Journal of Surgical Research (2008) 150(1):78-84.

Colonic Vascular Conductance Increased by Daikenchuto via Calcitonin Gene-Related Peptide and Receptor-Activity Modifying Protein 1

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Title: Colonic vascular conductance increased by Daikenchuto via calcitonin gene-related peptide and receptor-activity modifying protein 1

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ABSTRACT

Background. Daikencyuto (DKT) is a traditional Japanese medicine (Kampo) and is a mixture of extract powders from dried Japanese pepper, processed ginger, ginseng radix and maltose powder, and has been used as the treatment of paralytic ileus. DKT may increase gastrointestinal motility by an up-regulation of the calcitonin gene-related peptide (CGRP). CGRP is also the most powerful vasoactive substance. In the present study, we investigated whether DKT has any effect on the colonic blood flow (CBF) in rats.

Materials and Methods. Experiments were performed on fasted anesthetized and artificially ventilated Wistar rats. Systemic mean arterial blood pressure (MAP) and heart rate (HR) were recorded. Red blood cell flux in CBF was measured using non-contact laser tissue blood flowmetry, and colonic vascular conductance (CVC) was calculated as the ratio of flux to MAP. We examined four key physiological mechanisms underlying the response using blocker drugs: CGRP1 receptor blocker ($CGRP_{8-37}$), nitric oxide synthase inhibitor (L-NAME), vasoactive intestinal polypeptide (VIP) receptor blocker ([4-Cl-DPhe6, Leu17]-VIP), and substance P (SP) receptor blocker (spantide). RT-PCR was employed for the detection of mRNA of calcitonin receptor-like receptor (CRLR), receptor-activity modifying protein 1 (RAMP1), the component of CGRP 1

receptor and CGRP. After laparotomy, a cannula was inserted into the proximal colon to administer the DKT and to measure CVC at the distal colon.

Results. Intracolonal administration of DKT (10, 100 and 300 mg/kg) increased CVC (basal CVC, 0.10 mL/mmHg) from the first 15-min observation period (0.14, 0.17 and 0.17 mL/mmHg , respectively) and with peak response at either 45 min (0.17 mL/mmHg by 10 mg/kg), or at 75 min and 60 min (0.23 and 0.21 mL/mmHg by 100 mg/kg and 300 mg/kg, respectively). $CGRP_{8-37}$ completely abolished the DKT-induced hyperemia, whereas L-NAME partially attenuated the DKT-induced hyperemia. [4-Cl-DPhe6, Leu17]-VIP and spantide did not affect the hyperemia. Japanese pepper significantly increased CVC at 45 min or later, whereas ginseng radix only showed significant increase at 15 min. RT-PCR showed that mRNA for CRLR, RAMP1 and CGRP were expressed in rat colon and upregulated by DKT.

Conclusions. The present study demonstrated that DKT increased CVC which was mainly mediated by CGRP and its receptor components.

Keywords: Daikenchuto; colonic blood flow; calcitonin gene-related peptide; calcitonin receptor-like receptor; receptor-activity modifying protein 1; vascular conductance; Japanese pepper; ginseng radix.

INTRODUCTION

Daikenchuto (DKT) is a traditional Japanese medicine (Kampo) and is a mixture of extract powders from dried Japanese pepper, processed ginger, ginseng radix and maltose powder, and has been used for the improvement of gastrointestinal motility, postoperative adhesion and paralytic ileus after abdominal surgery and its clinical efficacy is well established 1-5). DKT may increase gastrointestinal motility by an up-regulation of the calcitonin gene-related peptide (CGRP) as well as acetylcholine release and plasma motilin 6-8).

CGRP is also the most powerful vasodilator and its vasodilatory effects following stimulated-release from the extrinsic sensory innervation is considered to serve as an important protective mechanism for maintaining mucosal integrity 9-12). Because blood flow has to meet the relatively high metabolic needs of the gastrointestinal tract as well as provide both valuable buffering and a pathway for removal of toxins that may have entered tissue 13). Therefore, maintaining or increasing blood flow is thought to be a central element in protecting the gastrointestinal tract 14).

It has been demonstrated that calcitonin receptor-like receptor (CRLR), a receptor with seven transmembrane domains, matures to a Gs-protein-coupled CGRP receptor when co-expressed with single transmembrane domain receptor activity modifying

protein 1 (RAMP1) 15, 16). Therefore, to confirm the existence of a CGRP1 receptor, it is necessary to determine the existence of not only CRLR but also RAMP1.

In the present study, we investigated whether DKT has any effect on the colonic blood flow in rats and which component of DKT is responsible for DKT-induced hyperemia. We also investigated the mechanisms of DKT-induced hyperemia in colon by using blocker drugs pharmacologically and by using RT-PCR for presence of CGRP and its receptor components.

MATERIALS AND METHODS

Ethical approval of the experimental procedures used in this study was obtained from the Asahiakwa Medical College Animal Care and Use Committee. All animal procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Chemicals

DKT and each component of DKT (Japanese pepper, processed ginger, ginseng radix and maltose powder) were obtained from Tsumura Co. (Tokyo, Japan). CGRP (8–37), spantide, N^GNitro-L-arginine methyl ester (L-NAME) and Vasoactive intestinal polypeptide (VIP) receptor antagonist [4-Cl-DPhe6, Leu17]-VIP were obtained from Sigma Chemical Co.

