ORIGINAL ARTICLE

Intestinal, portal, and peripheral profiles of daikenchuto (TU-100)'s active ingredients after oral administration

Junko Watanabe[1], Noriko Kaifuchi[1], Hirotaka Kushida[1], Takashi Matsumoto[1], Miwako Fukutake[1], Mitsue Nishiyama[1], Masahiro Yamamoto[1] & Toru Kono[2,3]

[1]Tsumura Research Laboratories, Tsumura & Co., Ami, Ibaraki, Japan
[2]Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido, Japan
[3]Center for Clinical and Biomedical Research, Sapporo Higashi Tokushukai Hospital, Sapporo, Hokkaido, Japan

Abstract

A pharmaceutical grade Japanese traditional medicine, daikenchuto (TU-100), consisting of Japanese pepper, processed ginger, and ginseng, has been widely used for various intestinal disorders in Japan and now under development as a new therapeutic drug in the US. It is suggested that TU-100 ingredients exert pharmacological effects on intestines via two routes, from the luminal side before absorption and the peripheral blood stream after absorption. Therefore, in order to fully understand the pharmacological actions of TU-100, it is critically important to know the intraluminal amounts and forms of ingested TU-100 ingredients. In the present study, after administrating TU-100 to rats, the concentrations of TU-100 ingredients and their conjugates in the peripheral and portal blood and ileal contents were determined by LC-MS/MS. Next, TU-100 was administered to patients with ileostomy bags, but whose small intestines are diagnosed as healthy, and the ingredients/conjugates in the ileal effluent were analyzed. The results suggest that: (1) Pepper ingredients hydroxy-sanshools are rapidly absorbed and enter systemic circulation, (2) Ginseng ingredients ginsenosides are transported to the colon with the least absorption, (3) Ginger ingredients gingerols are absorbed and some conjugated in the small intestine and transported via the portal vein. While only a small amount of gingerols/gingerol conjugates enter systemic circulation, considerable amounts reappear in the small intestine. Thus, the effect of TU-100 on the intestines is believed to be a composite of multiple actions by multiple compounds supplied via multiple routes.

Abbreviations

6G, [6]-gingerol; 6S, [6]-shogaol; CK, compound K; GS, γ-sanshool; HAS, hydroxy-α sanshool; HBBS, Hanks' balanced salt solution; HBS, hydroxy-β sanshool; KCNK, two-pore domain potassium channel; Rb1, ginsenoside Rb_1; Rg1, ginsenoside Rg_1; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1; TU-100, daikenchuto.

Introduction

Herbal medicine is a practice as old as mankind and in recent years much research has been performed with the goal of integration of traditional medicines with modern medicinal practice. However, in spite of the explosion in the number of papers in this field, convincing evidences have not been obtained for traditional medicines. Most of clinical studies on these medicines are of insufficient quality and the elucidation of mechanism of action or definition of their indications is incomplete (Wachtel-Galor and Benzie 2011). A principal factor in this problem is

the extraordinary difficulties in quality control of herbal products, particularly fluctuations in their chemical composition with respect to active ingredients and impurities (Zhang et al. 2011).

Among various traditional medicines, traditional Japanese (Kampo) medicine occupies a unique position. In Japan, certain Kampo medicines have been manufactured as extract granules with standardized ingredients and specification levels for impurities such as heavy metal, pesticides, phytotoxins, and bacterial contamination under modern quality control (Kono et al. 2009). They have been approved by the Ministry of Health, Labour, and Welfare of Japan for many decades and used by over 80% of physicians with, and in a similar manner to, Western medicines. Some Kampo medicines have been prescribed to millions of patients and their empirical efficacy and safety are widely recognized. During the last decade, many double-blind, placebo-controlled studies have been started in order to submit these medicines for approval outside of Japan. Additionally, studies are ongoing as part of postmarketing investigations of adverse drug reactions or research of their molecular mechanisms of action. Among such medicines, daikenchuto (TU-100) is a subject of the most intensive research efforts. Several double-blind placebo-controlled clinical trials (Manabe et al. 2010; Iturrino et al. 2013; Okada et al. 2013; Shimada et al. 2014) as well as pharmacokinetic (PK) studies (Iwabu et al. 2010; Munekage et al. 2011, 2013) have been conducted in Japan and the US aimed at FDA approval of a new drug for various intestinal disorders.

The study of ADME of Kampo medicines is extraordinarily challenging. A single Kampo formulation consists of multiple constituents, each in minute amounts. Furthermore, most Kampo medicines are administered orally and exposed to gastric acid, intestinal fluid, pancreatic juice, bile, and intestinal microflora. Consequently, some ingredients are converted to other forms, which are often the real active components of the Kampo medicine (Yamamoto et al. 2000; Lee et al. 2002; Hasegawa 2004; Yim et al. 2004; Kim et al. 2008; Park et al. 2013). In addition, certain ingredients in Kampo medicine exert their effect without absorption into the blood, that is, by targeting cells/molecules from inside the lumen of the gut. Although the previous pharmacokinetic studies of TU-100 conducted in Japan and the US have clarified the blood concentration profiles of the major TU-100 ingredients in healthy volunteers after a single oral administration, the concentrations of these ingredients, and their metabolites in the gastrointestinal tract have not been investigated. Because intraluminal transient receptor potential ankyrin 1 (TRPA1), which presents abundantly in the intestinal epithelial cells (Kono et al. 2013) and enterochromaffin cells (Nozawa et al. 2009), has been

suggested to be a target of TU-100 (Kono et al. 2013), the elucidation of intraluminal concentrations of TU-100 ingredients and their metabolites is critical for a complete understanding of the mechanisms of action of TU-100.

In the present study, we analyzed the concentrations of TU-100 ingredients and their conjugates in rat and human luminal contents. In the animal experiments, the small intestines were divided into four portions and a time-dependent change in each portion was determined. The concentrations in portal and peripheral blood were also measured. In the human study, the ileal effluent was collected 4 h after TU-100 administration, from ileostomy bags of patients with colonic surgery, whose small intestines are shown to be normal by enteroscopy. The results of this study and the previous studies on blood pharmacokinetics of TU-100 ingredients/conjugates (Munekage et al. 2011, 2013) indicate they essentially take the same paths in rats and human. The present findings support the previous supposition that some ingredients of TU-100 exert their effect via direct action on the intestine from inside the lumen. A possible involvement of intraluminal ingredients in the pharmacological action is plausible for various herbal medicines and the analysis of intraluminal contents using ileostomy effluent would contribute to the elucidation of complex mode of action of mixed or combined oral medicines.

