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Original Article

Association Between the Cool Temperature-dependent Suppression of Colonic Peristalsis and Transient Receptor Potential Melastatin 8 Activation in Both a Randomized Clinical Trial and an Animal Model

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Background/Aims

Several studies have assessed the effect of cool temperature on colonic peristalsis. Transient receptor potential melastatin 8 (TRPM8) is a temperature-sensitive ion channel activated by mild cooling expressed in the colon. We examined the antispasmodic effect of cool temperature on colonic peristalsis in a prospective, randomized, single-blind trial and based on the video imaging and intraluminal pressure of the proximal colon in rats and TRPM8-deficient mice.

Methods

In the clinical trial, we randomly assigned a total of 94 patients scheduled to undergo colonoscopy to 2 groups: the mildly cool water (n = 47) and control (n = 47) groups. We used 20 mL of 15°C water for the mildly cool water. The primary outcome was the proportion of subjects with improved peristalsis after treatment. In the rodent proximal colon, we evaluated the intraluminal pressure and performed video imaging of the rodent proximal colon with cool water administration into the colonic lumen. Clinical trial registry website (Trial No. UMIN-CTR; UMIN000030725).

Results

In the randomized controlled trial, after treatment, the proportion of subjects with no peristalsis with cool water was significantly higher than that in the placebo group (44.7% vs 23.4%; $P < 0.05$). In the rodent colon model, cool temperature water was associated with a significant decrease in colonic peristalsis through its suppression of the ratio of peak frequency ($P < 0.05$). Cool temperature-treated TRPM8-deficient mice did not show a reduction in colonic peristalsis compared with wild-type mice.

Conclusion

For the first time, this study demonstrates that cool temperature-dependent suppression of colonic peristalsis may be associated with TRPM8 activation.

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Key Words

Animals; Colon; Peristalsis; Single-blind method; Temperature

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Introduction

Gastrointestinal peristalsis is a common problem for endoscopists. It can interfere with intubation and withdrawal, and it can make endoscopic therapy difficult. Antispasmodics, including hyoscine butylbromide, glucagon, and L-menthol, are used during endoscopy to suppress peristalsis.[1] Furthermore, gastrointestinal peristalsis may be involved in the pathophysiology of various diseases, functional dyspepsia, irritable bowel disorder (IBS), and other conditions.[2,3]

There are some studies that have evaluated the role of temperature on gastrointestinal peristalsis.[4-7] Regarding the upper gastrointestinal tract, it was reported that esophageal motility changes were affected by the temperature of the water bolus.[6] Using high-resolution manometry, it was demonstrated that cold water increased the duration and decreased the amplitude of esophageal body shrinkage in healthy individuals.[6] It was reported that, compared to the primary speed of gastric emptying of the control drink, that of the cold drink was significantly delayed.[4] Regarding the lower gastrointestinal tract, it was reported that warm water irrigation during colonoscopy significantly suppressed pain.[5] However, the mechanism through which temperature influences colonic peristalsis has been poorly documented.

Transient receptor potential melastatin 8 (TRPM8) and transient receptor potential ankyrin 1 (TRPA1) are thermally sensitive ion channels that can be activated by cold.[8,9] The temperature threshold of TRPM8 activation is in the range of 18-23°C.[10] TRPA1 can be activated by noxious cold, below 15°C.[9] Transient receptor potential (TRP) channels are expressed at sensory nerve endings. These channels receive stimuli and cause depolarization. TRPM8 and TRPA1 are reportedly expressed in the colon.[11-13] We found that the TRPM8 agonist menthol suppressed colonic peristalsis and abdominal pain in a randomized controlled trial.[1,14] Peppermint oil is reported to induce symptom relief in patients with IBS and to exert spasmolytic effects and inhibition of gastrointestinal contractility.[15,16] Furthermore, TRPM8 polymorphism reportedly has association with higher incidence of IBS.[17,18] It is reported that the gastrointestinal mucosa is sensitive to local cold temperature due to the augmented vagal TRPA1 expression and function in IBS.[19] It was reported that a TRPM8 agonist reduced the spontaneous contractions in the human colon and that a TRPA1 agonist induced colonic motility in rats.[12,13] However, how TRP channels TRPM8 and TRPA1, which can be activated by cold, affect gastrointestinal peristalsis has been poorly documented.

We hypothesize that the administration of cool temperature water to the colon would result in less peristalsis by activating TRPM8. We designed this study to examine the role of temperature on colonic peristalsis.

Materials and Methods

Study Design and Patients

This study was a randomized, single-blind, open, prospective trial. As shown in Figure 1, patients to be examined by colonoscopy for screening colonoscopy, the examination of confirming fecal occult blood screening test, a colonoscopy for endoscopic treatment of colorectal tumors, or a surveillance colonoscopy after endoscopic treatment of colorectal tumors were subject to registration. Patients were excluded if they had (1) severe organ failure, (2) symptoms suggesting possible colorectal stenosis or cancer, (3) a history of colectomy, and (4) known colorectal cancer, inflammatory bowel disease, or familial polyposis. To participate in this study, from all patients, we obtained written informed consent.

Procedures

We allocated patients to either the mildly cool water group or the room temperature water group randomly. Before the examination, we allocated the patients to these groups by dynamic balancing with the minimization method. Independent members created the randomization table. Although patients were blinded to the allocation, endoscopists were not.

We gave patients undergoing colonoscopy a low-fiber diet and 20 mL of sodium picosulfate hydrate (sodium picosulfate hydrate [CHOS]; Mylan Seiyaku, Tokyo, Japan) on the night before colonoscopy as preparatory medication. Before colonoscopy, 20 mL

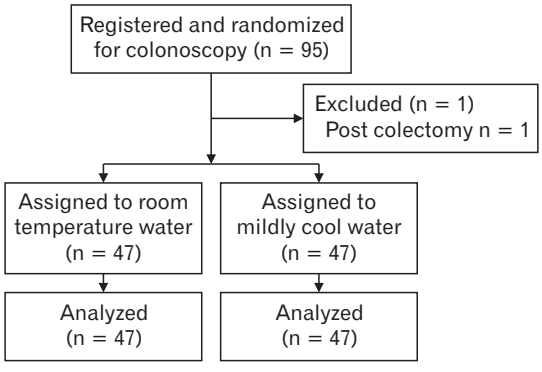

Figure 1. Patient allocation of clinical trial.

of dimeticon, 0.5 L of water, and 1 L of polyethylene glycol (Moviprep; EA Pharma, Tokyo, Japan) were used to clean the bowel in the morning. Pentazocine (Pentagin; Daiichi-Sankyo, Tokyo, Japan) or Midazolam (Dormicum 10 mg; Astellas Pharma, Tokyo, Japan) were administered to patients who wanted to be sedated during colonoscopy.