Animal preparation and Measurement of colonic blood flow

Male Sprague–Dawley rats (Charles River Japan Inc., Tsukuba, Japan) weighing 250–315 g were fasted overnight but given free access to water. After urethane (900 mg/kg i.p.), α-chloralose (60 mg/kg i.p.) and butorphanol (2 mg/kg i.m.) anesthesia, a tracheal y-shaped cannula was inserted through the small section of the trachea to ventilate artificially (tidal volume; 8-10 mL/kg, respiratory rate; 60 breaths/min) in

order to maintain a near-normal functional condition by a mechanical respirator (Respirator model SN 480-7, Shinano Co., Tokyo, Japan). The right femoral artery was cannulated and connected to a transducer (Colin BP508, Nihon Colin Co., Tokyo, Japan) to monitor systemic arterial blood pressure (AP) and heart rate (HR). Electromyograms (EM) were recorded with bipolar intramuscular needle electrodes. Body temperature was maintained at 37 ± 0.5 °C by a warming plate. After exposing the colon by a midline laparotomy, the cannula (25 G) was inserted via cecum into the proximal colon to facilitate injection of the drugs and to fix the proximal colon, the part of the distal colon (1 cm at length) was placed on wet absorbent cotton and filled with warmed physiological saline, then covered with saran wrap to keep warm, and prevent the tissue from becoming dehydrated.

Colonic blood flow was measured by non-contact laser tissue blood flowmetry (LaserMed, ALF21N, Advance Co., Tokyo, Japan) which is a well-characterized technique for the measurement of blood flow in the intestine. A fiberoptic probe was positioned against the surface (4 mm above) of the distal colon and secured inside the animal to prevent any movement of the tip of the probe during the course of the experiments. The penetration depth of the laser Doppler flowmetry system was 1 mm. Blood flow, AP, HR, and EM were continuously monitored on PowerLab data-sampling

unit (PowerLab8000, ADInstruments Co., Tokyo, Japan) and recorded on PC computer (Toshiba, Tokyo, Japan). Mean colonic vascular conductance (CVC) was calculated as the quotient of mean blood flow divided by mean AP and was expressed as mL/mmHg. CVC was used as a reliable index of colonic blood flow 17-20).

Experimental protocol

After an equilibration period of 45 min, each dose of DKT (10, 100, or 300 mg/kg), vehicle for DKT (maltose powder 800 mg/kg), or each component of DKT (Japanese pepper 20 mg/kg, processed ginger 50 mg/kg or ginseng radix 30 mg/kg), equal to the concentration in DKT (100 mg/kg), was administered intracolonically and its effect on CVC was monitored continuously for another 90 min. To determine the effects of antagonists on DKT-induced hyperemia, CGRP receptor antagonist CGRP (8–37) (45 μg/kg), substance P (SP) receptor antagonist spantide (100 μg/kg) and nitric oxide (NO) antagonist, L-NAME (200 μg/kg) were injected intravenously 15 min before the administration of the test drugs. VIP receptor antagonist [4-Cl-DPhe6, Leu17]-VIP (0.25 μg/kg/min) was infused into the femoral vein throughout the duration of the experiment. The doses of the antagonists were selected based on previous studies21-26).

RNA Extraction and RT-PCR

Total RNA was extracted from rat distal colon either vehicle for DKT or DKT administration (100 mg/kg) and homogenized colon tissues by Sepasol-RNA 1 (Nacalai Tesque Co., Kyoto, Japan) according to the manufacture's protocols. The quality and the quantity of the RNA were assessed at A 260/280, and all samples showed absorbency ratios ranging between 1.6 and 2.0. For reverse transcription (RT), 2 μg of total RNA was mixed with 3.0 nmol of random primer, 200 μM dNTP solution, and 10 U of AMV reverse transcriptase in the presence of 5 U of RNase inhibitor and placed in a thermal cycler for one cycle at 28 °C for 15 min, 42 °C for 30 min, 99 °C for 5 min, and 4 °C for 5 min.

The polymerase chain reaction (PCR) utilized primers specific to CGRP, CRLR, RAMP1 and glyceraldehydes 3-phosphate dehydrogenase (GAPDH) genes which were designed based on published cDNA sequences (GenBank). Details of the primers used are summarized in Table 1. The predicted sizes of the PCR products between two primers were 104 bp for CGRP, 504 bp for CRLR, 230 bp for RAMP1, and 249 bp for GAPDH, respectively, and which were used from previously reported 27, 28).

Commercially available one-step RT-PCR systems TaKaRa RNA PCR kit (AMV) Ver.3.0 (TakaRa-Biomedicals, Tokyo, Japan) was used, and all technical procedures

were undertaken as specified by the manufacturer. It comprised a total volume of 25 µl consisting of 12.5 µl 2X buffer II, 5 mM $MgCl_2$, 0.5 µM of PE1 et PE2, 1 mM of each dNTPs, 0.4 U of RNAsin (40 U/µl), 0.5 U of AMV Rtase XL (5 U/µl), and 0.5 U of AMV-Optimized taq. The RT-amplification was carried out as follows: 30 min at 50 °C for the reverse transcription, denaturation for 5 min at 85 °C and then a succession of 40 cycles as follows: 30 s at 85 °C, 40 s at 65 °C, and 90 s at 72 °C. Amplification took place after 5 min at 72 °C.