Materials and Methods

Chemicals, reagents and authentic standards

TU-100, Tsumura Daikenchuto Extract Granules, was manufactured by Tsumura & Co. (Ami, Ibaraki, Japan). Fifteen grams of Tsumura Daikenchuto extract granules contains 1.25 g of a dried extract prepared from a mixture of three herbs (5.0 g of processed ginger, 3.0 g of ginseng, and 2.0 g of Japanese pepper) and 10.0 g of maltose. Acetonitrile and acetic acid of HPLC grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). β-Glucuronidase (EC 3.2.1.31) was obtained from Sigma–Aldrich (St. Louis, MO). Hanks' balanced salt solution (HBBS) was purchased from GIBCO/Life Technologies (Grand Island, NY). The following authentic standards and internal standards were isolated or synthesized by Tsumura & Co.: hydroxy-α-sanshool (HAS), hydroxy-β-sanshool (HBS), γ-sanshool (GS), [6]-gingerol (6G), [6]-shogaol (6S), [10]-gingerol (10G), [10]- shogaol (10S), ginsenoside Rg$_1$ (Rg1), ginsenoside Rb$_1$ (Rb1), and schizandrin. Nonivamide was obtained from Alexis Biochemicals (San Diego, CA). Asiaticoside was purchased from Extrasynthese (Lyon, France). All of these authentic standards were reliably identified by spec-

troscopic methods such as NMR, mass, and IR spectrometry. The structural formulas of the authentic standards are provided in the previous paper (Iwabu et al. 2010).

Animals experimental design, administration, and sample collection

Male Sprague Dawley rats (7 weeks of age, 274.2–317.7 g BW) were purchased from Charles River Laboratories Japan Inc. (Yokohama, Japan) and housed in an air-conditioned room with free access to commercial chow and tap water. They were fasted for 16 h before the experiment. For collection of peripheral blood, jugular vein catheterization was performed 2 days before the experiment. For collection of portal blood, we purchased rats that had undergone hepatic portal vein catheterization. TU-100 drug substance (a powder made from an extract of the mixture of three herbs) was administered orally to rats ($n = 5$) at the dose of 0.3 g/kg body weight with 2.4 g/kg body weight of maltose as an additive. At 0.083, 0.25, 0.5, 1, 2, 4, 8, 24 h, blood was collected and plasma fractions were prepared and stored at −80°C until analysis. The preliminary experiments suggested that blood concentrations of 6G/6S would be very low, often below detection limit. Therefore, we used a higher dose of TU-100 (0.9 g/kg TU-100 + 7.2 g/kg maltose) in experiments examining 6G/6S. For sampling of luminal contents, at 0.083, 0.25, 0.5, 1, 2, 4 h after administration of 0.3 g/kg TU-100 + 2.4 g/kg maltose rats ($n = 3$) were sacrificed and the small intestine was ligated at both ends. The small intestine was isolated and ligated at three loci dividing it in quarters. Luminal contents from each portion were collected with cold water to a final volume of 10 mL. The luminal contents solutions were stored at −80°C until analysis.

Study subjects and adverse effects in clinical trial

This study was conducted at Asahikawa Medical University in accordance with the ethical principles of the Declaration of Helsinki. The study was approved by the ethical committee of Asahikawa Medical University (approval number: 821). Before participation, all subjects were informed about the study verbally and in writing prior to signing a written informed consent. Patients with strangulating intestinal obstruction, serious coexisting disease (ex. hepatic disorder, kidney disorder, cardiac disorder, hematological disorder, or metabolic disorder), women who were lactating or pregnant or hoping to become pregnant were excluded from the study. Patients who had been administered TU-100 or any Japanese traditional herbal medicine within 4 weeks before this study were

also excluded. A total number of 10 subjects (two males and eight females) were included in this study. Average subject age, body weight, and BMI was 46.0 ± 18.9, 51.4 ± 6.7, and 20.5 ± 1.3 kg/m^2 (mean $\pm$ SD). Two of these subjects underwent surgical removal of the diseased region of the colon due to ulcerative colitis, two due to Crohn's disease, one due to familial adenomatous polyposis and the other four due to colon cancer. Their small intestines exhibited no sign of aberration by small bowel enteroscopy. Ileostomies were performed in all patients at least 2 weeks before participation. In the morning, the stoma bags attached on the night before was removed and the ileal effluent was collected. After attaching a new stoma bag, the patient took 5 g of TU-100 drug product (corresponds to 0.42 g of TU-100 drug substance), ate breakfast with no limitation to the meal and recorded what they had eaten. Four hours after ingestion of the TU-100, the ileostomy effluent was collected and frozen at −80°C until use. There were no adverse experiences during the study.

Pretreatment procedure for rat plasma, ileal contents, and human ileal effluents

Rat plasma and luminal content samples were extracted using a protein precipitation procedure. Two hundred microliters of acetonitrile, 100 μL of methanol and 10 μL of the internal standard solution were added to 100 μL of rat plasma. The mixtures were centrifuged at 12,100g for 5 min at room temperature. Three hundred microliters of each of the resulting upper layers were evaporated to dryness under a nitrogen flow at 37°C. In cases of the β-glucuronidase treatment plasma samples, the volumes were reduced to a half. The dried residue was reconstituted in 75 μL of 30% (vol/vol) acetonitrile.

For rat ileal contents, to 20 μL of the β-glucuronidase nontreatment or treatment samples, 140 μL of HBSS containing 30% acetonitrile, and 40 μL of the internal standard solution were added. The mixtures were centrifuged at 12,100g for 5 min at room temperature. Twenty or ten microliters of the solution were injected into LC/MS/MS system for analysis.

For human ileal effluents, liquid–liquid extraction was adopted as the extraction procedure. To 100 mg of each human ileal samples, 200 μL of a mixture of ammonium acetate buffer solution (pH 5.0) and polyethylene glycol (25:1), 1000 μL of methanol, 100 μL of the 30% (vol/vol) acetonitrile, and 100 μL of the internal standard solution were added and mixed. The resulting mixtures were centrifuged (10,000g, 5 min, 25°C). To 300 μL of each of the resulting upper layers, 100 μL of purified water was added, the mixtures were filtered, and the resulting solutions were used for LC-MS/MS analysis.

Enzyme treatment

Ammonium acetate buffer (pH 6.0) and β-glucuronidase (final 0.1 U/μL) were added to the plasma and intraluminal fluid samples. The mixtures were incubated at 37°C for 1.5 h or 4 h. Subsequently, a sample for LC-MS/MS analysis was prepared as described under Pretreatment Procedure for human ileal effluents. Incubation in the enzyme solution in the present protocol, in which plasma or intraluminal fluid was contained, has been found to result in the deconjugation of both glucuronide- and sulfate conjugates of 6S and 6G.