In these patients, we used light sources and colonoscopes (Evis Lucera Elite, CVL-290SL, Evis PCF-H290ZI; Olympus Medical Systems, Tokyo, Japan). We supplied CO_2 using a CO_2 tube and insufflator (MAJ-1742 and UCR; Olympus Medical Systems).

By 1 of the same group of 7 colonoscopists, the colonoscopy procedures were performed: 3 highly qualified colonoscopists who had performed more than 3000 colonoscopies and 4 less experienced colonoscopists who had performed less than 500 colonoscopies. In every case, we inserted the colonoscope to the cecum as fast as we could without searching lesions, and the colonoscopists then began the study examination. Next, 20 mL of water at a temperature of 15°C, as a TRPM8 agonist, was administered in the mildly cool water group. In the room temperature water group, 20 mL of water at room temperature of 25°C was administered. During colonoscopy, via the biopsy channel of the endoscope with a length of 120 cm, we sprayed directly on the cecum 20 mL of water at a temperature of 15°C or at room temperature of 25°C in a prefilled syringe. We pushed the residual liquid out by air in this way. We used water at a temperature of 15°C as a TRPM8 agonist.

Outcome Assessment

We evaluated colonic peristalsis by an examiner using a 4-level rating of colonoscopy according to whether or not peristalsis causes interference with colonoscopy after applying the assigned administration as follows: for colonoscopy, (0) no peristalsis, (1) mild peristalsis, (2) moderate peristalsis, or (3) severe peristalsis. These classifications were also used in a previous study.[20] The investigator scored colonic peristalsis for 1 minute before treatment and for 1 minute after treatment.

We evaluated abdominal pain using the following 4-grade scale: (0) no pain, (1) mild pain, (2) moderate pain, or (3) severe pain, during the insertion of the colonoscope and its withdrawal. We used this scale in our previous report.[1] Independent staff members recorded the abdominal pain score which was reported by every patient.

We evaluated the quality of the bowel preparation for colonoscopy by the Aronchick Scale, as follows: (1) excellent, (2) good, (3) fair, (4) poor, or (5) inadequate.

Outcome Parameters

The difference in the ratio of patients with no peristalsis after administration for colonoscopy was the primary outcome. The interval to the improvement of peristalsis after treatment for colonoscopy was the secondary outcome.

Sample Size Calculation

We reported that the ratio of patients with no colonic peristalsis (grade 0) after treatment was 71.2% in an L-menthol group and 30.9% in a placebo group.[1] In the present study, we calculated the sample size be sufficient to show an improvement in peristalsis in 40% of the patients in the mildly cool water group in comparison to the placebo group. Our power analysis indicated that we needed more than 40 patients in each group, assuming a 1% significance level and a statistical power of 80% using 2-sided equivalence. Therefore, we estimated that a total of 90 patients would be needed.

Drugs and Chemicals

We purchased all chemicals from Wako Pure Chemical Corporation (Osaka, Japan).

Animals

We obtained Sprague-Dawley rats at 7-12 weeks of age and C57BL/6J mice at 6-8 weeks of age from Shimizu Laboratory Supplies Co, Ltd. (Kyoto, Japan). TRPM8 green fluorescent protein (GFP) transgenic animals were generated as described in the references (Professor Ardem Patapoutian) and were bred in the National Institute for Physiological Sciences, National Institutes of Natural Sciences, Okazaki, Japan (Professor Makoto Tominaga).[21] All mice were genotyped before use in experiments. We housed the animals at 22°C under a 12-hour light/dark cycle and allowed them access to food and water. They had *ad libitum* access to drinking water.

The Measurement of Intraluminal Pressure of Isolated Rat and Mouse Proximal Colon Tract

We fasted rats and mice overnight and we euthanized them by decapitation before the removal of their colon. In an organ bath (100 mL volume), we placed a 2-cm to 3-cm segment of the proximal colon, and we perfused an organ bath with Krebs solution (34-35°C, 3.5 mL/min). We securely attached with string the oral and aboral ends of the proximal colon segment to the saline input and output ports, respectively. In the colon, we set a Mikro-Tip catheter pressure transducer (SPR-524, Millar Instruments, Houston, TX, USA) to monitor the intraluminal pressure (cmH_2O). By elevat-

ing the drain tube, we caused shrinking by loading an intraluminal pressure to ~4 cmH_2O. By a data acquisition and analysis system (MP100, BIOPAC Systems, Goleta, CA, USA), we evaluated the intraluminal pressure waves. We macroscopically observed motility through video images (HDC-HS 100-K, Panasonic, Osaka, Japan). We added 2.5 mL of saline at room temperature (25°C), saline at a temperature of 15°C, saline at a temperature of 5°C and 0.8% L-menthol solution (MINCLEA; Nihon Seiyaku, Tokyo, Japan) at room temperature (25°C) into the intraluminal tract via the drain tube for 25 seconds. We used saline at a temperature of 15°C and 5°C as a candidate for TRPM8 agonists. A TRPA1 antagonist (HC-030031; FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan) was added to the Krebs solution in the organ bath (serosal side). We calculated the peak frequency (PF) as the number of high-amplitude pressure peaks (> 8 mm on spatiotemporal mapping) per minute within a certain period of time. We also calculated the area under the curve (AUC) above the minimum value of the pressure during a defined period. We calculated the peak pressure amplitude (PPA) as the average pressure of the peaks minus the minimum value during a defined period. We calculated PPA, PF, and the AUC for each period as the ratio to before drug administration.

Spatiotemporal Mapping

According to a previously described method before, Spatiotemporal mappings with image-based representations of motions were created.[22,23] Along the length of the colon (image y-axis), for each video frame (image x-axis), we calculated the colon width (coded as image intensity, black to white) at each point using the ImageJ software program. As shown in Figure 2, broad relaxation was represented as the white area. The transmitting shrinkages were expressed as dark colored diagonal stripes.