The relative concentrations of CGRP, CRLR and RAMP1 mRNA were determined by densitometric analysis of the ethidium bromide-stained reaction products using Sigma Gel analysis system. The results were expressed as the ratio of the densitometric reading for CGRP, CRLR or RAMP1 to GAPDH mRNA from the same tissue.

Statistical analysis

All data are expressed as mean ± SEM. Comparisons between multiple groups were made by two-way analysis of variance (ANOVA) followed by Duncan's post hoc test. Values of $p<0.05$ were considered significant.

RESULTS

Effects of DKT on CVC

In rats treated with vehicle for DKT, CVC did not change significantly during the 90 min period compared to the baseline, although there was a slight tendency for it to decrease (Figure 1). Intracolonal administration of DKT (10, 100 and 300 mg/kg) increased basal CVC (0.10 ± 0.01) from the first 15-min observation period (0.14 ± 0.01, 0.17 ± 0.01, 0.17 ± 0.01 mL/mmHg, respectively) and with peak response at either 45 min (0.17 ± 0.02 mL/mmHg by 10 mg/kg), or 75 min and 60 min (0.23 ± 0.03 mL/mmHg and 0.21 ± 0.02 mL/mmHg by 100 mg/kg and 300 mg/kg, respectively), and returned to the 15-min level by 90 min after DKT administration (10, 100, 300 mg/kg, 0.13 ± 0.02, 0.16 ± 0.02, 0.16 ± 0.02 mL/mmHg, respectively) (Figure 1). The maximum effect of DKT was observed in the 100 mg/kg group with peak response at 75 min which did not change significantly compared with the 300 mg/kg group (Figure 1). The time course study of the CVC after DKT administration showed very similar patterns among the three different doses of DKT-treated groups at each time during the experimental period (Figure 1). The time course study of mean AP showed very similar patterns among the experimental groups. Mean AP was not affected by dose differences during the experimental period before and the 0–90 min period after either DKT,

although in the 100 mg/kg group was a slight lower than 100 mg/kg group at each observed time or vehicle administration (Table 2).

Effects of antagonists on DKT-induced hyperemia

DKT (100mg/kg) significantly increased CVC. CGRP receptor antagonist CGRP (8–37) completely abolished DKT-induced hyperemia, whereas L-NAME partially attenuated the hyperemic response (Figure 2a). However, VIP receptor antagonist [4-Cl-DPhe6, Leu17]-VIP and substance P (SP) receptor antagonist spantide did not affect the hyperemic response (Figure 2b).

Effects of each component of DKT on CVC

Japanese pepper, processed ginger, ginseng radix and maltose powder are components of DKT and each dose is an equal to the concentration in DKT (100 mg/kg). Japanese pepper significantly increased CVC at 45 min or later, whereas ginseng radix only showed significant increase at 15 min (Figure 3). However, ginger and maltose powder did not showed significant increase during the experimental period before and the 0–90 min period (Figure 3).

DKT-induced changes in CGRP, CRLR and RAMP1 mRNA

Agarose gel electrophoresis of the RT-PCR products from the rat colon with the primers for CGRP, CRLR, RAMP1, and GAPDH showed single bands of 102 bp, 504 bp, 230 bp, and 249 bp, respectively (Figure 4a). Sequence analysis of these RT-PCR products revealed that they were identical to each cDNA from the libraries.

Semi-quantification of the CGRP, CRLR and RAMP1 mRNA corrected by mRNA for GAPDH revealed significant increase of CGRP, CRLR and RAMP1 transcripts in the rat colon 45 min after DKT administration, compared to those transcripts in the control rat colon (Figure 4b).

DISCUSSION

The present study first demonstrated that intracolonal administration of DKT increased CVC in rats. Moreover, this study showed that DKT did not affect mean AP, even when DKT was administered at the highest dose. This finding suggests that the increase in CVC by DKT results from a local vasodilator effect in intestinal blood vessels rather than being secondary to changes in systemic blood pressure.

Nervous mechanisms are important for the regulation of gastrointestinal blood flow. A number of neuropeptides such as CGRP, VIP and SP, have been localized immunohistochemically in sensory nerves innervating various viscera, including the gastrointestinal tract 29-33). Exogenous application of these peptides has been shown to dilate arterioles 34-36). The present study showed that the CGRP receptor antagonist, CGRP (8–37), completely abolished DKT-induced hyperemia, whereas the VIP receptor antagonist, [4-Cl-DPhe6, Leu17]-VIP, and SP receptor antagonist did not attenuate the hyperemic response. The pharmacological study suggests that DKT-induced hyperemia of the rat colon is mediated by CGRP, but neither by VIP nor SP release. The pharmacological basis for the DKT effect on CVC in the present study is supported by a previous report. Nagano et al. have reported that DKT causes increase in plasma CGRP after a single oral administration in healthy humans and all subjects feel a warm

sensation in their abdomens 37).