LC-MS/MS analysis

LC-MS/MS analyses were conducted using the API 5000 system (AB SCIEX, Tokyo, Japan) equipped with a 1200 series HPLC system (Agilent Technologies, Inc., Santa Clara, CA), and Analyst Software (version 1.4.2; AB SCIEX) was used for analysis of TU-100 ingredients in human ileal effluents. The column for LC-MS/MS analyses was a XBridge TM C18 (2.5 μm, 50 × 2.1 mm i.d.; Nihon Waters K.K., Tokyo, Japan). An API 4000 system (AB SCIEX, Tokyo, Japan) equipped with an Agilent 1100 series HPLC system (Agilent Technologies, Inc., Santa Clara, CA) was used for analysis of TU-100 ingredients in rat plasma and luminal content solutions. The column for LC-MS/MS analyses was a YMC-Pack ODA-AQ (3 μm, 150 × 2.0 mm i.d.; 3 μm, 50 × 2.0 mm i.d.; YMC Co., Ltd., Kyoto, Japan). The detailed conditions for analyses and methods of determination of concentrations of TU-100 ingredients and conjugates were described in the previous paper (Iwabu et al. 2010).
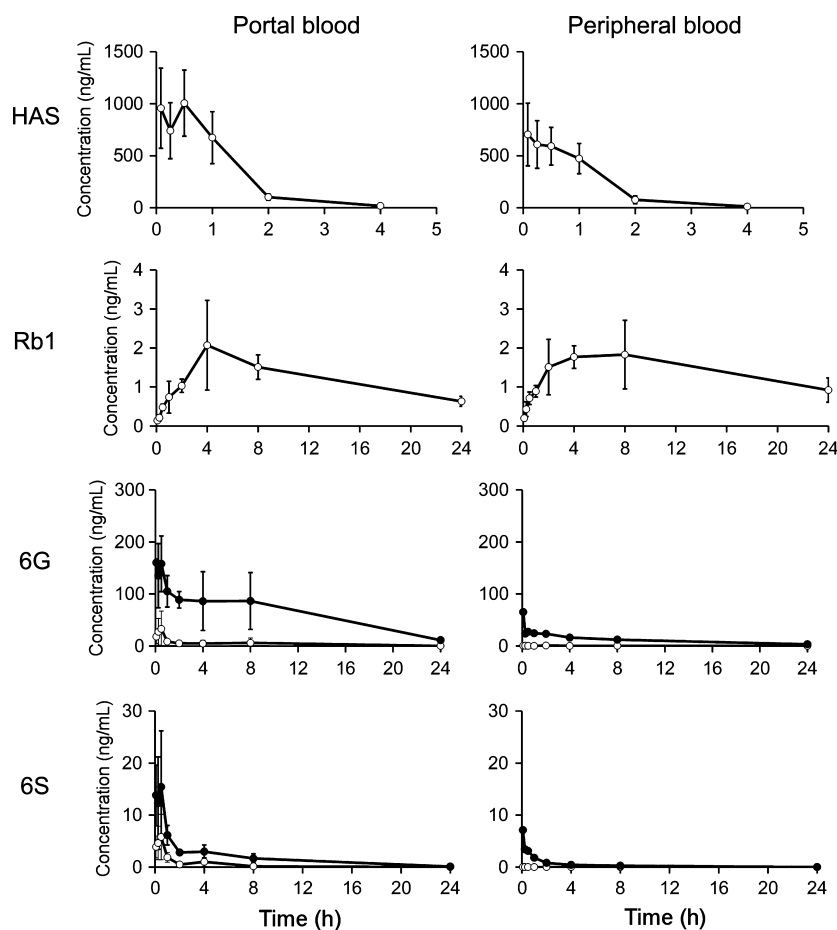

Figure 1. Time-dependent changes in plasma concentrations of major TU-100 ingredients (HAS, Rb1, 6G, and 6S) in portal and peripheral blood. Portal and peripheral blood were collected at the indicated times after oral administration of TU-100 and the blood concentrations were determined. The graphs of 6G and 6S in peripheral blood were the results by 0.9 g/kg TU-100 administration. Other graphs were by 0.3 g/kg TU-100 administration. Values represent means ± SD, $n = 5$. Closed circles represent values after β-glucuronidase treatment. Open circles represent values without treatment. Pharmacokinetic parameters are demonstrated in Tables 1 (portal blood) and 2 (peripheral blood). HAS, hydroxy-α sanshool; Rb1, ginsenoside Rb$_1$; 6G, [6]-gingerol; 6S, [6]-shogaol; TU-100, daikenchuto.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated using Phoenix WinNonlin (version 6.3; Certara L.P.St. Louis, MO, CA). Experimentally observed values of maximum concentration (C_{max}) and time to maximum concentration (t_{max}) after TJ-100 administration were used for the analysis. The area under the plasma concentration-time curve from zero to time t ($AUC_{0–last}$) was calculated from time 0 to the last detected time. Apparent elimination half-life ($t_{1/2}$) was calculated divided by $\log_e 2/k_e$ where k_e is the terminal elimination rate constant. C_{max} and $AUC_{0–last}$ are presented as the mean $\pm$ SD. The apparent elimination half-life and t_{max} are presented as the median with range.

Results

Profile of TU-100 ingredients and conjugates in portal and peripheral blood of rats

Firstly, we measured the concentrations of TU-100 ingredients and conjugates in the portal and peripheral blood of rats after a single oral administration of TU-100 (Fig. 1). Pharmacokinetic parameters are demonstrated in Tables 1 (portal blood) and 2 (peripheral blood). For the

estimation of the amounts of conjugates, which had been found mainly in sulfate- and glucuronide-forms in the previous paper (Iwabu et al. 2010), the samples were enzymatically digested with β-glucuronidase. The concentrations of HAS were immediately measured and after 30 min (portal blood) or 5 min (peripheral blood) observed to decrease reaching almost 0 within 5 h. The concentrations in portal and peripheral blood were similar suggesting HAS absorbed from the small intestine quickly entered systemic circulation. Comparable results were obtained for HBS, but at a lower range of concentrations (data not shown). The concentrations of Rb1 in the peripheral and portal blood are both in a similar range and increased gradually to a maximum at about 4 h, before slowly decreasing, although significant levels of Rb1 were still present even after 24 h. However, the concentrations of Rb1 were quite low. The concentrations of Rg1 were too low to obtain reliable data. The profiles of time-dependent changes in the concentrations of peripheral blood HAS, HBS, and Rb1 in rats are similar to those of human (Munekage et al. 2011). Treatment with β-glucuronidase had no effect on the concentrations of HAS, HBS, and Rb1 indicating these ingredients were unconjugated (data not shown) like those in human (Iwabu et al. 2010). Substantial amounts of 6G were detected in the portal blood but the majority was already conju-

Table 1. Pharmacokinetic parameters for components of TJ-100 (portal blood).

