Increased Tone of the Human Colon Muscle by Bisacodyl In Vitro

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Background/Aims
Although bisacodyl is a widely administered laxative, its underlying mechanism of action remains generally unknown. This study focuses on investigating the effects of bisacodyl on the human colon muscle contraction, and elucidating its mechanism of action.

Methods
Sigmoid colon muscle strips (20 longitudinal and 18 circular muscles) were obtained from 20 subjects who underwent colectomy for colon cancer. Isometric force measurements were calculated in response to electrical field stimulation (EFS, 0.3 milliseconds in trains of 10 Hz for 20 seconds, 150 V). Peak and nadir (tone) during and after EFS, were measured in a controlled state, and after sequential addition of bisacodyl (1 µM), atropine (1 µM), N-nitro-L-arginine (L-NNA, 100 µM), MRS2500 (1 µM), and tetrodotoxin (TTX, 1 µM) to the organ bath.

Results
Transient phasic contractions were observed during EFS, and after cessation of EFS. In the longitudinal muscles, nadir during EFS, and tone after EFS, significantly increased after addition of bisacodyl, and persisted after sequential addition of atropine, L-NNA, MRS2500, and TTX, indicating a direct action of bisacodyl on the smooth muscle. In the second experiment, pretreatment of TTX abolished EFS-induced phasic contractions. Although no phasic contraction was produced after perfusion of bisacodyl, tone was increased, thereby supporting evidence of a direct mechanism of action of bisacodyl on the colon smooth muscle.

Conclusions
Bisacodyl increases the tone of longitudinal muscle in the human sigmoid colon through a direct action on the smooth muscle. Further study is warranted to investigate the neural mechanism of action of bisacodyl.

(J Neurogastroenterol Motil 2018;24:317-323)

Key Words
Bisacodyl; Colon; Humans; Muscle contraction; Physiology
Introduction

Bisacodyl is a diphenylmethane stimulant laxative, which is frequently administered for treating functional constipation, bowel preparation before colonoscopy, and pharmacologic provocation during colon manometry.\textsuperscript{1-3} Bisacodyl induces high-amplitude propagating contractions (HAPCs), which is the colonic propulsive motor activity.\textsuperscript{3-5} However, the underlying mechanism of action of bisacodyl on the colon is largely unknown.

Guidelines for medical management of chronic constipation recommend bisacodyl as the second line treatment, when bulk or osmotic laxatives are ineffective, for improving bowel movement and stool consistency.\textsuperscript{1-3} Although it is unlikely that chronic use of bisacodyl is harmful to the colon, it could induce abdominal discomfort and cramping pain.\textsuperscript{5,6} In addition, new drugs such as lubiprostone, linaclotide, and prucalopride have been developed, and could be administered when conventional laxatives fail to manage constipation, and irritable bowel syndrome with constipation.\textsuperscript{5-12} Thus, bisacodyl needs to be efficiently administered with a consideration of its mechanism of action.

This study aims to investigate the effects of bisacodyl on the human colon muscle contraction and to elucidate its mechanism of action.

Materials and Methods

Subjects and Tissues

All patients provided written informed consent, before inclusion in this study. The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Samsung Medical Center, Seoul, South Korea (No. 2012-02-031, 20th March 2012). Sigmoid colon tissues were collected from 20 subjects (14 males, median age 62 years, age range 50-77 years) who underwent colectomy for colon cancer at Samsung Medical Center from October 2016 to March 2017. Shortly after colectomy, colon smooth muscle specimens were collected from regions free of macroscopic evidence of cancer infiltration. The specimens were collected in cold oxygenated modified Krebs-Ringer bicarbonate solution (97% O\textsubscript{2} and 3% CO\textsubscript{2}), and immediately transported to the laboratory. After specimens were pinned to the base of a Sylgard Silicone Elastomer dish, the mucosa was extracted via sharp dissection. Then, thin strips of tissues were cut in parallel to the muscle fibers in 2 × 10 mm slices.