This observation also suggests that the hyperemic response to DKT mostly results from the upregulation of CGRP release and/or the upregulation of CGRP receptor. The results from the present study by RT-PCR revealed that DKT had an up-regulatory effect on CGRP and CGRP receptor. In addition, an increase in CVC was observed from immediately after DKT administration. This indicated that CGRP release might first be up-regulated by DKT, and then up-regulated RAMP1 might develop up-regulation of CGRP receptor followed by CGRP up-regulation. We believe that the present study have first discovered a part of the mechanism of the clinical effect of DKT. However, it is necessary for proving our proposal that further examination of CGRP and CGRP receptors protein level to rule out the possibility that the protein synthesis may not change even through the expression of mRNA increases.

DKT may be used as a CGRP up-regulator for intestine. Intestinal tissue CGRP is diminished in human and experimental Crohn's disease, and has been reported as a therapeutic and protective peptide 38-40). Therefore, we hypothesize that DKT has therapeutic effect on Crohn's disease via up-regulation of tissue CGRP and its receptor component. Next step of our investigations will be conducted focusing on the effect of

DKT on Crohn's disease.

As a possibility that CGRP indirectly increased CVC, it had been known that CGRP triggered NO release via CGRP[1] receptor 41, 42). So, it was assumed that the indirect action of CGRP via NO might develop the increasing effect of CVC by DKT. The results obtained from the present study showed that administration of NO antagonist could not inhibit an increase in CVC for 15 min after administration of DKT but that it significantly inhibited increase in VC in part 45 min or later. This indicated that the indirect CVC increasing effect by NO was involved as the mechanism in part in the CVC increasing effect 45 min or later after DKT administration. This indicated also that NO was not involved in the mechanism of increasing CVC for 15 min after administration of DKT and that two different steps might exist in the mechanism of increasing CVC by DKT.

This study found that Japanese pepper and ginseng radix showed the most potent effect in DKT-induced hyperemia. Ginseng radix is mainly responsible for the increase in CVC induced by DKT at 15 min, whereas Japanese pepper is mainly responsible for the increase in CVC induced by DKT at 45 min and later. This observation suggests that the mechanism of the hyperemic response to Japanese pepper is different from ginseng radix-induced hyperemia. Further detailed investigation on the

precise mechanisms of both Japanese pepper- and ginseng radix-induced colonic hyperemia remains as a task in the future.

Postoperative ileus remains a source of morbidity and major determinant of length of stay after abdominal operation. Its economic burden in the United States health-care system is estimated to surpass $ 1 billion 43). Postoperative ileus involves an inhibition of bowel transit as well as an adhesive intestinal obstruction, which are caused by an impairment of motility. However, other mechanisms such as the reduced intestinal blood flow may contribute as well. In fact, several studies described that intestinal ischemia causes adhesion formation 44-46). Thus, the present finding that DKT produced an increase of intestinal blood flow as well as an improvement of motility may contribute to its clinical usefulness for prevention the postoperative ileus. This hypothesis is supported by a recent report showing that DKT inhibited experimentally induced intestinal postoperative adhesion formation in rat 5). Moreover, in Japan, DKT has been used as the treatment of postoperative ileus and its efficacy is well established 1, 4). However, precise mechanism of the prevention from postoperative adhesion formation by DKT is still unknown.

In conclusion, the present study demonstrated that DKT produced an increase in CVC. Furthermore, the present results also suggest that DKT-induced hyperemia is

mainly mediated by CGRP and its receptor components.

ACKNOWLEDGMENTS

T. Kono and T. Koseki contributed equally to this manuscript. This work is partly supported by a grant-in-aid from the Ministry of Education, Science, and Culture of Japan (no. 17790904).

Table 1. Oligonucleotide Sequences

Gene (base pair)	Primer sequence (5' → 3')
CGRP (102 bp)	
Forward	GTG TCA CTG CCC AGA AGA GAT C
Reverse	CAA AGT TGT CCT TCA CCA CAC C
CRLR (504 bp)	
Forward	CAG CAG GAA CCG AGT CAA TG
Reverse	CAA ACA CAG CCA CCA CAA TG
RAMP-1 (230 bp)	
Forward	ACT GGG GAA AGA CCA TAG GGA G
Reverse	AGT CAT GAG CAG TGT GAC CGT A
GAPDH (249 bp)	
Forward	TGA TGA CAT CAA GAA GGT GGT GAA G
Reverse	TCC TTG GAG GCC ATG TAG GCC AT

Table 2. Changes in mean arterial blood pressure (mm Hg)