TU-100 components	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h × ng/mL)	AUC_{all} (h × ng/mL)
HAS	4.23 ± 1.08	0.292 ± 0.241	1060 ± 349	1400 ± 421	1400 ± 421
Rb1	N.C.[1]	6.00 ± 2.31	2.10 ± 1.13	28.7 ± 6.29	28.7 ± 6.29
6G	1.79[2]	1.31 ± 1.80	38.3 ± 31.8	59.7 ± 40.4	59.7 ± 40.4
Treated 6G	7.10 ± 1.71	0.292 ± 0.295	181 ± 21.9	1290 ± 696	1290 ± 696
6S	3.21 ± 3.30	0.333 ± 0.204	6.30 ± 4.20	7.44 ± 5.45	7.44 ± 5.45
Treated 6S	3.80 ± 0.982	0.292 ± 0.295	16.4 ± 9.50	40.6 ± 19.5	40.6 ± 19.5

Value represents mean ± SD ($n = 5$). HAS, hydroxy-α sanshool; Rb1, ginsenoside Rb$_1$; 6G, [6]-gingerol; 6S, [6]-shogaol; TU-100, daikenchuto.
[1]Not calculated due to insufficient time point data.
[2]The mean of two mice. For 1 mouse, $t_{1/2}$ could not be calculated.

Table 2. Pharmacokinetic parameters for components of TJ-100 (peripheral blood).

TU-100 components	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h × ng/mL)	AUC_{all} (h × ng/mL)
HAS	3.01 ± 2.90	0.283 ± 0.209	778 ± 276	951 ± 264	951 ± 264
Rb1	N.C.[1]	4.80 ± 1.79	2.12 ± 0.759	34.3 ± 12.0	34.3 ± 12.0
6G	N.C.[1]	0.466 ± 0.857	0.676 ± 0.479	0.974 ± 0.839	0.974 ± 0.839
Treated 6G	5.49[2]	0.08[2]	65.2[2]	236[2]	236[2]
6S	N.C.[3]	N.C.[3]	N.C.[3]	N.C.[3]	N.C.[3]
Treated 6S	2.95[2]	0.08[2]	7.11[2]	7.10[2]	7.10[2]

Value represents mean ± SD. $N = 5$ except for "Treated 6G" and "Treated 6S". HAS, hydroxy-α sanshool; Rb1, ginsenoside Rb$_1$; 6G, [6]-gingerol; 6S, [6]-shogaol; TU-100, daikenchuto.
[1]N.C.: Not calculated due to insufficient time point data.
[2]The mean of two mice.
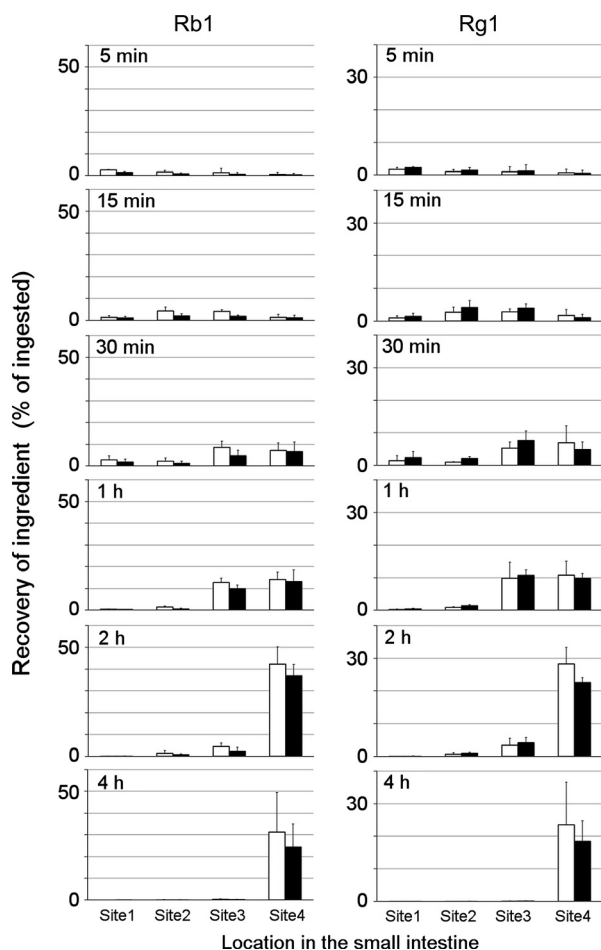[3]6S was not detected in any of five mice at any time points.

Figure 2. Time-dependent changes in intraluminal concentrations of ginseng ingredients Rb1 (left panels) and Rg1 (right panels) in various loci of rat small intestine. A small intestine was cut into four pieces (#1-4, from oral to anal side) at the indicated times after oral administration of TU-100 (1 g/kg BW), and the luminal concentrations of ingredients were determined as described in Materials and Methods. The percentage of the amount of each ingredient detected in various loci relative to that contained in the dose of administered TU-100 was calculated. Values represent means ± SD, $n = 3$. Closed columns represent values after β-glucuronidase treatment. Open columns represent values without treatment. Rb1, ginsenoside Rb$_1$; Rg1, ginsenoside Rg$_1$; TU-100, daikenchuto.

Figure 3. Time-dependent changes of intraluminal concentrations of ginger ingredients 6G (left panels) and 6S (right panels) in various loci of rat small intestine. A small intestine was cut into four pieces (#1–4, from oral to anal side) at the indicated times after oral administration of TU-100 (1 g/kg BW), and the luminal concentrations of ingredients were determined as described in Materials and Methods. The percentage of the amount of each ingredient detected in various loci relative to that contained in the dose of administered TU-100 was calculated. Values represent means ± SD, $n = 3$. Closed columns represent values after β-glucuronidase treatment. Open columns represent values without treatment. 6G, [6]-gingerol; 6S, [6]-shogaol; TU-100, daikenchuto;.

gated, suggesting conjugation occurred predominantly in the small intestine. The peak concentration of 6G and its conjugates in portal blood was about one-fifth of that of HAS, which is similar to the ratio of these compounds in the TU-100 drug substance, suggesting the absorption efficiency of HAS and 6G are similar. However, the concentration of 6G in the peripheral blood was considerably lower than that in portal blood indicating only a small portion of this ingredient entered systemic circulation via the liver. The concentrations of 6S and its conjugates are both below 20 ng/mL in the portal blood, which are
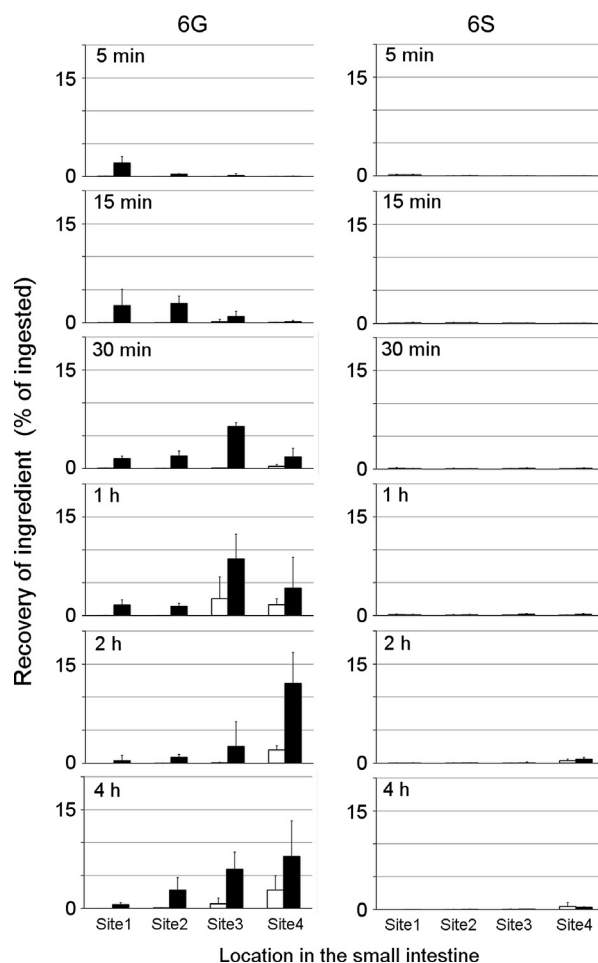
much lower than those of 6G and its conjugates in spite of the fact that larger amounts of 6S are contained in the TU-100 drug substance.

