due to the heavy patient burden: fecal collection, additional blood tests, and DKT medication for approximately 1 month. A study in a large number of patients is necessary to clarify the postoperative effects of DKT in patients following laparoscopic colectomy. Another limitation was a bias of open-label design, indicating a possibility that unblinding assessors may result in performance bias. To the best of our knowledge, this is the first study to investigate the postoperative effects of DKT on metabolites and gut microbiome in humans. The results of the metabolome and gut microbiome analyses varied substantially among the individuals. We perceive that the results of this study will help to plan the study design for future clinical trials.

5. Conclusions

We conducted a prospective randomized study to investigate the effect of DKT on abdominal symptoms following laparoscopic colectomy in patients with left-sided colon cancer. Although DKT administration did not improve the abdominal symptoms following laparoscopic colectomy, this study revealed DKT administration could have some effects on metabolites and gut microbiome postoperatively. The effects of DKT on metabolites and gut microbiome have to be further investigated.

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Author contributions

K. Kawada and Y. Sakai: Conception of the study; **K. Kawada, K. Hida, N. Hoshino, and T. Wada**: Design, and/or refine the protocol; **K. Hanada, T. Wada, N. Hoshino, M. Okamoto, W. Hirata, R. Mizuno, Y. Itatani, S. Inamoto, M. Yoshitomi and T. Watanabe**: Acquisition of the data and sample; **K. Hanada and K. Kawada**: Analysis and interpretation of the data, Drafting the manuscript; **All authors**: Review, and/or revision of the manuscript, Approve the manuscript; **K. Obama and Y. Sakai**: Study supervision.

Competing interests

The authors have indicated that they have no other conflicts of interest regarding the content of this article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2021.111887.

References

[1] K.M. Augestad, C.P. Delaney, Postoperative ileus: impact of pharmacological treatment, laparoscopic surgery and enhanced recovery pathways, World J. Gastroenterol. 16 (2010) 2067–2074, https://doi.org/10.3748/wjg.v16.i17.2067.

[2] S. Iyer, W.B. Saunders, S. Stemkowski, Economic burden of postoperative ileus associated with colectomy in the United States, J. Manag Care Pharm. 15 (2009) 485–494, https://doi.org/10.18553/jmcp.2009.15.6.485.

[3] C.P. Delaney, P.W. Marcello, T. Sonoda, P. Wise, J. Bauer, L. Techner, Gastrointestinal recovery after laparoscopic colectomy: results of a prospective, observational, multicenter study, Surg. Endosc. 24 (2010) 653–661, https://doi.org/10.1007/s00464-009-0652-7.

[4] M. Kraft, R. Maclaren, W. Du, G. Owens, Alvimopan (entereg) for the management of postoperative ileus in patients undergoing bowel resection, P T. 35 (2010) 44–49.

[5] T.A. Brown, J. McDonald, W. Williard, A prospective, randomized, double-blinded, placebo-controlled trial of cisapride after colorectal surgery, Am. J. Surg. 177 (1999) 399–401, https://doi.org/10.1016/s0002-9610(99)00071-9.

[6] W.E. Longo, A.M. Vernava 3rd, Prokinetic agents for lower gastrointestinal motility disorders, Dis. Colon Rectum 36 (1993) 696–708, https://doi.org/10.1007/BF02238599.

[7] T. Itoh, J. Yamakawa, M. Mai, N. Yamaguchi, T. Kanda, The effect of the herbal medicine dai-kenchu-to on post-operative ileus, J. Int. Med. Res. 30 (2002) 428–432, https://doi.org/10.1177/147323000203000410.

[8] T. Hayakawa, Y. Kase, K. Saito, K. Hashimoto, A. Ishige, Y. Komatsu, H. Sasaki, Effects of Dai-kenchu-to on intestinal obstruction following laparotomy, J. Smooth Muscle Res. 35 (1999) 47–54, https://doi.org/10.1540/jsmr.35.47.