Organ Chamber Experiment

Experiments were performed in vitro with strips of both longitudinal (n = 20) and circular (n = 18) muscles. The mechanical activities were recorded, as changes in isometric force using standard organ bath techniques, previously described in another study conducted by our laboratory.\textsuperscript{13-16} Isolated muscle strips were attached to a fixed mount, and to a Fort 10 isometric strain gauge (UC3-Gould Instruments, Paris, France; FT03-GFASS, Warwick, RI, USA). The strips were immersed in organ baths maintained at 37 ± 0.5°C with oxygenated Krebs-Ringer bicarbonate solution. After stabilization for 60 minutes, electrical field stimulation (EFS) of intramural nerves was carried to attached strips by 2 platinum ring electrodes with an optimal frequency and duration (0.3 milliseconds in trains of 10 Hz for 20 seconds, 150 V). The electrodes were connected to a GRASS S88 (GRASS Instruments, Quincy, MA, USA) stimulator.

Experimental Protocol and Measurement

We measured EFS-induced contractile responses in a control state and after the administering of bisacodyl (1 µM; Merck KGaA, Darmstadt, Germany). To understand the cholinergic, purinergic, and nitric roles in the EFS-induced response under bisacodyl perfusion, atropine (a muscarinic antagonist, 1 µM), N-nitro-L-arginine (L-NNA; a nitric oxide synthase inhibitor, 100 µM), and MR2500 (a purinergic P\textsubscript{2Y}\textsubscript{1} antagonist, 1 µM), were added in a sequential order to the organ bath. Thus, tetrodotoxin (TTX, 1 µM) was applied to inhibit any nerve-mediated contraction. Muscle strips (13 longitudinal and 12 circular muscles) were perfused with each drug for at least 20 minutes after EFS, and had a recovery time for 5 minutes before perfusion of the next drug (Fig. 1).

Peak (the highest value) and nadir (the lowest value) amplitudes of both circular and longitudinal muscle strips were measured with a passive tension of 0.7 g during EFS of 20 seconds. After cessation of EFS, post-stimulus rebound (PSR) responses were produced as reported in the previous study.\textsuperscript{17} Thus, we also measured peak and tone of PSR. PSR tone was defined as the value 2 minutes after the initiation of EFS. In addition, late (stabilized) tone was measured as the value just before perfusion of the next drug (Fig. 2).

To test the direct effect of bisacodyl on the colon smooth muscle, muscle strips (7 longitudinal and 6 circular muscles) were immersed in TTX-containing organ bath, and were stimulated before and after additional perfusion of bisacodyl (Fig. 3).
Effects of Bisacodyl

Vol. 24, No. 2   April, 2018 (317-323)

Statistical Methods

Data are expressed as mean ± SD. The Wilcoxon signed-rank test was used to evaluate the effects of each drug by compare values to the previous one. P-values less than 0.05 were considered significant. Statistical analysis was performed using SPSS version 21 (IBM Corp, Armonk, NY, USA).

Results

Effects of Bisacodyl on the Phasic Contraction During Stimulation

Phasic contractions were observed by EFS (Fig. 2). After addition of atropine, EFS elicited early phasic relaxation and delayed contraction following cessation of EFS (Fig. 1). In longitudinal muscles (n = 13), the peak decreased from 1.55 ± 0.72 g to 1.18 ± 0.49 g after perfusion of bisacodyl (Fig. 4A). Peak values decreased further to 0.61 ± 0.24 g after perfusion of atropine and increased to 0.71 ± 0.20 g after addition of L-NNA. Subsequent addition of MRS2500 and TTX did not affect peak values. These observations indicate that bisacodyl decreases the peak contraction in the sigmoid colon longitudinal muscle, and cholinergic and nitrergic pathways are active in the peak contraction under bisacodyl perfusion. However, the nadir increased from 0.28 ± 0.22 g to 0.82 ± 0.25 g after perfusion of bisacodyl (Fig. 4B). Increased nadir persisted after sequential addition of atropine, L-NNA, MRS2500, and TTX, indicating that bisacodyl increases the tone in the sigmoid colon longitudinal muscle through direct action on the smooth muscle (Fig. 4B). On the other hand, bisacodyl did not show significant effects on the peak and nadir of the colonic circular muscle (n = 12) (Fig. 4C and 4D).