Time-dependent appearance and disappearance of TU-100 ingredients in various loci of small intestines of rats

Next, we collected intestinal contents of rats at various times after oral administration of TU-100. Small intestines were divided into quarters (sites 1, 2, 3, and 4 were

roughly estimated as duodenum, upper jejunum, lower jejunum, and ileum, respectively) and the concentrations of TU-100 ingredients in the contents of the respective portion were measured. The levels of HAS and HBS in all sites in all rats and at all time points were below 10 μg/site which correspond to <1% of the ingested amount (data not shown). The results of Rb1 and Rg1 are shown in Figure 2. Rb1 and RG1 appeared at 30 min and 1 h at the level of 10% of the ingested amount in site 3 and 4, and at 2 h and 4 h at nearly 50% (Rb1) and 30% (Rg1) in site 4. These data indicate that Rb1 and Rg1 were slowly discharged from the stomach and accumulated in the ileum. Treatment with β-glucuronidase did not have a significant effect on the amounts of Rb1 and Rg1, suggesting most of these ingredients were not conjugated. The behaviors of the two ginger ingredients, 6G and 6S are very dissimilar as shown in Figure 3. Unconjugated 6G was found in far smaller amount compared to that of conjugated 6G. At 5 and 15 min, conjugated 6G was found in sites 1 and 2 at the level of 2–3% of the ingested amount. At 30 min, 6G apparently disappeared from the small intestine in all sites (i.e., they are below 2% of the ingested amount in all sites). However, since 30 min, substantial amounts of conjugated 6G appeared in sites 3 and 4 (around 10% of the ingested amount in sum total for these two sites). At 2 h, the amount of 6G in site 4 was very much greater than compared to the other sites. After 1 h, unconjugated 6G at the level of 2–3% of the ingested amount appeared in sites 3 and/or 4. In contrast, 6S and its conjugates, were virtually absent in all sites of all rats, at all time points (Fig. 3). Table 3 summarizes the contents of TU-100 ingredients in the ileum of rat at 4 h.

TU-100 ingredients and conjugates in human ileal effluent

Human ileal effluent was collected for 4 h after TU-100 administration. The results are presented in Table 4. Trace amounts of HAS, HBS, and their conjugates were detected in the ileal contents. A considerable amount of another pepper ingredient GS was found to remain in the intestinal tract despite having previously been shown to be absorbed rapidly (Munekage et al. 2011), although the difference from the ingested amount was notable among individuals. As this ingredient has no hydroxyl group it is not supposed to be a substrate for conjugating enzymes. In contrast, the substantial amounts of ginsenosides were detected in the ileal effluent. For ginger ingredients, the contrasting results were obtained for shogaols and gingerols. Shogaols, both conjugated and unconjugated were detected only in trace amounts while substantial amounts of gingerol conjugates were detected in the ileum. No TU-100 ingredients/conjugates were

Table 3. Recovery of TU-100 ingredients found in the rat ileal (site 4) contents (%)[1].

	HAS		HBS		Rb1		Rg1		6G		10G		6S		10S	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
2 h																
Mean	0.02	0.02	0.01	0.00	42.30	36.98	28.31	22.59	2.01	12.05	1.51	9.92	0.35	0.60	0.19	0.15
SD	0.01	0.02	0.00	0.01	7.89	11.80	5.01	7.99	0.62	3.56	1.77	7.17	0.29	0.09	0.04	0.15
4 h																
Mean	0.02	0.04	0.02	0.01	31.18	24.45	23.49	18.50	2.79	7.90	3.51	2.93	0.46	0.34	0.07	0.09
SD	0.03	0.01	0.01	0.02	18.29	10.57	13.07	6.25	2.17	5.37	2.27	1.99	0.60	0.11	0.07	0.09

Treated/untreated refer to the treatment with -glucuronidase. 6G, [6]-gingerol; 10G, [10]-gingerol; HAS, hydroxy- sanshool; HBS, hydroxy- sanshool; Rb1, ginsenoside Rb1; Rg1, ginsenoside Rg1; TU-100, daikenchuto.
[1]Value represents (the amount of each ingredient in the ileal contents)/(the amount of the ingredient contained in ingested TU-100) 100, $n = 3$.

Table 4. Recovery of TU-100 ingredients found in the human ileal fluid.

Herbs	Japanese pepper			Ginseng		Ginger							
Ingredients	HAS	HBS	GS	Rb1	Rg1	6G		10G		6S		10S	
Amount contained in ingested TU-100 (ng/5 g TU-100)	2,800,000	505,000	204,000	685,000	685,000	558,000		176,200		760,000		197,500	
β-glucuronidase treatment	−	−	−	−	−	−	+	−	+	−	+	−	+
Amount in the ileum (ng/ileum)													
Min	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q	B.L.Q
Max	10,700	B.L.Q	13,9000	53,1000	78,1000	3870	76,100	2040	16,100	1250	5710	5020	6970
Median	B.L.Q	B.L.Q	8380	405,000	62,7000	B.L.Q	41,200	B.L.Q	7630	B.L.Q	3000	B.L.Q	1550
% of ingested TU-100[1]													
Max	0.4	B.L.Q	68.1	77.5	114.0	B.L.Q	13.6	B.L.Q	9.1	B.L.Q	0.8	B.L.Q	3.5
Median	B.L.Q	B.L.Q	4.1	59.1	91.5	B.L.Q	7.4	B.L.Q	4.3	B.L.Q	0.4	B.L.Q	0.8

B.L.Q. means "below the limit of quantitation". 10G, [10]-gingerol; 10S, [10]- shogaol; 6G, [6]-gingerol; 6S, [6]-shogaol; GS, γ-sanshool; HAS, hydroxy-α sanshool; HBS, hydroxy-β sanshool; Rb1, ginsenoside Rb$_1$; Rg1, ginsenoside Rg$_1$; TU-100, daikenchuto.
[1]Value represents (the amount of each ingredient in the ileal fluid)/(the amount of the ingredient contained in ingested TU-100) × 100.

virtually detected in the ileal effluents collected before TU-100 administration.