		Post treatment (min)						
Drugs	Dose (mg/kg)	Basal	15	30	45	60	75	90
Vehicle		113.3 ± 6.7	109.5 ± 5.7	107.2 ± 5.7	105.8 ± 5.8	103.1 ± 5.7	102.7 ± 8.7	99.8* ± 8.6
DKT	10	112.1 ± 6.2	108.2 ± 6.4	107.4 ± 5.7	106.2 ± 6.3	103.8 ± 5.2	102.4. ± 7.7	101.3 ± 7.5
	100	112.3 ± 6.2	109.6 ± 5.1	107.8 ± 6.4	105.7 ± 7.0	104.1 ± 7.8	103.2 ± 8.3	101.1 ± 7.3
	300	112.7 ± 6.1	108.4 ± 5.4	107.0 ± 6.6	106.3 ± 6.2	104.3 ± 6.6	102.9 ± 7.1	99.7* ± 8.7
CGRP antagonis		108.2 ± 6.4	105.0 ± 6.2	103.5 ± 7.1	102.2 ± 6.3	98.9 ± 6.3	97.5 ± 7.3	92.4* ± 8.3
SP antagonist		111.8 ± 9.5	105.3 ± 10.4	105.5 ± 11.4	101.3 ± 10.2	98.2 ± 9.7	97.8 ± 12.7	92.3* ± 12.9
VIP antagonist		125.7 ± 12.7	122.3 ± 11.5	124.7 ± 14.2	124.9 ± 20.4	115.5 ± 18.3	110.9 ± 16.8	97.5* ± 16.9
NO antagonist		126.5 ± 7.2	131.8 ± 11.6	124.7 ± 11.7	119.8 ± 12.3	118.7 ± 9.7	117.9 ± 9.8	111.5 ± 9.7

Mean arterial blood pressure was determined during the 15 min period before and the 0–90 min period after the administration of test drugs. vehicle; vehicle for DKT (maltose powder), DKT; Daikenchuto (10, 100, 300 mg/kg), CGRP antagonist; CGRP receptor antagonist CGRP (8–37) (45 μg/kg), SP antagonist; substance P (SP) receptor antagonist spantide (100 μg/kg), NO antagonist, VIP receptor antagonist, , [4-Cl-DPhe6, Leu17]-VIP(15 μg/kg/hr), nitric oxide (NO) antagonist, L-NAME (200 μg/kg)
Data are mean ± SEM; N = 8. *P<0.05 vs. basal

Figure 1. Effects of Daikenchuto (DKT) on colonic vascular conductance (CVC) in rats. The rats received an intracolonal injection of vehicle or various doses of DKT (10, 100 and 300 mg/kg). CVC was calculated every 15 min after the administration of either DKT or vehicle. Values are expressed as the quotient of mean blood flow divided by mean AP and was expressed as mL/mmHg. Data are mean ± SEM; N = 8. *P<0.01 vs. all DKT, †P<0.01 vs. 100 mg/kg and 300 mg/kg, respectively.

Figure 2. Effects of antagonists on DKT-induced hyperemia

a. Daikenchuto (DKT, 100 mg/kg) significantly increased colonic vascular conductance (CVC) (closed circle). Calcitonin gene-related peptide (CGRP)1 receptor antagonist completely suppressed the DKT hyperemia (closed triangle). Nitric Oxide (NO) blocker, N^{G}Nitro-L-arginine methyl ester (L-NAME), also, but only partially, suppressed CVC between at 45 min and at 60 min (open circle).
b. Daikenchuto (DKT, 100 mg/kg) significantly increased colonic vascular conductance (CVC) (closed circle). Vasoactive intestinal polypeptide (VIP) receptor antagonist, [4-Cl-DPhe6, Leu17]-VIP and substance P (SP) receptor antagonist, spantide did not affect the hyperemic response (open square and open triangle, respectively).

CVC is expressed as the quotient of mean blood flow divided by mean AP and was expressed as mL/mmHg. Data are mean ± SEM; N = 8. *P<0.01 vs. Vehicle + DKT, †P<0.05 vs. Vehicle + DKT

CGRP1ra; CGRP1 receptor antagonist CGRP (8–37) (45 μg/kg), SPra; SP receptor antagonist spantide (100 μg/kg), VIPra; VIP receptor antagonist, [4-Cl-DPhe6, Leu17]-VIP (15 μg/kg/hr), N^GNitro-L-arginine methyl ester; L-NAME (200 μg/kg).

Figure 3. Effect of DKT's components, Japanese pepper, processed ginger, ginseng radix and maltose powder on colonic vascular conductance (CVC)

The rats received an intracolonal injection of Japanese pepper (20 mg/kg) , processed ginger (50 mg/kg), ginseng radix (30 mg/kg) or maltose powder (800 mg/kg). CVC was calculated every 15 min after the administration of each component. CVC is expressed as the quotient of mean blood flow divided by mean AP and was expressed as mL/mmHg. Data are mean ± SEM; N = 6. *P<0.01 vs. basal CVC, †P<0.01 vs. basal CVC, respectively.

Figure 4. Evaluation of RNA from the rat colon after DKT administration.

The left panel shows representative photos of gel electrophoresis of RT-PCR products

and a molecular marker (M), 100 bp DNA ladder, are shown in (A). Relative levels of mRNA for CGRP (102 bp) (B), CRLR (504 bp) (C), and RAMP1 (230 bp) (D) in the rat colon 45 min after vehicle or DKT administration as described in Material and Methods, respectively. The right panel exhibits colonic RNA products from the rat colon of vehicle control or Daikenchuto (DKT). Densitometric analysis of PCR products of CRLR, RAMP1, and CGRP in the control and DKT-treated rats, respectively. The intensity of each band was normalized to that of the corresponding band of GAPDH. The data are presented as mean ± SE (n = 4). Bars show SEM. Compared with control, *$p < 0.05$.