[9] T. Kono, Y. Omiya, Y. Hira, A. Kaneko, S. Chiba, T. Suzuki, M. Noguchi, T. Watanabe, Daikenchuto (TU-100) ameliorates colon microvascular dysfunction via endogenous adrenomedullin in Crohn's disease rat model, J. Gastroenterol. 46 (2011) 1187–1196, https://doi.org/10.1007/s00535-011-0438-2.

[10] T. Kono, T. Koseki, S. Chiba, Y. Ebisawa, N. Chisato, J. Iwamoto, S. Kasai, Colonic vascular conductance increased by Daikenchuto via calcitonin gene-related peptide and receptor-activity modifying protein 1, J. Surg. Res. 150 (2008) 78–84, https://doi.org/10.1016/j.jss.2008.02.057.

[11] H. Fukuda, C. Chen, C. Mantyh, K. Ludwig, T.N. Pappas, T. Takahashi, The herbal medicine, Dai-Kenchu-to, accelerates delayed gastrointestinal transit after the operation in rats, J. Surg. Res 131 (2006) 290–295, https://doi.org/10.1016/j.jss.2005.09.018.

[12] N. Manabe, M. Camilleri, A. Rao, B.S. Wong, D. Burton, I. Busciglio, A. R. Zinsmeister, K. Haruma, Effect of daikenchuto (TU-100) on gastrointestinal and colonic transit in humans, Am. J. Physiol. Gastrointest. Liver Physiol. 298 (2010) G970–G975, https://doi.org/10.1152/ajpgi.00043.2010.

[13] T. Wada, K. Kawada, K. Hirai, K. Toda, M. Iwamoto, S. Hasegawa, Y. Sakai, Enhanced anastomotic healing by Daikenchuto (TJ-100) in rats, Sci. Rep. 8 (2018) 1091, https://doi.org/10.1038/s41598-018-19550-4.

[14] M.J. Wood, N.H. Hyman, G.M. Mawe, The effects of daikenchuto (DKT) on propulsive motility in the colon, J. Surg. Res. 164 (2010) 84–90, https://doi.org/10.1016/j.jss.2009.03.068.

[15] J.Y. Koo, Y. Jang, H. Cho, C.H. Lee, K.H. Jang, Y.H. Chang, J. Shin, U. Oh, Hydroxy-alpha-sanshool activates TRPV1 and TRPA1 in sensory neurons, Eur. J. Neurosci. 26 (2007) 1139–1147, https://doi.org/10.1111/j.1460-9568.2007.05743.x.

[16] M. Munekage, K. Ichikawa, H. Kitagawa, K. Ishihara, H. Uehara, J. Watanabe, T. Kono, K. Hanazaki, Population pharmacokinetic analysis of daikenchuto, a traditional Japanese medicine (Kampo) in Japanese and US health volunteers, Drug Metab. Dispos. 41 (2013) 1256–1263, https://doi.org/10.1124/dmd.112.050112.

[17] M. Munekage, H. Kitagawa, K. Ichikawa, J. Watanabe, K. Aoki, T. Kono, K. Hanazaki, Pharmacokinetics of daikenchuto, a traditional Japanese medicine (kampo) after single oral administration to healthy Japanese volunteers, Drug Metab. Dispos. 39 (2011) 1784–1788, https://doi.org/10.1124/dmd.111.040097.

[18] K. Tsuchiya, K. Kubota, K. Ohbuchi, A. Kaneko, N. Ohno, A. Mase, H. Matsushima, M. Yamamoto, K. Miyano, Y. Uezono, T. Kono, Transient receptor potential ankyrin 1 agonists improve intestinal transit in a murine model of postoperative ileus, Neurogastroenterol. Motil. 28 (2016) 1792–1805, https://doi.org/10.1111/nmo.12877.

[19] K. Nozawa, E. Kawabata-Shoda, H. Doihara, R. Kojima, H. Okada, S. Mochizuki, Y. Sano, K. Inamura, H. Matsushime, T. Koizumi, T. Yokoyama, H. Ito, TRPA1 regulates gastrointestinal motility through serotonin release from enterochromaffin cells, Proc. Natl. Acad. Sci. USA 106 (2009) 3408–3413, https://doi.org/10.1073/pnas.0805323106.