Discussion

Various chemical compounds have been isolated from herbal plants and their pharmacological activities investigated over the past many decades. However, the results of such studies may mislead the investigation of mechanisms of action of herbal prescriptions due to the lack of ADME data. For examples, a vast large number of studies have demonstrated the potent antioxidant activity of various flavonoids (Terao 2009; Landete 2012). However, the pharmacological effects of the herbal medicines containing such flavonoids are not always explained by this activity, as most of these flavonoids are present in the blood as biologically inactive conjugated forms (Hollman et al. 2011; Hollman 2014). In order to investigate the mechanisms of action of herbal medicines, it is critically important to clarify the absorption of their pharmacological active components.

It is well recognized that many herbal medicines exert their pharmacological activity only after conversion of their ingredients to bioactive forms by the action of the gastrointestinal contents. The previous PK studies for TU-100 (Munekage et al. 2011, 2013) revealed that the major ingredients derived from pepper (hydroxysanshools) are absorbed rapidly and efficiently into the blood stream while ginsenosides from ginseng are absorbed slowly and only in small amounts. The conversion of ginsenosides to their metabolites by intestinal bacteria and succeeding absorption have been suggested to be critical for the various pharmacological effects of ginseng (Lu et al. 2009; Yang et al.

2014). The major ingredients of ginger, shogaols, and gingerols were detected in the blood within 15 min after TU-100 administration, but, their blood concentrations were at a trace level. HAS, shogaols, and gingerols in TU-100 have been shown to exert some of their pharmacological activities via stimulation of TRPA1 (Kono et al. 2013), which is present in the epithelial and enterochromaffin cells in the luminal surface of intestinal epithelium. Thus, although the blood concentrations of ginseng and ginger ingredients are low, the intraluminal content of these ingredients might be more important for the exertion of their pharmacological activities.

Japanese pepper and ginseng ingredients

In the present study, only trace amounts of HAS, HBS, and their conjugates have been detected in the rat and human ileal contents. This may be reasonable because HAS and HBS have been shown to be absorbed very rapidly and efficiently. Administration of HAS has been reported to ameliorate postoperative ileus (Tokita et al. 2007) and postoperative obstructive adhesion (Tokita et al. 2011) in rats and a possible involvement of ion channels such as TRPV1, TRPA1 (Koo et al. 2007; Riera et al. 2009), and KCNK (Bautista et al. 2008) in the intestinal tissues has been suggested. The primary route of supply of HAS and HBS to the intestine is thus suggested to be via systemic circulation. In contrast, a considerable amount of Rb1 and Rg1, 59.1% and 91.5% (median) of ingested amount in human, 42.3% and 28.3% (mean) in rat, respectively, have been found in the ileal contents. This is in accordance with the previous reports obtained from animal experiments, which indicate that significant amounts of ginsenosides remain in the intestine

(Hasegawa 2004). The present data are, as far as we know, the first direct demonstration that a similar phenomenon occurs in human. Ginsenosides are then transported into the colon, and encounter colonic bacteria. Some human enteric bacteria are known to have the ability to deglycosilate ginsenosides to lipophilic metabolites such as compound K(CK) and ginosenosides Rh, and F1 (Shen et al. 2013). CK has been reported to have antitumor, anti-inflammatory, antiallergic and antistress actions (Wakabayashi et al. 1997; Bae et al. 2002; Park et al. 2004; Tachikawa and Kudo 2004). Ginsenoside Rh_1 and F1 have also been suggested to have some biological activities such as inhibition of nuclear factor κ B (Dong et al. 2014) and β-site secretase 1 (Karpagam et al. 2013). Thus, the pharmacological effects of ginseng are largely influenced by these bacterial metabolites.

Gingerols

The journey of ginger ingredients (gingerols and shogaols) after intake of TU-100 is more complicated. Both gingerols and shogaols were detected in the blood within 15 min after administration (Munekage et al. 2011), however, the concentrations were only of a trace amount. About ten times higher concentrations of 6G and its conjugates were detected in the portal blood in rats suggesting only a small portion of absorbed 6G/6G conjugates entered systemic circulation. Human ileal effluents as well as rat ileal contents contain only a trace amount of unconjugated gingerols but abundant gingerol conjugates. Furthermore, the higher concentrations of 6G conjugates over unconjugated 6G were observed both in peripheral and portal blood in rats. These data have led us to the following hypothesis: (1) 6G is conjugated mainly in the small intestine. (2) After absorption and conjugation, 6G, at least partly, returns to the ileum via bile secretion.

Shogaols

6S and their conjugates were virtually absent in ileal contents. The portal blood contains 6S and its conjugates at levels of approximately one tenth of those of 6G/6G conjugates, despite the fact that the amounts of 6S in the original drug were larger than those of 6G. We have previously reported that, when isotope-labeled 6S was administered orally, about 90% of the isotope labels were recovered in the urine suggesting 6S may be absorbed efficiently (Asami et al. 2010). It has been reported that absorbed shogaols (including 6S) were rapidly converted into a variety of forms in mice and human (Chen et al. 2012). Considering the paper reporting that shogaols might accumulate in the intestinal epithelial cells (Nievergelt et al. 2010), it is plausible that rapidly absorbed shogaols may be transformed to various forms in the
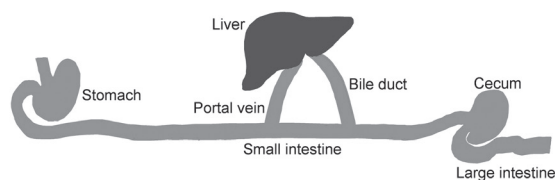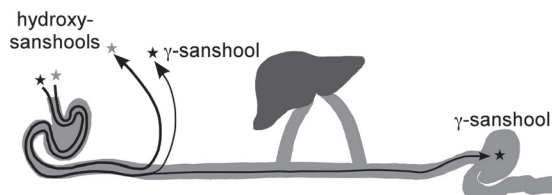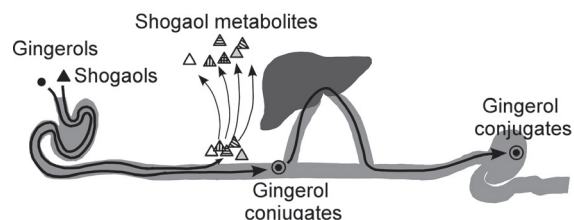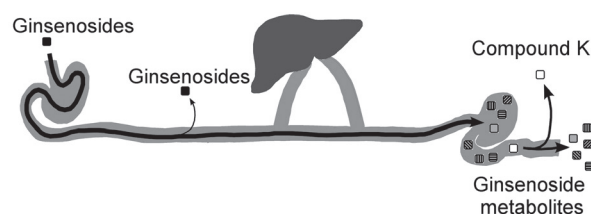

Figure 4. The diagrammatic hypothesis of the journey of TU-100 ingredients after ingestion. TU-100, daikenchuto.

intestine and transported to the portal and thereafter peripheral blood. In this case, the present approach cannot reveal the whereabouts of such metabolites because it can detect only 6S and its glucuronide and sulfate conjugates.