Immunocytochemical Detection of Transient Receptor Potential Melastatin 8 Expression in the Mouse Colon

We detected colonic expression of TRPM8 using *TRPM8*^{GFP} transgenic mice. We euthanized mice, and excised their colons and rinsed with phosphate-buffered saline (PBS). The colons were not fixed and snap frozen in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek Japan, Tokyo, Japan). Optimal cutting temperature-embedded tissue was sectioned with a cryostat at 10 μm slices and mounted on CREST slides. We stored the slides at –80°C. We dried the frozen slide at room temperature for 30 minutes and then at room temperature for 30 minutes, after which we washed them with deionized water for 30 seconds. We washed slide-mounted sections 3 times with PBS for 5 minutes. Slides were then blocked (Protein Block; Abcam, Cambridge, UK), and the samples were incubated with rabbit polyclonal anti-GFP antibody (1:400 dilution; Abcam) and goat polyclonal anti-calcitonin gene-related peptide (CGRP) antibody (1:200 dilution; Abcam) in PBS and incubated at 4°C overnight. Sections were washed 3 times with PBS containing 0.1% Tween 20, followed by incubation with the secondary Donkey anti-Rabbit IgG, Alexa Fluor 488 (1:1000 dilution; Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and Donkey anti-Goat IgG, Alexa Fluor 594 (1:1000 dilution; Invitrogen, Thermo Fisher Scientific) for an additional hour. Sections were washed 3 times with PBS containing 0.1% Tween 20 and coverslipped with DAPI-Fluormount-G (Southern Biotechnology Associates Inc, Birmingham, AL, USA). We acquired digital images using a Keyence BZ-X810 microscope with the BZ-X800 viewer (KEYENCE, Osaka, Japan).

Statement of Ethics

The study was approved by the Clinical Ethics Committee on Human Experiments of Fukuchiyama City Hospital (IRB Registration No. 29-8, June 19, 2017). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 1964 Declaration of Helsinki and later versions. This study followed the CONSORT guidelines and is registered in the University Hospital Medical Network Clinical Trials Registry (UMIN-CTR, UMIN 000030725). Informed consent to be included in the study, or the equivalent, was obtained from all patients. All animal experiments were approved by the Institutional Animal Care and Use Committee of Kyoto Prefectural University of Medicine (Kyoto, Japan) under Assurance Number M2020-94, M2020-95, M2020-151 and performed according to the institutional guidelines for the care and use of laboratory animals, which is accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Statistical Methods

The median, mean, range, and 95% confidence intervals were summarized in the quantitative data. We compared patient characteristics and the details of the colonoscopy examinations by the chi-squared test, chi-squared test with Yates' correction, unpaired *t* test, and the Mann–Whitney *U* test. Using the chi-squared test, we compared the ratio of patients with no peristalsis after administration. We compared the changes in the peristalsis scores of patients

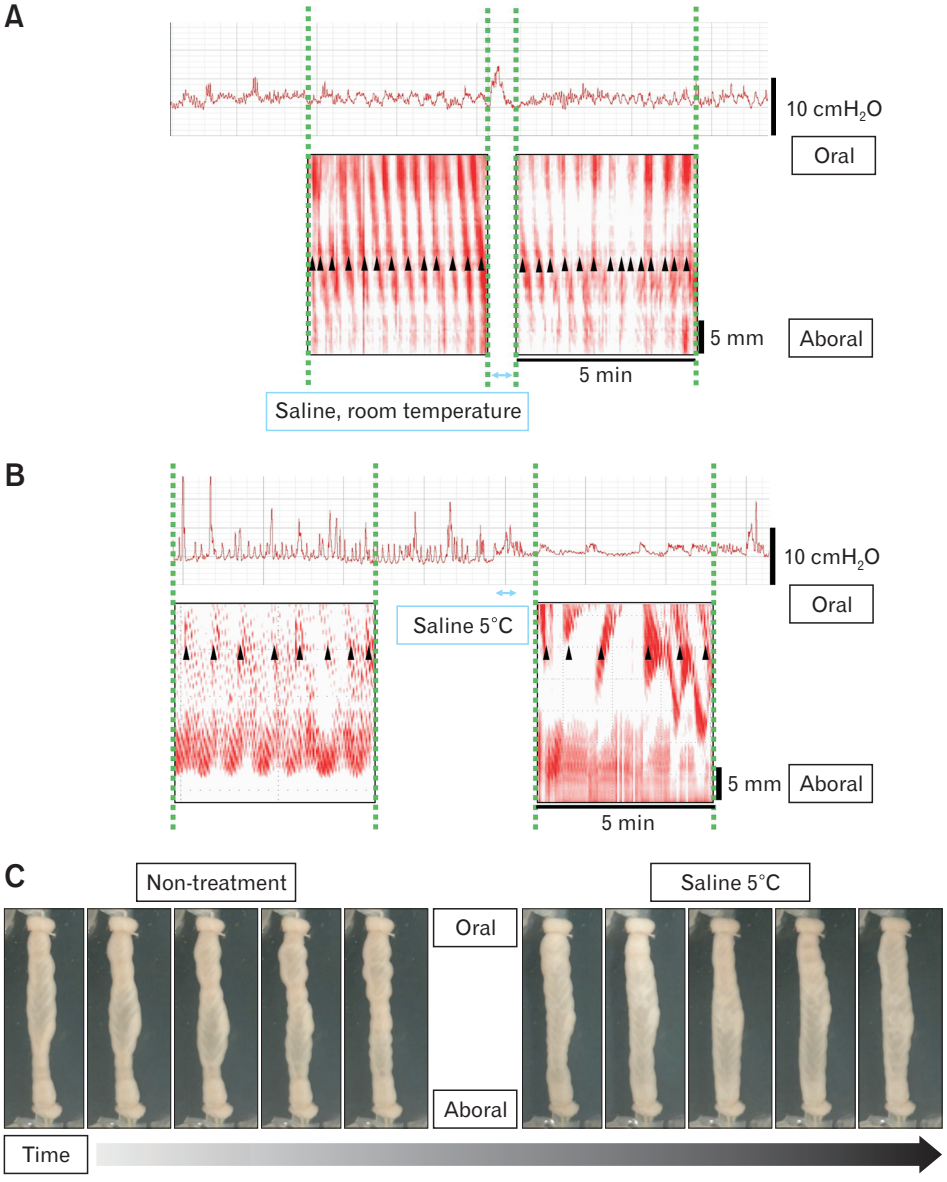

Figure 2. Reduction of colonic motor activity and intraluminal high-amplitude pressure by cold saline in rats. (A) The colonic motility and intraluminal high-amplitude pressure after administration of room temperature saline. (B) A typical pattern of reduction of colonic motility and intraluminal high-amplitude pressure induced by 5°C saline. (C) Serial photographs of colonic motor activity. Left: motility before treatment. Right: motility after treatment with 5°C saline.

after administration using the Wilcoxon signed rank test. We evaluated statistical significance between 2 groups by unpaired *t* test and among 3 groups by a one-way analysis of variance (ANOVA) or Kruskal-Wallis test. We performed all analyses using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA) on a Windows-based computer.