Effects of Bisacodyl on the Post-stimulus Rebound and Tone

After cessation of EFS, PSR was produced (Fig. 2). Effects of bisacodyl on the PSR were similar to that during stimulation. In longitudinal muscles (n = 13), the PSR peak decreased from 1.29 ± 0.63 g to 0.60 ± 0.58 g after perfusion of bisacodyl (Fig. 5A). The PSR peak decreased further to 0.08 ± 0.54 g, after perfusion of atropine and increased to 0.20 ± 0.46 g after addition of L-NNA. Subsequent addition of MRS2500 and TTX did not affect the PSR peak. These observations indicate that bisacodyl decreases the PSR peak contraction in the sigmoid colon longitudinal muscle, and cholinergic and nitrergic pathways are active in the PSR peak contraction under bisacodyl perfusion. However, PSR tone in-
creased from 0.41 ± 0.54 g to 0.73 ± 0.26 g after perfusion of bisacodyl (Fig. 5B). Increased PSR tone persisted after sequential addition of atropine, L-NNA, MRS2500, and TTX, indicating that bisacodyl increases the PSR tone in the sigmoid colon longitudinal muscle through direct action on the smooth muscle (Fig. 5B). Bisacodyl did not significant affect the post-stimulus peak and tone of the colonic circular muscle (n = 12) (Fig. 5C and 5D).

Effects of Bisacodyl on the Late Tone

Late tone increased from 0.26 ± 0.22 g to 0.80 ± 0.24 g after perfusion of bisacodyl (Fig. 6A). Increased PSR tone persisted after sequential addition of atropine, L-NNA, MRS2500, and TTX, indicating that bisacodyl increases the PSR tone in the sigmoid colon longitudinal muscle through direct action on the smooth muscle (Fig. 5B). Bisacodyl did not significant affect the post-stimulus peak and tone of the colonic circular muscle (n = 12) (Fig. 5C and 5D).

Effects of Bisacodyl on the Longitudinal Muscle Under Pre-treatment of Tetrodotoxin

Pre-treatment of TTX abolished EFS-induced phasic contraction (Fig. 3). No EFS-induced phasic contraction was produced after perfusion of bisacodyl. However, the tone increased from 0.140 ± 0.141 g to 0.809 ± 0.641 g after perfusion of bisacodyl in the longitudinal muscle (n = 7), and from 0.167 ± 0.074 g to 0.423 ± 0.200 g in the circular muscle (n = 6), indicating a direct mechanism of action of bisacodyl on the colson muscles.

The amount of increased tone and time latency to the maximal tone after perfusion of bisacodyl was compared between with and without pre-treatment of TTX. There were no differences in the longitudinal and circular muscles through direct action on the smooth muscle.
**Figure 5.** Post-stimulus rebound (PSR) peak and tone after serial administration of bisacodyl, atropine, N-nitro-L-arginine (L-NNA), MRS2500, and tetrodotoxin (TTX). (A) PSR peak in the longitudinal muscle. (B) PSR tone in the longitudinal muscle. (C) PSR peak in the circular muscle. (D) PSR tone in the circular muscle. The Wilcoxon signed-rank test was used to evaluate the effects of each drug by compare values to the previous one: *P < 0.05 and **P < 0.01.

**Figure 6.** Late tone after serial administration of bisacodyl, atropine, N-nitro-L-arginine (L-NNA), MRS2500, and tetrodotoxin (TTX). (A) Late tone in the longitudinal muscle. (B) Late tone in the circular muscle. The Wilcoxon signed-rank test was used to evaluate the effects of each drug by compare values to the previous one: *P < 0.05 and **P < 0.01.
longitudinal muscle. Latency was longer with pre-treatment of TTX than without in the circular muscle (39.8 minutes vs 26.8 minutes), while there was no difference regarding the amount of increased tone.