The fate of TU-100 ingredients in the intestines, liver and systemic circulation

The diagrammatic hypothesis of the fate of TU-100 ingredients after ingestion is shown in Figure 4. Together with known pharmacological activities, it is summarized as follows. After TU-100 ingestion, HAS, HBS, shogaols, and gingerols are rapidly absorbed in the small intestine. It is not plausible for the ingredients to be absorbed from the stomach because a direct in situ injection of TU-100 ingredient into cardiac and pylorus

ligated stomach had no effect on gastric concentrations of these ingredients (unpubl. obs., HK, JW, MY and TK). They all have an agonistic activity to TRPA1 and TRPV1 (Riera et al. 2009), and so may stimulate TRPA1 in intestinal epithelial cells and entrenchromaffin cells, enhancing intestinal blood flow and motility, respectively. In addition, they may also stimulate TRPV1/TRPA1 in the enteric and sensory nerves in the gut wall. Absorbed HAS and HBS are rapidly distributed to the whole body via systemic circulation (Fig. 4B). HAS and HBS are known to inhibit certain KCNK channels which might be involved in the intestinal motility and visceral pain perception. We have observed that intraperitoneal as well as oral administration of HAS rapidly enhances defecation via stimulation of colonic motility (Kubota et al. 2015). After intraluminal stimulation of TRPA1 in intestinal epithelium, absorbed shogaols are rapidly converted to various forms presumably in the small intestine (Fig. 4C). Some of these metabolites have a biological activity such as antitumor (Chen et al. 2012), however, it is unclear whether these metabolites play a role in the TU-100 pharmacology. Gingerols may also stimulate intestinal TRPV1 and TRPA1, and be conjugated mainly in the small intestine, as well as absorbed and transported via the portal vein. Some of them might return to the ileum in the bile (Fig. 4C). It is an intriguing question whether gingerol conjugates are reactivated by encountering colonic microflora, which are known to have deconjugation activities for various conjugated compounds (Crozier et al. 2010). Further, the presence of high concentrations of HAS, HBS, 6G/6G conjugates, and conceivably 6S metabolites in the portal vein may be related to TU-100′s beneficial effects on liver (Ogasawara et al. 2008; Narita et al. 2009). Ginseng ingredients such as ginsenosides are absorbed very slowly and the majority of them are transported to the terminal ileum (Fig. 4D). Subsequent encounter with colonic microflora probably results in the conversion of ginsenosides to range of compounds including compound K, ginsenosides Rh_1 and F1, which have various pharmacological activities.

In conclusion, we have measured the concentrations of TU-100 ingredients and conjugates in the ileum of humans and rats. By a combination of the findings from previous plasma PK studies, and the present study, we have proposed a more comprehensive view of TU-100′s pharmacological actions in which multiple ingredients/metabolites are supposed to exert their action toward multiple sites over different time schedules. Although further study is still required to fully elucidate the mechanisms of the pharmacological action of TU-100, the present study lays the basis for these future investigations.

Author Contributions

Disclosure

References

Asami A, Shimada T, Mizuhara Y, Asano T, Takeda S, Aburada T, et al. (2010). Pharmacokinetics of [6]-shogaol, a pungent ingredient of Zingiber officinale Roscoe (Part I). J Nat Med 64: 281–287.

Bae EA, Choo MK, Park EK, Park SY, Shin HY, Kim DH (2002). Metabolism of ginsenoside R(c) by human intestinal bacteria and its related antiallergic activity. Biol Pharm Bull 25: 743–747.

Bautista DM, Sigal YM, Milstein AD, Garrison JL, Zorn JA, Tsuruda PR, et al. (2008). Pungent agents from Szechuan peppers excite sensory neurons by inhibiting two-pore potassium channels. Nat Neurosci 11: 772–779.

Chen H, Lv L, Soroka D, Warin RF, Parks TA, Hu Y, et al. (2012). Metabolism of [6]-shogaol in mice and in cancer cells. Drug Metab Dispos 40: 742–753.

Crozier A, Del Rio D, Clifford MN (2010). Bioavailability of dietary flavonoids and phenolic compounds. Mol Aspects Med 31: 446–467.

Dong L, Cheng B, Luo Y, Zhang N, Duan H, Jiang M, et al. (2014). Identification of nuclear factor-kappaB inhibitors and beta2 adrenergic receptor agonists in Chinese medicinal preparation Fuzilizhong pills using UPLC with quadrupole time-of-flight MS. Phytochem Anal 25: 113–121.

Hasegawa H (2004). Proof of the mysterious efficacy of ginseng: basic and clinical trials: metabolic activation of ginsenoside: deglycosylation by intestinal bacteria and esterification with fatty acid. J Pharmacol Sci 95: 153–157.

Hollman PC (2014). Unravelling of the health effects of polyphenols is a complex puzzle complicated by metabolism. Arch Biochem Biophys 559: 100–105.

Hollman PC, Cassidy A, Comte B, Heinonen M, Richelle M, Richling E, et al. (2011). The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. J Nutr 141: 989S–1009S.

Iturrino J, Camilleri M, Wong BS, Linker Nord SJ, Burton D, Zinsmeister AR (2013). Randomised clinical trial: the effects of daikenchuto, TU-100, on gastrointestinal and colonic transit, anorectal and bowel function in female patients with functional constipation. Aliment Pharmacol Ther 37: 776–785.

Iwabu J, Watanabe J, Hirakura K, Ozaki Y, Hanazaki K (2010). Profiling of the compounds absorbed in human plasma and urine after oral administration of a traditional Japanese (Kampo) medicine, daikenchuto. Drug Metab Dispos 38: 2040–2048.

Karpagam V, Sathishkumar N, Sathiyamoorthy S, Rasappan P, Shila S, Kim YJ, et al. (2013). Identification of BACE1 inhibitors from Panax ginseng saponins-An Insilco approach. Comput Biol Med 43: 1037–1044.

Kim YS, Kim JJ, Cho KH, Jung WS, Moon SK, Park EK, et al. (2008). Biotransformation of ginsenoside Rb1, crocin, amygdalin, geniposide, puerarin, ginsenoside Re, hesperidin, poncirin, glycyrrhizin, and baicalin by human fecal microflora and its relation to cytotoxicity against tumor cells. J Microbiol Biotechnol 18: 1109–1114.

Kono T, Kanematsu T, Kitajima M (2009). Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines: is it time yet? Surgery 146: 837–840.