Results

Patient Baseline Characteristics and Compliance With the Protocol

A total of 95 patients who underwent colonoscopy were registered in this study between October 2017 and March 2018. We assigned the patients equally and randomly to 2 groups: the mildly cool water (15°C) and room temperature (25°C) groups. One patient was excluded from enrollment. Thus, the study population included a total of 94 patients: 47 patients were randomly assigned

to the mildly cool water group and 47 patients were assigned to the room temperature (25°C) group (Fig. 1). In Table 1, we summarize the baseline data of the patients. In the baseline data of the 2 groups, no significant differences were found. All 94 colonoscopic examinations were completed.

Colonic Peristalsis

Table 2 summarizes the grade of colonic peristalsis before and after treatment in both groups. We converted the evaluated colonic peristalsis grades into a numerical score. The peristalsis score of the patients who were administered mildly cool water was significantly decreased compared to before the administration ($P = 0.002$, after administration, Wilcoxon signed rank test). After administration, the ratio of patients who did not show any peristalsis (grade 0) was 44.7% in the mildly cool water group and 23.4% in the room temperature water group ($P < 0.05$, chi-squared test) (Fig. 3).

Table 1. Baseline Characteristics of All Patients

Characteristics	Room temperature water (n = 47)	Mildly cool water (n = 47)	*P*-value
Age (yr)	68 (34-86)	66 (21-83)	0.849
Sex (male/female)	29/18	31/16	0.668
Indication			0.507
Screening	23 (48.9)	19 (40.4)	
FOBT positive results	7 (14.9)	11 (23.4)	
EMR	10 (21.3)	7 (14.9)	
Surveillance	7 (14.9)	10 (21.3)	
Bowel preparation			
% rated (excellent, good or fair)	44 (93.7)/3 (6.3)	43 (91.5)/4 (8.5)	0.694
Insertion time·median (sec)	472 (192-1423)	539 (217-1455)	0.167
Observation time·median (sec)	746 (382-3800)	763 (416-1998)	0.865
Pain during insertion			
None/mild/moderate/severe	11 (23.4)/29 (61.7)/6 (12.8)/1 (12.8)	10 (21.3)/27 (57.4)/9 (19.1)/1 (2.2)	0.869
Pain during colonoscopy			
None/mild/moderate/severe	31 (66.0)/13 (27.7)/3 (6.3)/0 (0.0)	29 (61.7)/15 (31.9)/3 (6.4)/0 (0.0)	-
Colonoscopists			
Experts/trainee	31 (66.0)/16 (34.0)	26 (55.3)/21 (44.7)	0.291
Underlying disease			
Prostatic hyperplasia	1 (2.1)	0 (0.0)	0.315
Glaucoma	0 (0.0)	1 (2.1)	0.315
Arrhythmia	1 (2.1)	0 (0.0)	0.315

FOBT, fecal occult blood test; EMR, endoscopic mucosal resection.
Data are presented as median (range), n, or n (%).

Table 2. Grades of Peristalsis Before and After Treatment

Medication	Peristalsis grade				*P*-value	Interval to the improvement of peristalsis (sec)
	0	1	2	3		
Room temperature water (n = 47)						
Before treatment	13 (27.7)	24 (51.1)	10 (21.2)	0 (0.0)	0.688	-
After treatment	11 (23.4)	26 (55.3)	10 (21.3)	0 (0.0)		
Mildly cool water (n = 47)						
Before treatment	13 (27.7)	20 (42.6)	13 (27.7)	1 (2.0)	0.002	42.7
After treatment	21 (44.7)	22 (46.8)	4 (8.5)	0 (0.0)		

Data are presented as n (%).
Wilcoxon signed rank test.

Adverse Events

We observed no bleeding or symptoms after endoscopy in any of the patients.

The Evaluation of Rat Colonic Motor Activity by Video Imaging and Intraluminal Pressure Peaks, and the Effect of Cool Water and Menthol

By video image recording and intraluminal pressure measurement, we analyzed the motility of isolated segments of the rat proximal colon. High-amplitude pressure peaks were observed after the beginning of the experiment. The administration of room temperature saline into the colon from the oral side had no effect on either the intraluminal pressure or the spatiotemporal mapping (Fig. 2A).

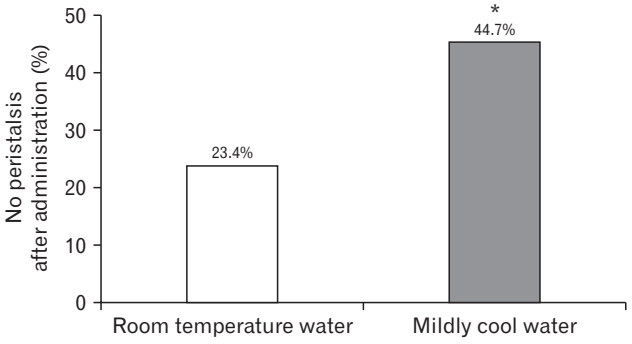

Figure 3. In clinical trial, the ratio of patients who did not show any peristalsis (grade 0) after mildly cool water or room temperature water was administered as a direct spray to the colonic mucosa. *$P < 0.05$ in comparison to room temperature water (chi-squared test).

The administration of cold saline into the colon from the oral side induced low-amplitude periodic pressure peaks (Fig. 2B). Serial photographs of the mobility of the unmedicated colon (left) and the colon treated with cool saline (right) are shown in Figure 2C. After the administration of cool saline, the spastic colon started to relax. In Figure 4 (Supplementary Videos 1-3), the suppression of the pressure peak pattern, PF, AUC, and PPA are shown. The suppression of PF in the group treated with 5°C saline was significantly lower in comparison to the room temperature water groups ($P < 0.05$ after treatment, Kruskal-Wallis test) (Fig. 4A). The administration of 5°C saline, 15°C saline and room temperature saline had no significant effect on the suppression of PPA or the AUC (one-way ANOVA) (Fig. 4B and 4C). The administration of menthol into the colon from the oral side had effect on either the intraluminal pressure (Supplementary Figure) or the spatiotemporal mapping (data are not shown). In Supplementary Figure, the suppression of the pressure peak pattern, PF, AUC, and PPA is shown. The suppression of PF and PPA in the group treated with menthol was significantly lower in comparison to the room temperature water groups ($P < 0.01$ after treatment, unpaired *t* test) (Supplementary Figure A and C). The administration of menthol and room temperature saline had no significant effect on the suppression of the AUC (unpaired *t* test) (Supplementary Figure B).