[20] T. Kono, A. Kaneko, Y. Omiya, K. Ohbuchi, N. Ohno, M. Yamamoto, Epithelial transient receptor potential ankyrin 1 (TRPA1)-dependent adrenomedullin upregulates blood flow in rat small intestine, Am. J. Physiol. Gastrointest. Liver Physiol. 304 (2013) G428–G436, https://doi.org/10.1152/ajpgi.00356.2012.

[21] T. Kono, A. Kaneko, Y. Hira, T. Suzuki, N. Chisato, N. Ohtake, N. Miura, T. Watanabe, Anti-colitis and -adhesion effects of daikenchuto via endogenous adrenomedullin enhancement in Crohn's disease mouse model, J. Crohns Colitis 4 (2010) 161–170, https://doi.org/10.1016/j.crohns.2009.09.006.

[22] K. Yoshikawa, M. Shimada, M. Nishioka, N. Kurita, T. Iwata, S. Morimoto, T. Miyatani, M. Komatsu, H. Kashihara, C. Mikami, The effects of the Kampo medicine (Japanese herbal medicine) "Daikenchuto" on the surgical inflammatory response following laparoscopic colorectal resection, Surg. Today 42 (2012) 646–651, https://doi.org/10.1007/s00595-011-0094-4.

[23] M. Shimada, Y. Morine, H. Nagano, E. Hatano, T. Kaiho, M. Miyazaki, T. Kono, T. Kamiyama, S. Morita, J. Sakamoto, M. Kusano, S. Saji, T. Kanematsu, M. Kitajima, Effect of TU-100, a traditional Japanese medicine, administered after hepatic resection in patients with liver cancer: a multi-center, phase III trial (JFMC40-1001), Int J. Clin. Oncol. 20 (2015) 95–104, https://doi.org/10.1007/s10147-014-0678-2.

[24] Y. Akamaru, T. Takahashi, T. Nishida, T. Omori, K. Nishikawa, S. Mikata, N. Yamamura, S. Miyazaki, H. Noro, S. Takiguchi, M. Mori, Y. Doki, Effects of daikenchuto, a Japanese herb, on intestinal motility after total gastrectomy: a prospective randomized trial, J. Gastrointest. Surg. 19 (2015) 467–472, https://doi.org/10.1007/s11605-014-2730-y.

[25] H. Katsuno, K. Maeda, T. Kaiho, K. Kunieda, K. Funahashi, J. Sakamoto, T. Kono, H. Hasegawa, Y. Furukawa, Y. Imazu, S. Morita, M. Watanabe, Clinical efficacy of Daikenchuto for gastrointestinal dysfunction following colon surgery: a randomized, double-blind, multicenter, placebo-controlled study (JFMC39-0902), Jpn J. Clin. Oncol. 45 (2015) 650–656, https://doi.org/10.1093/jjco/hyv056.

[26] K. Yoshikawa, M. Shimada, G. Wakabayashi, K. Ishida, T. Kaiho, Y. Kitagawa, J. Sakamoto, N. Shiraishi, K. Koeda, E. Mochiki, Y. Saikawa, K. Yamaguchi, M. Watanabe, S. Morita, S. Kitano, S. Saji, T. Kanematsu, M. Kitajima, Effect of daikenchuto, a traditional Japanese herbal medicine, after total gastrectomy for gastric cancer: a multicenter, randomized, double-blind, placebo-controlled, phase II trial, J. Am. Coll. Surg. 221 (2015) 571–578, https://doi.org/10.1016/j.jamcollsurg.2015.03.004.

[27] K. Okada, M. Kawai, S. Hirono, T. Fujii, Y. Kodera, M. Sho, Y. Nakajima, S. Satoi, A. H. Kwon, Y. Shimizu, Y. Ambo, N. Kondo, Y. Murakami, J. Ohuchida, H. Eguchi, H. Nagano, M.S. Oba, S. Morita, J. Sakamoto, H. Yamaue, … JAPAN-PD Investigators. Evaluation of the efficacy of daikenchuto (TJ -100) for the prevention of paralytic ileus after pancreaticoduodenectomy: a multicenter, double-blind, randomized, placebo-controlled trial, Surgery 159 (2016) 1333–1341, https://doi.org/10.1016/j.surg.2015.11.019.