Discussion

Bisacodyl is a potent stimulus laxative. Although bisacodyl has safety concerns relative to long-term use, it appears to be effective in practice. However, the underlying mechanism of action of bisacodyl on the colon remains to be elucidated, especially in humans. If the effects of bisacodyl on the human colon are well defined and its mechanism of action is elucidated, we could administer bisacodyl more effectively for patients with constipation. Thus, we investigated the effects of bisacodyl on human colon muscle contraction, using an organ bath technique, and elucidated its mechanism of action. Bisacodyl directly increases the tone of sigmoid colon muscles, while it decreases the peak contraction of the sigmoid longitudinal muscle, which probably is able to facilitate colonic propulsion.

In an early German study, bisacodyl increased the tone of isolated muscle strips of the human colon. The effect of bisacodyl could not be prevented by TTX, and abolished by pre-treatment with verapamil, antagonizing calcium influx into the muscle cell, showing similar results to the current study: On the contrary, an increase in the sporadic spiking activity during stimulation by bisacodyl has been reported in humans. This electromechanical coupling vanishes with the application of Lidocaine onto the colon mucosa indicating that bisacodyl acts on the mucosal nerve endings. An experimental animal study revealed that bisacodyl inhibits water absorption by increasing the secretion of prostaglandin E₂ from macrophages in the colon mucosa. However, prostaglandin E₂ induces longitudinal smooth muscle contraction via the EP₁ receptor in the human colon. Thus, the direct effect on the colon smooth muscle, and an activation of mucosal endings of myenteric and submucosal nerves have been suggested as a mechanism of action of bisacodyl.

Colonic HAPC is an important mechanism for facilitating the transit of colonic contents over long distances, and often precedes defecation. Intraluminal infusion of bisacodyl can elicit HAPC in humans. Thus, bisacodyl is administered for a pharmacologic provocation to assess colonic neuromuscular function integrity. In a recent study, a higher dose of bisacodyl during colonic manometry increased the number of propagated HAPCs. This implies that an activation of mucosal nerve endings may contribute more to the mechanism of action of bisacodyl, compared to a direct effect on the colon smooth muscle, because the intrinsic neural reflex within the colonic myenteric plexus has been suggested as a potential underlying mechanism of HAPC. However, the tonic contraction of the longitudinal muscle by a direct effect of bisacodyl on the smooth muscle could reduce colon elongation, which improves the spatiotemporal-coordinated pattern of HAPCs. Thus, bisacodyl would improve HAPCs in terms of facilitating the transit of colon contents. From the practical point of view, bisacodyl could be effectively administered for managing constipation in patients with neuropathy.

In the present study, it took about 60 minutes to reach the maximal response (increased basal tone of colon muscle strips) of bisacodyl. Interestingly, the potentiated tone lasted more than 2 hours (Fig. 1). The effect of bisacodyl gradually reduced and nearly abolished at 2 hours by washout (data not shown). Although it is uncertain how much the direct effect of bisacodyl is involved in practical use, we could expect its persistent direct effect on the colon when the concentration of bisacodyl is maintained.

In conclusion, the present study provides evidence that a direct action of bisacodyl on the smooth muscle increases the tone of longitudinal muscle in the human sigmoid colon, suggesting that bisacodyl could be effectively administered for managing constipation in patients with neuropathy. Further study is warranted to investigate the neural mechanism of action of bisacodyl.

Financial support: None.

Conflicts of interest: None.

Author contributions: Yang Won Min contributed to the data analysis and interpretation, and drafted the manuscript; Eun-ju Ko and Ji Yeon Lee performed experiments; Jeong Hwan Kim contributed to the data interpretation and edited the manuscript; Hee...
Cheol Kim and Woo Yong Lee contributed to the data collection; Poong-Lyul Rhee designed and coordinated the study, contributed to the data interpretation, and edited the manuscript; and all the authors approved the final version of the manuscript.

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