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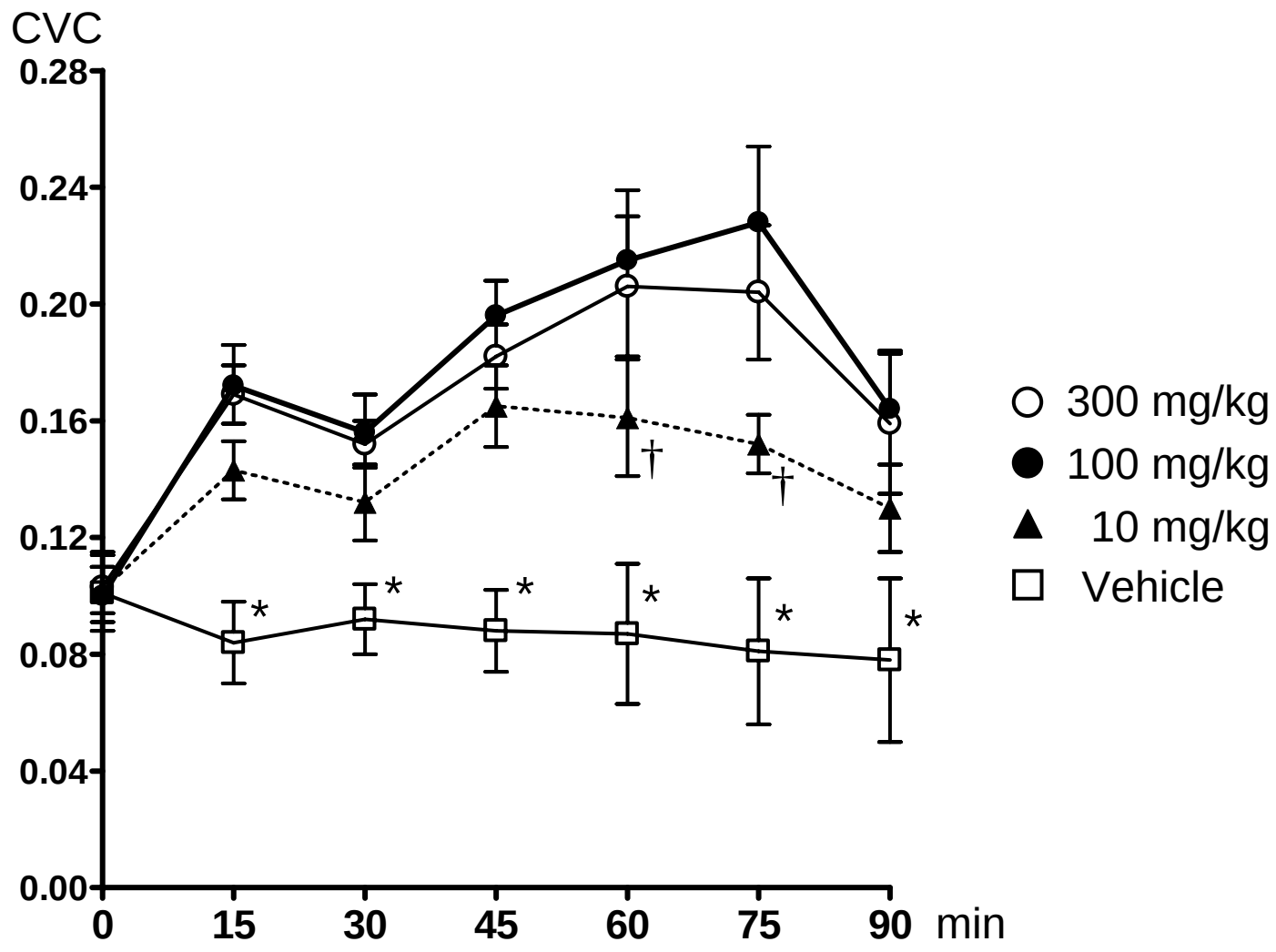
Figure 1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0.16
0.12
0.08
0.04
0.00
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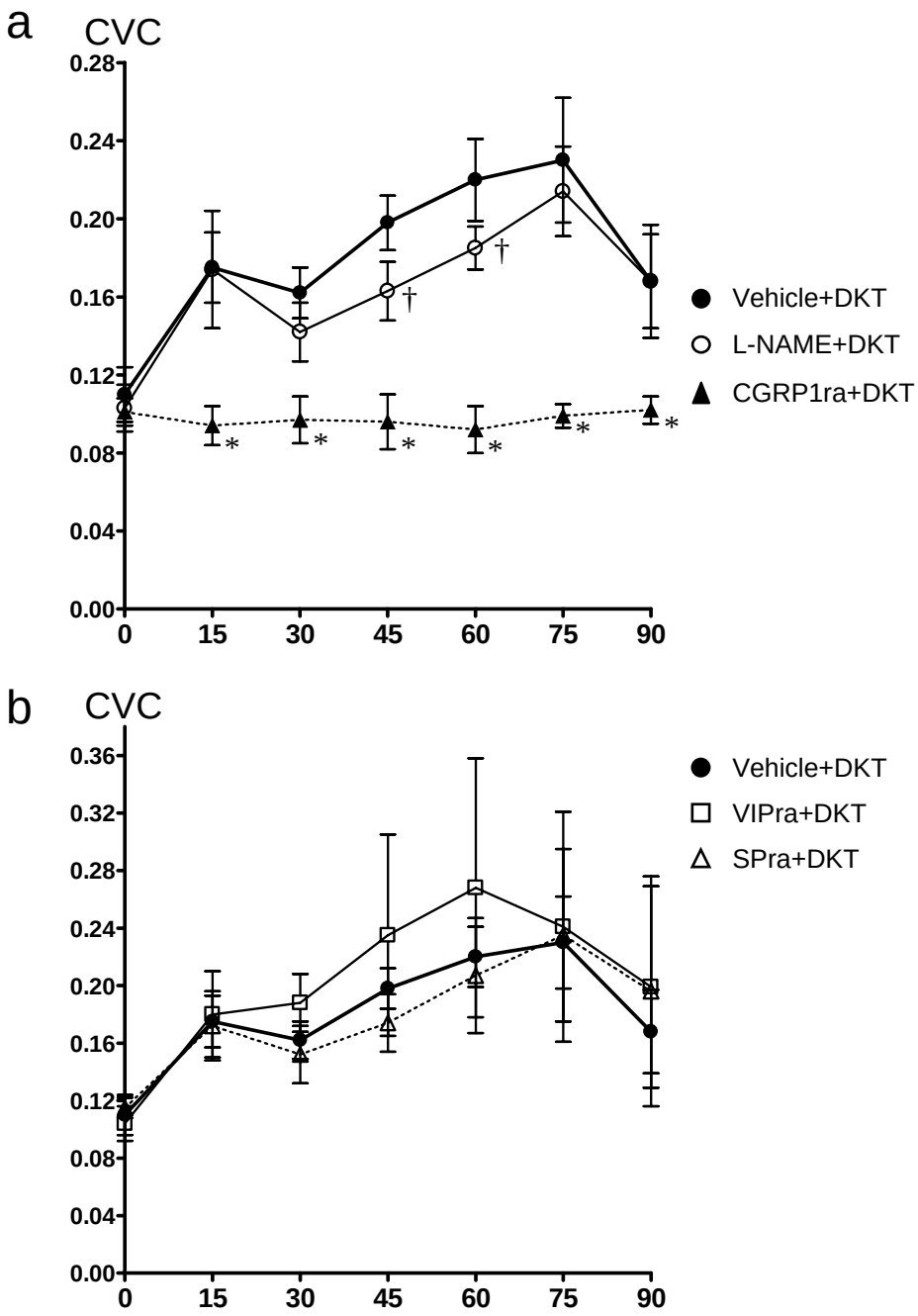
30
45
60
75
90
min
300 mg/kg
100 mg/kg
10 mg/kg
Vehicle
*
†