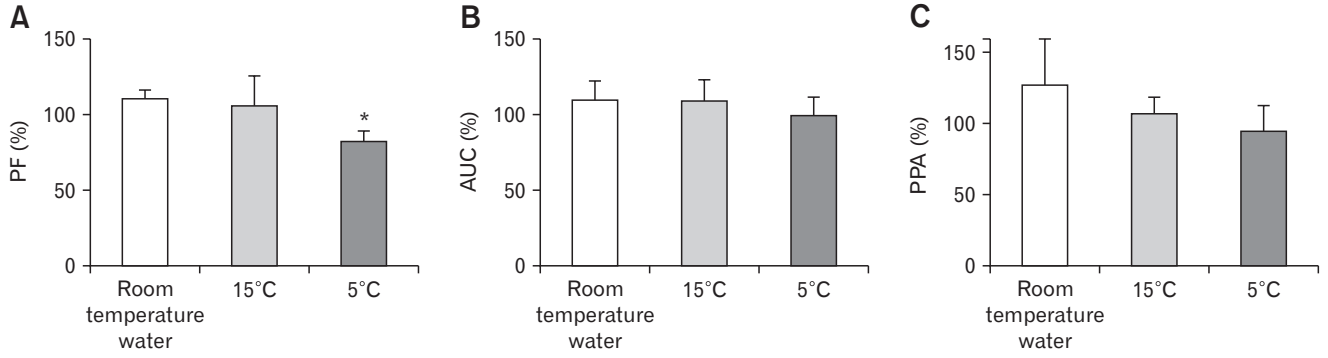

Figure 4. Suppression of peak frequency (PF) after the administration of 5°C saline in rats. (A) Decreased ratio of PF in high-amplitude pressure following the administration of 5°C saline. To quantify the decrease in high-amplitude pressure induced by 5°C saline, the ratio of contraction frequency before and after administration with a length of more than 8 mm was calculated as the %PF. *$P < 0.05$ vs room temperature. (B) The area under the curve (AUC) at high-amplitude pressure with 5°C saline. The AUC was calculated before and after drug administration using the lowest intraluminal pressure value as the baseline. There were no significant differences among the 3 groups. (C) The results of the peak pressure amplitude (PPA) at high-amplitude pressure with 5°C saline. The difference between PPA and the lowest intraluminal pressure was defined as PPA, and the ratio of PPA before and after medication was calculated as % PPA. There was no significant difference among the 3 groups (n = 5).