[28] M. Wakasugi, Y. Suzuki, M. Tei, S. Ueshima, H. Akamatsu, T. Nishida, Effects of Daikenchuto on postoperative gastrointestinal motility in colorectal carcinoma patients with abdominal pain and distension: a prospective, randomized trial, Surg. Today 50 (2020) 1524–1529, https://doi.org/10.1007/s00595-020-02052-0.

[29] K. Yoshikawa, M. Shimada, T. Kuwahara, H. Hirakawa, N. Kurita, H. Sato, T. Utsunomiya, T. Iwata, T. Miyatani, J. Higashijima, H. Kashihara, C. Takasu, N. Matsumoto, H. Nakayama-Imaohji, Effect of Kampo medicine "Dai-kenchu-to" on microbiome in the intestine of the rats with fast stress, J. Med. Investig. 60 (2013) 221–227, https://doi.org/10.2152/jmi.60.221.

[30] T. Hasebe, N. Ueno, M.W. Musch, A. Nadimpalli, A. Kaneko, N. Kaifuchi, J. Watanabe, M. Yamamoto, T. Kono, Y. Inaba, M. Fujiya, Y. Kohgo, E.B. Chang, Daikenchuto (TU-100) shapes gut microbiota architecture and increases the production of ginsenoside metabolite compound K, Pharm. Res Perspect. 4 (2016) 00215, https://doi.org/10.1002/prp2.215.

[31] C.M. Dejea, E.C. Wick, E.M. Hechenbleikner, J.R. White, J.L. Mark Welch, B. J. Rossetti, S.N. Peterson, E.C. Snesrud, G.G. Borisy, M. Lazarev, E. Stein, J. Vadivelu, A.C. Roslani, A.A. Malik, J.W. Wanyiri, K.L. Goh, I. Thevambiga, K. Fu, F. Wan, N. Llosa, F. Housseau, K. Romans, X. Wu, F.M. McAllister, S. Wu, B. Vogelstein, K.W. Kinzler, D.M. Pardoll, C.L. Sears, Microbiota organization is a distinct feature of proximal colorectal cancers, Proc. Natl. Acad. Sci. USA 111 (2014) 18321–18326, https://doi.org/10.1073/pnas.1406199111.

[32] B. Flemer, D.B. Lynch, J.M. Brown, I.B. Jeffery, F.J. Ryan, M.J. Claesson, M. O'Riordain, F. Shanahan, P.W. O'Toole, Tumour-associated and non-tumour-associated microbiota in colorectal cancer, Gut 66 (2017) 633–643, https://doi.org/10.1136/gutjnl-2015-309595.

[33] N. Hoshino, K. Kawada, K. Hida, T. Wada, R. Takahashi, M. Yoshitomi, Y. Sakai, Effect of Daikenchuto (TJ-100) on gastrointestinal symptoms following laparoscopic colectomy in patients with colon cancer: study protocol for a randomized controlled trial, Trials 18 (2017) 553, https://doi.org/10.1186/s13063-017-2307-7.

[34] G.A. Hawker, S. Mian, T. Kendzerska, M. French, Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP), Arthritis Care Res. Hoboken 63 (2011) S240–S252, https://doi.org/10.1002/acr.20543.

[35] E. Eypasch, J.I. Williams, S. Wood-Dauphinee, B.M. Ure, C. Schmülling, E. Neugebauer, H. Troidl, Gastrointestinal Quality of Life Index: development, validation and application of a new instrument, Br. J. Surg. 82 (1995) 216–222, https://doi.org/10.1002/bjs.1800820229.