Figure 2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75
90
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*
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Vehicle+DKT
L-NAME+DKT
CGRP1ra+DKT
b
CVC
0.36
0.32
0.28
0.24
0.20
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0.12
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75
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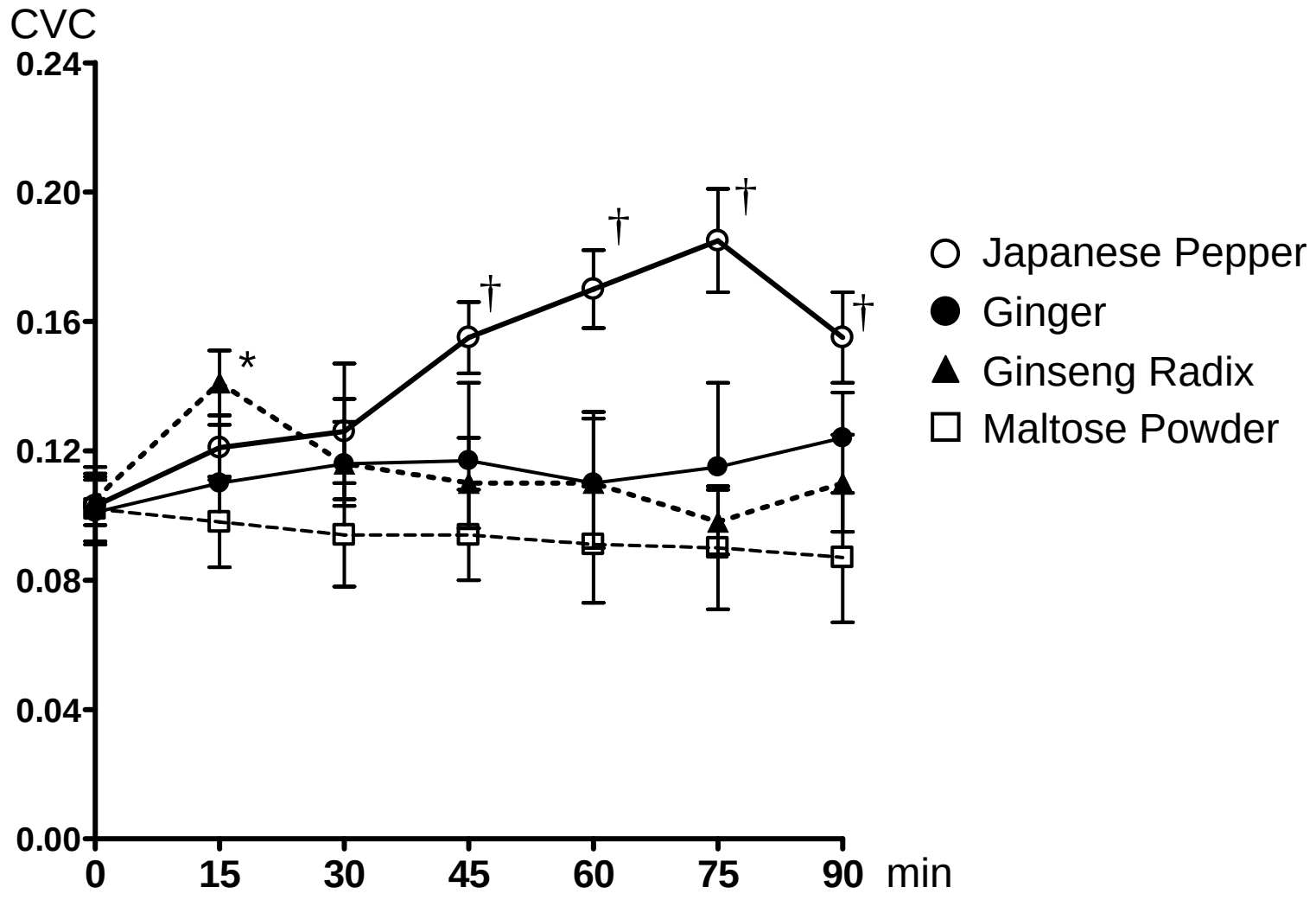
Vehicle+DKT
VIPra+DKT
SPra+DKT