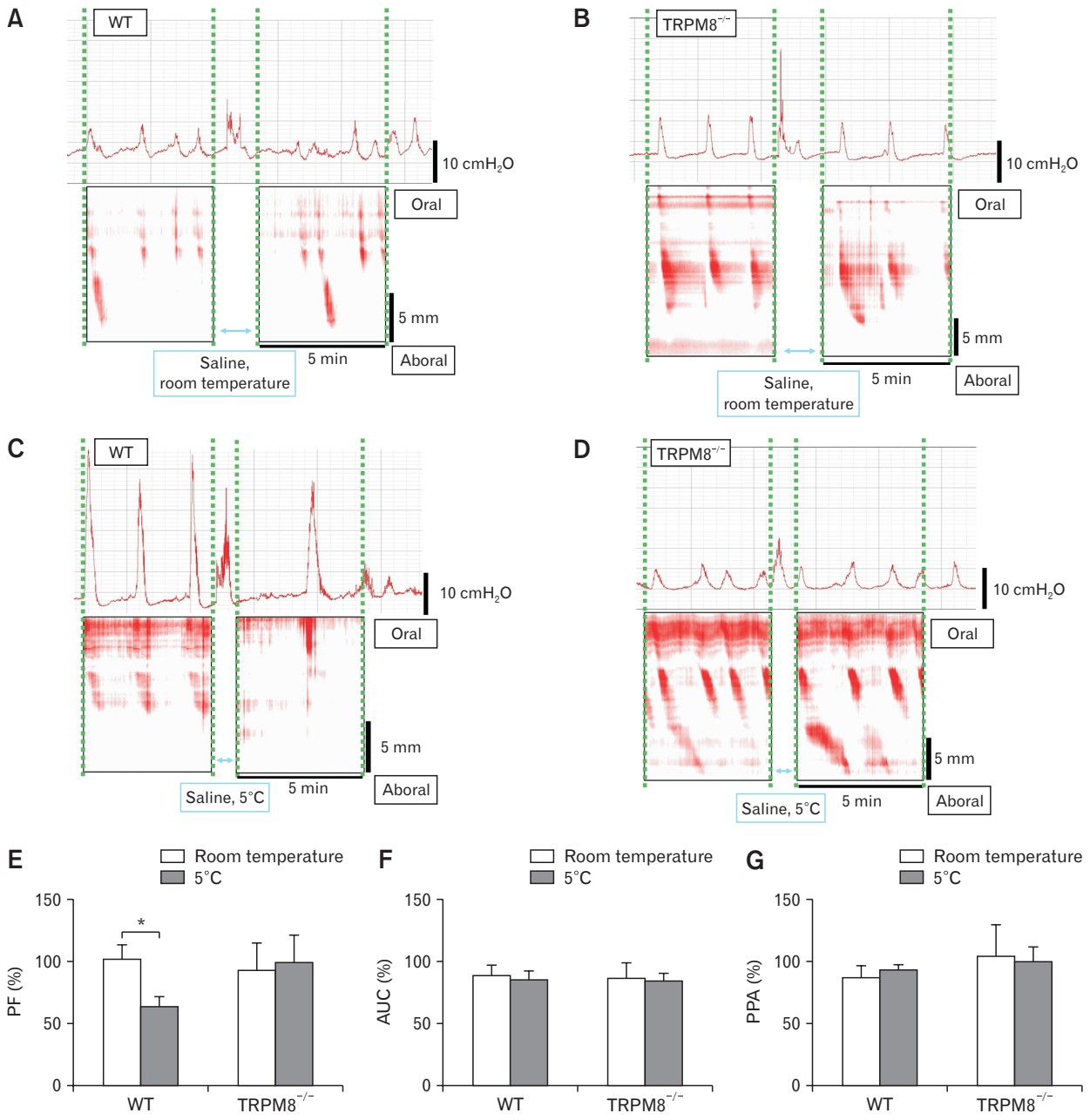

Figure 5. Colonic motility in wild type (WT) mice and transient receptor potential melastatin 8 (TRPM8)-deficient (TRPM8$^{-/-}$) mice. The intraluminal high-amplitude pressure and colonic motor activity before and after administration of 5°C saline in wild type (WT) mice. A typical pattern of reduction of colonic motor activity and intraluminal high-amplitude pressure induced by 5°C saline. (D) The colonic motility and intraluminal high-amplitude pressure before and after administration of 5°C saline in transient receptor potential melastatin 8-deficient (TRPM8)$^{-/-}$ mice. (E) Decreased ratio of peak frequency (PF) in high-amplitude pressure following administration of 5°C saline in WT mice. To quantify the decrease in high-amplitude pressure induced by 5°C saline, the ratio of contraction frequency before and after administration with a length of more than 8 mm was calculated as the %PF. *$P < 0.05$ vs room temperature (25°C) of wild type mice. (F) The area under the curve (AUC) at high-amplitude pressure with 5°C saline. The AUC was calculated before and after saline administration using the lowest intraluminal pressure value as the baseline. There were no significant differences between the 2 groups. (G) The results of the peak pressure amplitude (PPA) at high-amplitude pressure with 5°C saline. The difference between PPA and the lowest intraluminal pressure was defined as PPA, and the ratio of PPA before and after medication was calculated as %PPA. There were no significant differences between the 2 groups (n = 6).

The Evaluation of Transient Receptor Potential Melastatin 8-deficient and Wild-type Mouse Colonic Motor Activity by Video Imaging and Intraluminal Pressure Peaks and the Effect of Cool Water

We analyzed the motility of isolated segments of the proximal colon from TRPM8-deficient and wild-type mice by video image recording and intraluminal pressure measurement. In Figure 5, the intraluminal pressure, spatiotemporal mapping, pressure peak pattern, PPA, PF, and AUC are shown. In both TRPM8-deficient and wild-type mice, after the experiment was started, high-amplitude pressure peaks were observed. The administration of room temperature (25°C) saline into the colon from the oral side had no effect on either the intraluminal pressure or the spatiotemporal mapping in either TRPM8-deficient or wild-type mice (Fig. 5, Supplementary Videos 4-7). The administration of room temperature (25°C) saline into the colon from the oral side did not induce low-amplitude periodic pressure peaks in either wild-type mice (Fig. 5A) or TRPM8-deficient mice (Fig. 5B). The administration of cold (5°C) saline into the colon from the oral side induced low-amplitude periodic pressure peaks in wild-type mice (Fig. 5C) but not in TRPM8-deficient mice (Fig. 5D). After the administration of cool saline, the spastic colon started to relax in wild-type mice (Fig. 5C) but not in TRPM8-deficient mice (Fig. 5D). In wild-type mice, the inhibition of PF in the group administered saline at 5°C was significantly lower than that in the group administered with room temperature saline, but no significant difference was observed in TRPM8-deficient mice ($P < 0.05$) (Fig. 5E). The administration of 5°C saline and room temperature saline did not have a significant effect on the inhibitory effect of the AUC (Fig. 5F) or PPA (Fig. 5G) in either TRPM8-deficient or wild-type mice.

The Evaluation of Rat Colonic Motor Activity With or Without a Transient Receptor Potential Ankyrin 1 Inhibitor by Video Imaging and Intraluminal Pressure Peaks and the Effect of Cool Water

We analyzed the motility of isolated segments of the rat proximal colon with or without a TRPA1 inhibitor by video image recording and intraluminal pressure measurement. Figure 6 shows the pressure peak pattern, PPA, PF, and AUC. After the experiment was started, high-amplitude pressure peaks were observed in the rat proximal colon both with and without TRPA1 inhibitor (Fig. 6). The administration of room temperature (25°C) saline into the colon from the oral side had no effect on the intraluminal pressure in the rat proximal colon with or without the TRPA1 inhibitor (Fig. 6). The administration of cold (5°C) saline into the colon from the oral side induced low-amplitude periodic pressure peaks in the rat proximal colon both with and without the TRPA1 inhibitor (Fig. 6). After the administration of cold (5°C) saline, the spastic colon started to relax in both the rat proximal colon, both with and without the TRPA1 inhibitor (Fig. 6). In the rat proximal colon, both with and without the TRPA1 inhibitor, the inhibition of PF in the group that received saline at 5°C was significantly lower than that in the group that received room temperature (25°C) saline ($P < 0.01$) (Fig.

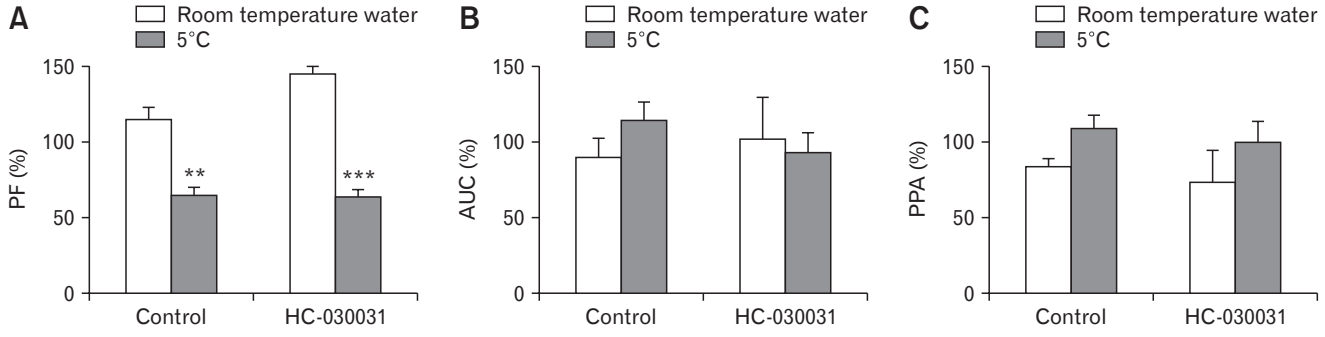

Figure 6. Colonic motility in rats without or with the administration of a transient receptor potential ankyrin 1 (TRPA1) inhibitor. (A) Decreased ratio of peak frequency (PF) in high-amplitude pressure following the administration of 5°C saline in rats without administration of a TRPA1 inhibitor. To quantify the decrease in high-amplitude pressure induced by 5°C saline, the ratio of contraction frequency before and after administration with a length of more than 8 mm was calculated as the %PF. **$P < 0.01$ vs room temperature (25°C) without a TRPA1 inhibitor, ***$P < 0.001$ vs room temperature (25°C) with a TRPA1 inhibitor. (B) The area under the curve (AUC) at high-amplitude pressure with 5°C saline. The AUC was calculated before and after administration of saline using the lowest intraluminal pressure value as the baseline. There were no significant differences between the 2 groups. (C) Results of the peak pressure amplitude (PPA) at high-amplitude pressure with 5°C saline. The difference between the PPA and the lowest intraluminal pressure was defined as the PPA, and the ratio of the PPAs before and after medication was calculated as the %PPA. There were no significant differences between the 2 groups (n = 3).