[36] T. Onodera, N. Goseki, G. Kosaki, Prognostic nutritional index in gastrointestinal surgery of malnourished cancer patients, Nihon Geka Gakkai Zasshi 85 (1984) 1001–1005.
[37] J. Ignacio de Ulíbarri, A. González-Madroño, N.G.P. de Villar, P. González, B. González, A. Mancha, F. Rodríguez, G. Fernández, CONUT: a tool for controlling nutritional status. First validation in a hospital population, Nutr. Hosp. 20 (2005) 38–45.
[38] H. Kitagawa, K. Ohbuchi, M. Munekage, K. Fujisawa, Y. Kawanishi, T. Namikawa, H. Kushida, T. Matsumoto, C. Shimobori, A. Nishi, C. Sadakane, J. Watanabe, M. Yamamoto, K. Hanazaki, Data on metabolic profiling of healthy human subjects' plasma before and after administration of the Japanese Kampo medicine maoto, Data Brief 22 (2018) 359–364, https://doi.org/10.1016/j.dib.2018.11.116.
[39] E. Takeo, R. Sasano, S. Shimma, T. Bamba, E. Fukusaki, Solid-phase analytical derivatization for gas-chromatography-mass-spectrometry-based metabolomics, J. Biosci. Bioeng. 124 (2017) 700–706, https://doi.org/10.1016/j.jbiosc.2017.07.006.
[40] Y. Furusawa, Y. Obata, S. Fukuda, T.A. Endo, G. Nakato, D. Takahashi, Y. Nakanishi, C. Uetake, K. Kato, T. Kato, M. Takahashi, N.N. Fukuda, S. Murakami, E. Miyauchi, S. Hino, K. Atarashi, S. Onawa, Y. Fujimura, T. Lockett, J.M. Clarke, H. Ohno, Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells, Nature 504 (2013) 446–450, https://doi.org/10.1038/nature12721.
[41] J.G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F.D. Bushman, E. K. Costello, N. Fierer, A.G. Peña, J.K. Goodrich, J.I. Gordon, G.A. Huttley, S. T. Kelley, D. Knights, J.E. Koenig, R.E. Ley, C.A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, R. Knight, QIIME allows analysis of high-throughput community sequencing data, Nat. Methods 7 (2010) 335–336, https://doi.org/10.1038/nmeth.f.303.
[42] H. Hata, T. Yamaguchi, S. Hasegawa, A. Nomura, K. Hida, R. Nishitai, S. Yamanokuchi, T. Yamanaka, Y. Sakai, Oral and parenteral versus parenteral antibiotic prophylaxis in elective laparoscopic colorectal surgery (JMTO PREV 07-01): A phase 3, multicenter, open-label, randomized trial, Ann. Surg. 263 (2016) 1085–1091, https://doi.org/10.1097/SLA.0000000000001581.
[43] S.K. Hyoju, R.E. Klabbers, M. Aaron, M.A. Krezalek, A. Zaborin, M. Wiegerinck, N. H. Hyman, O. Zaborina, H. Van Goor, J.C. Alverdy, Oral polyphosphate suppresses bacterial collagenase production and prevents anastomotic leak due to Serratia marcescens and Pseudomonas aeruginosa, Ann. Surg. 267 (2018) 1112–1118, https://doi.org/10.1097/SLA.0000000000002167.
[44] R. Hajjar, M.M. Santos, F. Dagbert, C.S. Richard, Current evidence on the relation between gut microbiota and intestinal anastomotic leak in colorectal surgery, Am. J. Surg. 218 (2019) 1000–1007, https://doi.org/10.1016/j.amjsurg.2019.07.001.
[45] K. Mima, Y. Sakamoto, K. Kosumi, Y. Ogata, K. Miyake, Y. Hiyoshi, T. Ishimoto, M. Iwatsuki, Y. Baba, S. Iwagami, Y. Miyamoto, N. Yoshida, S. Ogino, H. Baba, Mucosal cancer-associated microbes and anastomotic leakage after resection of colorectal carcinoma, Surg. Oncol. 32 (2020) 63–68, https://doi.org/10.1016/j.suronc.2019.11.005.
[46] H. Tilg, T.E. Adolph, R.R. Gerner, A.R. Moschen, The intestinal microbiota in colorectal cancer, Cancer Cell 33 (2018) 954–964, https://doi.org/10.1016/j.ccell.2018.03.004.