Figure 3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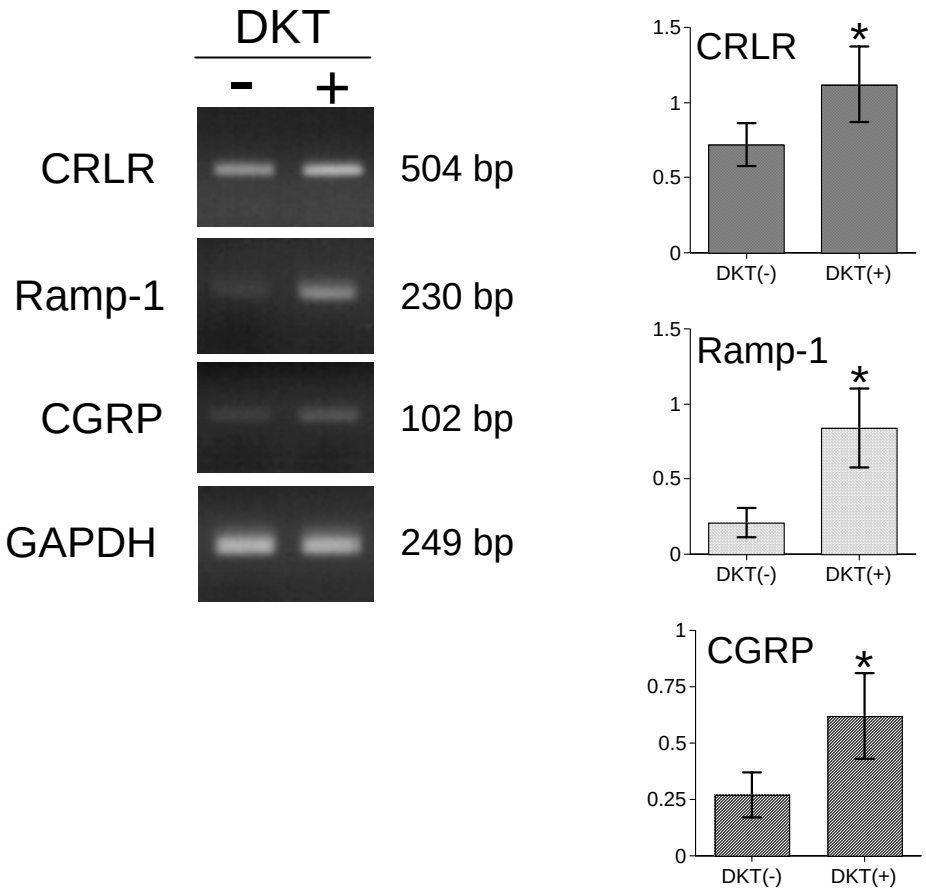
Japanese Pepper
Ginger
Ginseng Radix
Maltose Powder

Figure 4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DKT(+)
*
Ramp-1
1.5
1
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0
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DKT(+)
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CGRP
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0.25
0
DKT(-)
DKT(+)
*