6A). The administration of 5°C saline and room temperature (25°C) saline did not have a significant effect on the inhibitory effect of the AUC (Fig. 6B) or PPA (Fig. 6C) in the rat proximal colon with or without the TRPA1 inhibitor.

The Transient Receptor Potential Melastatin 8 and Calcitonin Gene-related Peptide Expression in the Colon

We used the transgenic mice in which the expression of the GFP reporter was driven by the TRPM8 transcriptional promoter (TRPM8GFP) to monitor the expression of TRPM8. Digital imaging of GFP-stained colonic sections derived from TRPM8 GFP mice revealed abundant TRPM8 expression in the mouse colon (Fig. 7A-D). GFP was observed in the luminal propria, submucosal plexus and myenteric plexus (Fig. 7C, blue arrows). Co-staining with CGRP revealed that the expression of TRPM8 was closely associated with peptidergic neurons (Fig. 7E-G). CGRP was observed in neuronal like structures (Fig. 7G, white arrows).

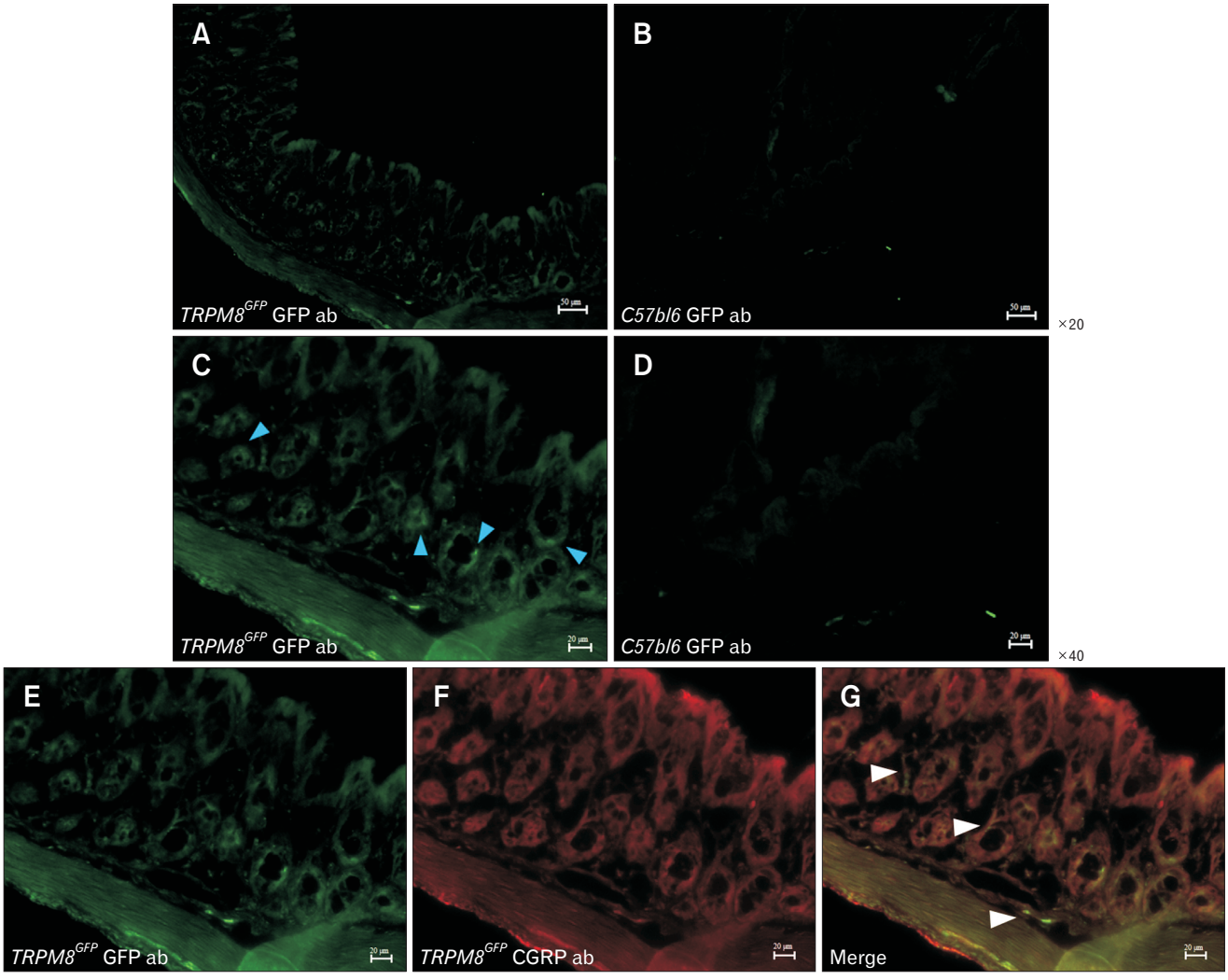

Figure 7. Transient receptor potential melastatin 8 (TRPM8) localization in the TRPM8 green fluorescent protein (GFP) mouse colon. (A) GFP-targeted polyclonal antibody staining in the TRPM8 GFP mouse colon. (B) GFP staining in C57BL/6J mice. No detectable signal was observed. (C) Enlarged (×2 zoom) view of GFP staining in c57bl6 mice. No detectable signal was observed. (D) Enlarged (×2 zoom) view of a section of the TRPM8 GFP mouse colon. (E) Enlarged (×2 zoom) view of a section of the TRPM8 GFP mouse colon. (E) Calcitonin gene-related peptide (CGRP) staining in the TRPM8 GFP mouse colon. (G) Merged image showing TRPM8 and CGRP expressing cells in the TRPM8 GFP mouse colon.

Discussion

For the first time, we examined the effects of cool temperature on colonic peristalsis in a randomized, single-blind, placebo-controlled trial, and based on intraluminal pressure and video imaging of the proximal colon of rats and TRPM8-deficient mice. The results in clinical trial showed that the use of mildly cool temperature significantly reduced peristalsis in the colon. The results in the animal model showed that the use of the cool temperature water may be associated with a significant decrease in the PF of colonic peristalsis through TRPM8 activation.