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

Koo JY, Jang Y, Cho H, Lee CH, Jang KH, Chang YH, et al. (2007). Hydroxy-alpha-sanshool activates TRPV1 and TRPA1 in sensory neurons. Eur J Neuorsci 26: 1139–1147.

Kubota K, Ohtake N, Ohbuchi K, Mase A, Imamura S, Sudo Y, et al. (2015). Hydroxy-alpha sanshool induces colonic motor activity in rat proximal colon: a possible involvement of KCNK9. Am J Physiol Gastrointest Liver Physiol 308: G579–G590.

Landete JM (2012). Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. Crit Rev Food Sci Nutr 52: 936–948.

Lee DS, Kim YS, Ko CN, Cho KH, Bae HS, Lee KS, et al. (2002). Fecal metabolic activities of herbal components to bioactive compounds. Arch Pharmacal Res 25: 165–169.

Lu JM, Yao Q, Chen C (2009). Ginseng compounds: an update on their molecular mechanisms and medical applications. Curr Vasc Pharmacol 7: 293–302.

Manabe N, Camilleri M, Rao A, Wong BS, Burton D, Busciglio I, et al. (2010). Effect of daikenchuto (TU-100) on gastrointestinal and colonic transit in humans. Am J Physiol Gastrointest Liver Physiol 298: G970–G975.

Munekage M, Kitagawa H, Ichikawa K, Watanabe J, Aoki K, Kono T, et al. (2011). Pharmacokinetics of daikenchuto, a traditional Japanese medicine (Kampo) after single oral administration to healthy Japanese volunteers. Drug Metab Dispos 39: 1784–1788.

Munekage M, Ichikawa K, Kitagawa H, Ishihara K, Uehara H, Watanabe J, et al. (2013). Population pharmacokinetic analysis of daikenchuto, a traditional Japanese medicine (Kampo) in Japanese and US health volunteers. Drug Metab Dispos 41: 1256–1263.

Narita M, Hatano E, Tamaki N, Yamanaka K, Yanagida A, Nagata H, et al. (2009). Dai-kenchu-to attenuates rat sinusoidal obstruction syndrome by inhibiting the accumulation of neutrophils in the liver. J Gastroenterol Hepatol 24: 1051–1057.

Nievergelt A, Huonker P, Schoop R, Altmann KH, Gertsch J (2010). Identification of serotonin 5-HT1A receptor partial agonists in ginger. Bioorg Med Chem 18: 3345–3351.

Nozawa K, Kawabata-Shoda E, Doihara H, Kojima R, Okada H, Mochizuki S, et al. (2009). TRPA1 regulates gastrointestinal motility through serotonin release from enterochromaffin cells. Proc Natl Acad Sci USA 106: 3408–3413.

Ogasawara T, Morine Y, Ikemoto T, Imura S, Fujii M, Soejima Y, et al. (2008). Influence of Dai-kenchu-to (DKT) on human portal blood flow. Hepatogastroenterology 55: 574–577.

Okada K, Kawai M, Uesaka K, Kodera Y, Nagano H, Murakami Y, et al. (2013). Effect of Daikenchuto (TJ-100) on postoperative bowel motility and on prevention of paralytic ileus after pancreaticoduodenectomy: a multicenter, randomized, placebo-controlled phase II trial (the JAPAN-PD study). Jpn J Clin Oncol 43: 436–438.

Park EK, Choo MK, Han MJ, Kim DH (2004). Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities. Int Arch Allergy Immunol 133: 113–120.

Park HY, Choi HD, Eom H, Choi I (2013). Enzymatic modification enhances the protective activity of citrus flavonoids against alcohol-induced liver disease. Food Chem 139: 231–240.

Riera CE, Menozzi-Smarrito C, Affolter M, Michlig S, Munari C, Robert F, et al. (2009). Compounds from Sichuan and Melegueta peppers activate, covalently and non-covalently, TRPA1 and TRPV1 channels. Br J Pharmacol 157: 1398–1409.

Shen H, Leung WI, Ruan JQ, Li SL, Lei JP, Wang YT, et al. (2013). Biotransformation of ginsenoside Rb1 via the gypenoside pathway by human gut bacteria. Chin Med 8: 22.

Shimada M, Morine Y, Nagano H, Hatano E, Kaiho T, Miyazaki M, et al. (2015). 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: 95–104.

Tachikawa E, Kudo K (2004). Proof of the mysterious efficacy of ginseng: basic and clinical trials: suppression of adrenal medullary function in vitro by ginseng. J Pharmacol Sci 95: 140–144.

Terao J (2009). Dietary flavonoids as antioxidants. Forum Nutr 61: 87–94.

Tokita Y, Yuzurihara M, Sakaguchi M, Satoh K, Kase Y (2007). The pharmacological effects of Daikenchuto, a traditional herbal medicine, on delayed gastrointestinal transit in rat postoperative ileus. J Pharmacol Sci 104: 303–310.

Tokita Y, Yamamoto M, Satoh K, Nishiyama M, Iizuka S, Imamura S, et al. (2011). Possible involvement of the transient receptor potential vanilloid type 1 channel in postoperative adhesive obstruction and its prevention by a Kampo (traditional Japanese) medicine, daikenchuto. J Pharmacol Sci 115: 75–83.

Wachtel-Galor S, Benzie IFF (2011). Herbal medicine: an introduction to its history, usage, regulation, current trends, and research needs. *in* I. F. F. Benzie and S. Wachtel-Galor, eds. Herbal medicine: biomolecular and clinical aspects. Llc, Boca Raton, FL, 2nd edition, Chapter 1.

Wakabayashi C, Hasegawa H, Murata J, Saiki I (1997). In vivo antimetastatic action of ginseng protopanaxadiol saponins is based on their intestinal bacterial metabolites after oral administration. Oncol Res 9: 411–417.

Yamamoto M, Miura N, Ohtake N, Amagaya S, Ishige A, Sasaki H, et al. (2000). Genipin, a metabolite derived from the herbal medicine Inchin-ko-to, and suppression of Fas-induced lethal liver apoptosis in mice. Gastroenterology 118: 380–389.

Yang XD, Yang YY, Ouyang DS, Yang GP (2015). A review of biotransformation and pharmacology of ginsenoside compound K. Fitoterapia 100: 208–20.

Yim JS, Kim YS, Moon SK, Cho KH, Bae HS, Kim JJ, et al. (2004). Metabolic activities of ginsenoside Rb1, baicalin, glycyrrhizin and geniposide to their bioactive compounds by human intestinal microflora. Biol Pharm Bull 27: 1580–1583.

Zhang AL, Changli Xue C, Fong HHS (2011). Integration of Herbal Medicine into Evidence-Based Clinical Practice: Current Status and Issues. *in* I. F. F. Benzie and S. Wachtel-Galor, eds. Herbal medicine: biomolecular and clinical aspects. Llc, Boca Raton, FL. 2nd edition, chapter 22