Previous studies showed that warm water irrigation of the colon induced significantly less discomfort in comparison to room temperature water during colonoscopy.[5] A significant relationship was found between the difference in intragastric temperature and the difference in gastric ejection rate after taking cold and control liquids.[4] Cold temperature water caused an increase in the duration and a decrease in the amplitude of esophageal body contraction.[6] Cold activates TRPM8 and TRPA1 which are thermally sensitive ion channels.[8,9] In a mouse model of colitis, it was reported that TRPM8 expressed in the colon and activation of TRPM8 suppressed the inflammatory response, partly by reducing the neuropeptides release.[11] It is reported that TRPM8 receptors are expressed in the human colon and that ligand-dependent TRPM8 activation can reduce colonic motility.[13] The TRPA1 agonist, allyl isothiocyanate, is reported to induce colonic motility of rats.[12] However, the data regarding how the low temperature of irrigated water is associated with colonic peristalsis and the correlation between TRPM8 and TRPA1 activation and colonic peristalsis are limited.

In the present study, as expected, the use of cool temperature water was associated with a significant reduction in colorectal peristalsis during colonoscopy. The administration of cool temperature water was associated with a significant reduction in colorectal peristalsis via the modulation of the PF in the rat with and without TRPA1 antagonist and wild-type mouse colon models, but not in TRPM8-deficient mouse colon models. The results of this study are consistent with those of previous studies on changes in esophageal body contraction duration and peristalsis. As expected, the suppression of colonic peristalsis by the administration of cool water to the colon prolonged the duration and resulted in decreased colonic peristalsis through TRPM8 activation.

Antispasmodic agents, including hyoscine butylbromide, glucagon, L-menthol, and lidocaine, are used to treat the colon. Among these, hyoscine butylbromide exerts similar antimuscarinic anticholinergic effects.[24] In vivo and in vitro studies suggest that glucagon may be activated via the production of cyclic adenosine-3',5'-monophosphate, through a neuronal effect, by the release of catecholamine from the adrenal medulla, or by a combination of these mechanisms. L-menthol directly inhibits gastrointestinal smooth muscle contractility.[15] Lidocaine hydrochloride is thought to affect the mucosal nerves of colonic motility, thereby producing an antispasmodic effect.[25] The temperature was associated with colonic peristalsis. Our present study showed that the cool temperature regulated colonic motility by suppressing the PF of the colon. It was reported that a TRPM8 receptor agonist, (2S,5R)-2-Isopropyl-N(4-methoxyphenyl)-5-methylcyclohexanecarboximide (WS-12), [1-[Dialkyl-phosphinoyl]-alkane (DAPA) 2-5, 1-[Diisopropyl-phosphinoyl]-alkane (DIPA) 1-7, DIPA 1-8, DIPA 1-9, DIPA 1-10 induced the reduction of spontaneous contractions of human colon circular muscle.[13,15] Therefore, our results suggest that TRPM8 activation by cool temperature was associated with the mechanism through which the PF of the colon was suppressed. In a previous study and our own study, TRPM8 was expressed in the sensory neurons of the colon,[11,13,26] thus, low temperature could activate the sensory input. It was reported that ligand-dependent TRPM8 activation is able to reduce the spontaneous contractions in the human colon, probably through the opening of the large-conductance Ca^{2+}-dependent K^+-channels.[13] Therefore, in our study, TRPM8 activation by low temperature may reduce the colonic peristalsis through the opening of the large-conductance Ca^{2+}-dependent K^+-channels. However, it appears that Na^+ channels and A-type K^+ channels are expressed in neuronal cells and extremely sensitive to temperature, and that T-type Ca^+ channels are also sensitive to temperature. The activation of these channels can be shifted to the positive potential by low temperature and result in decreased the pacemaker activity in the colon. It has been reported that gastrointestinal peristalsis may be involved in the pathophysiology of various diseases, and research on this potential drug discovery target is progressing. Thus, the mechanism of suppression of colonic peristalsis by cool temperature should be further investigated.

In our clinical study, mildly cool (15°C) water significantly suppressed colonic peristalsis. However, in the rat colon model, 15°C water did not suppress colonic peristalsis, while 5°C water significantly suppressed colonic peristalsis. In the clinical study, via the biopsy channel of the endoscope, we sprayed 20 mL water directly on the cecum within just 1 second. In the rat colon model, 2.5 mL saline was administered into the colon via the tube within 25 seconds. The injection speed in the rat model was much slower than that in the clinical study. Although we could not measure the precise tem-

perature of water or saline at the colon, the temperature of the 5°C saline was expected to be higher than 5°C when it reached colon of the animals and the temperature of the 15°C saline was expected to be higher than 15°C when it reached the colon of the animals, while the temperature of the sprayed 15°C water was expected to be almost equal. We therefore used water at a temperature of 15°C as a candidate TRPM8 agonist in our clinical study and used saline at temperatures of 5°C and 15°C as candidate TRPM8 agonists in the animal colon model. This may be the reason for the difference in the effective temperature between the clinical study and in the animal colon model. As TRPA1 could be activated by noxious cold (below 15°C), the solution of 5°C in the animal colon model can activate TRPA1. This was the reason why we used a TRPA1 antagonist in the animal colon model.

In the clinical study, the administration of 15°C water to the colonic mucosa did not lower the temperature of the colonic mucosa to 15°C because of the body temperature. In the rat model, the administration of 5°C water to the colonic did not lower the temperature of the rat colon to 5°C due to the presence of warm Krebs solution. A cool temperature regulated colonic peristalsis; thus, the gastrointestinal mucosa may have a system that responds to a highly diverse range of sensory stimuli, including nociceptive compounds, temperature, touch, pheromones, and osmolarity. It was reported that the TRP channels, TRPV1, TRPA1, and TRPM8, are expressed in the gastrointestinal tract.[11,12,27] It was reported that cold tolerance was regulated by mechanoreceptor-mediated circuit calculation in a worm model.[28] The biological effects of low temperature on the occurrence of slow wave motion and colon motility require further investigation.

We had some limitations in the present study. First, we undertook this study at a single center. Second, although the assigned teatment was blinded to patients, it was not blinded to colonoscopists. Third, the endoscopists evaluated the colonic peristalsis subjectively. Fourth, the temperature of the administered water and saline was not measured precisely at the colon mucosa in both the clinical study and the animal model.

In summary, our findings suggest that cool temperature was associated with a significant decrease in colonic peristalsis by suppressing the PF of the colon through TRPM8 activation. We showed for the first time that cool temperature was associated with a decrease in colonic peristalsis in both a clinical study and an animal model, and TRPM8 activation may be associated with colonic peristalsis.

Supplementary Materials

Note: To access the supplementary figure and videos mentioned in this article, visit the online version of *Journal of Neurogastroenterology and Motility* at http://www.jnmjournal.org/, and at https://doi.org/10.5056/jnm21198.

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Itoh: lead of supervision, writing of review, and editing. All authors had access to the study data and reviewed and approved the final manuscript.

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